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PRESYNAPTIC DEPOLARIZATION MEDIATES PRESYNAPTIC INHIBITION AT A SYNAPSE BETWEEN AN IDENTIFIED MECHANOSENSORY NEURONE AND GIANT INTERNEURONE 3 IN THE FIRST INSTAR COCKROACH, *PERIPLANETA AMERICANA*

BY JONATHAN M. BLAGBURN AND DAVID B. SATTELLE

AFRC Unit of Insect Neurophysiology and Pharmacology, Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, UK

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SUMMARY

Intracellular microelectrodes were used to study presynaptic inhibition at a cholinergic synapse between identified neurones: the lateral filiform hair sensory neurone (LFHSN) and giant interneurone 3 (GI 3) in the terminal ganglion of the first instar cockroach *Periplaneta americana*. The LFHSN–GI 3 synapse was shown to fulfil physiological criteria for monosynaptic transmission: the latency of the EPSPs was 1·4 ms and was constant during high-frequency firing of LFHSN; transmission was progressively and reversibly abolished by replacement of Ca^{2+} with Mg^{2+}.

Movement of the lateral filiform hair towards the cercal tip produced a burst of spikes in LFHSN and a burst of EPSPs in GI 3. Movement of the medial filiform hair towards the base of the cercus produced a burst of spikes in the medial filiform hair sensory neurone (MFHSN) and a burst of EPSPs in GI 2. EPSPs evoked in GI 3 by LFHSN spikes were inhibited during bursts of EPSPs in GI 2 which were evoked by MFHSN spikes. LFHSN was depolarized and its spikes were reduced in amplitude during spike bursts in MFHSN. Reduction in LFHSN spike amplitude reduced GI 3 EPSPs. This phenomenon was attributed, therefore, to presynaptic inhibition. The occurrence of presynaptic inhibition was dependent upon the degree of delayed rectification exhibited by the LFHSN axon. Hyperpolarization of LFHSN increased spike height, but did not increase the amplitude of GI 3 EPSPs.

The delay between the onset of MFHSN-evoked EPSPs in GI 2 and MFHSN-evoked depolarizations in LFHSN suggested that MFHSN does not synapse directly onto LFHSN. Neither depolarization nor hyperpolarization of GI 2 had any effect on MFHSN-mediated presynaptic inhibition of LFHSN–GI 3 transmission, therefore it was considered unlikely that GI 2 synapses onto LFHSN. Prolonged hyperpolarization lowered the LFHSN spike threshold and temporarily abolished presynaptic inhibition.

Bursts of spikes in LFHSN mediated presynaptic inhibition of MFHSN–GI 2 EPSPs. Mutual presynaptic inhibition by the FHSNs may have a functional significance in sharpening the boundaries of the GIs' directional sensitivities.

Key words: presynaptic inhibition, primary afferent depolarization, giant interneurones, cockroach.

INTRODUCTION

Presynaptic inhibition, a term coined by Frank & Fuortes (1957), has been described extensively in vertebrates (reviewed by Eccles, 1964; Schmidt, 1971; Ryall, 1978; Nicholl & Alger, 1979). It has also been reported in invertebrates: the neuromuscular junctions of crustaceans (Dudel & Kuffler, 1961; Nakajima, Tisdale & Henkart, 1973; Fuchs, 1977) and insects (Parnas & Grossman, 1973); the central nervous system of crustaceans (Kennedy, Calabrese & Wine, 1974; Bryan & Krasne, 1977), insects (Levine & Murphey, 1980; Pearson & Goodman, 1981; Hue & Callec, 1983), annelids (Nicholls & Wallace, 1978) and molluscs (Shapiro, Castellucci & Kandel, 1980*a*,*b*).

A variety of possible mechanisms of presynaptic inhibition have been proposed. The earliest of these (Eccles, 1964) correlates presynaptic depolarization (primary afferent depolarization) with a decrease in spike amplitude, resulting in turn in a reduction of the amount of transmitter released. Depolarization-induced suppression of neurotransmitter release has been demonstrated for the presynaptic terminal of the squid giant synapse (Miledi & Slater, 1966). In some preparations it can be shown directly that spike amplitude is reduced during presynaptic inhibition (Eccles, Schmidt & Willis, 1963; Kennedy *et al.* 1974; Levine & Murphey, 1980; Pearson & Goodman, 1981). However, in these cases it has not been demonstrated that the reduction in spike amplitude itself leads to a reduction of transmitter released.

In other preparations it is thought that a conductance increase results in suppression of the presynaptic spike height, irrespective of the sign of the potential change (Baxter & Bittner, 1981; Pearson & Goodman, 1981; Hue & Callec, 1983). Such conductance increases may be mediated by Cl^-, and the neurotransmitter involved is thought to be γ-aminobutyric acid (GABA) (Nishi, Minota & Karczmar, 1974; Adams & Brown, 1975; Gallagher, Higashi & Nishi, 1978; Pearson & Goodman, 1981; Hue & Callec, 1983). It has also been suggested that an increase in conductance may block the conduction of spikes through small-diameter branches of the presynaptic axon, resulting in presynaptic inhibition (Wall, 1964; Atwood, 1976).

More recent evidence, from *Aplysia* neuronal synapses, shows that the presynaptic Ca^{2+} current is reduced as a direct result of the action of the inhibitory neurotransmitter (Shapiro *et al.* 1980*a*,*b*). It has now been established that several neurotransmitters, e.g. noradrenaline, GABA, serotonin and certain peptides either modify Ca^{2+} currents or alter the duration of Ca^{2+} spikes in vertebrate and invertebrate neurones (Dunlap & Fischbach, 1978, 1981; Horn & McAfee, 1980; Bixby & Spitzer, 1983; Kandel & Schwartz, 1982; Leonard & Wickelgren, 1985).

Relatively few preparations in which presynaptic inhibition takes place allow microelectrode impalement of both pre- and postsynaptic neurones. Very few of these fulfil the condition that the presynaptic recording site is physically and/or electrically close to the site of neurotransmitter release.

We have recently described a preparation in which both the pre- and postsynaptic elements of a neuronal synapse are accessible to impalement by microelectrodes

(Blagburn, Beadle & Sattelle, 1986). These synapses, between the filiform hair sensory neurone (FHSN) and giant interneurone (GI) in the terminal abdominal ganglion play an essential part in the escape response of the first instar cockroach *Periplaneta americana* (Dagan & Volman, 1982). Acetylcholine is the likely neurotransmitter at these synapses (Sattelle, 1985). Presynaptic inhibition of unidentified cercal afferent fibres has been shown to occur in the adult insect (Hue & Callec, 1983).

The first instar terminal ganglion preparation is advantageous in that it allows living neurones to be seen with Nomarski optics, and the relatively large FHSN axons can be impaled at a distance estimated to be less than 5 μm from a major site of synaptic activity (see Blagburn *et al.* 1984). In this study we first ascertain if the physiological criteria for monosynaptic transmission (Berry & Pentreath, 1976) are fulfilled by the synapse between the lateral FHSN (LFHSN) and GI 3 in the first instar cockroach. Second, we investigate if, and how, presynaptic inhibition takes place at identifiable FHSN–GI synapses.

MATERIALS AND METHODS

Recently hatched, first instar cockroach nymphs (*Periplaneta americana*) were placed in a saline solution of the following composition (in mmol l^{-1}): NaCl, 150·0; KCl, 3·1; $CaCl_2$, 5·4; Hepes, 5·0; sucrose, 50·0; pH 7·2 (based on Callec & Sattelle, 1973). The animals were dissected as described previously (Blagburn *et al.* 1985*b*) to isolate the CNS and the cerci. The preparation was then anchored, ventral-side up, to a microscope slide using petroleum jelly. The connective tissue sheath around the terminal abdominal ganglion was softened by a brief (5 s) exposure to saline containing 1·0 mg ml^{-1} protease (Type XIV, Sigma, UK), then removed using fine forceps. The lateral filiform hair on the left cercus was immobilized with petroleum jelly, leaving the medial hair free to move. The right cercal nerve was crushed.

Isolated preparations were viewed with Nomarski optics, using a Zeiss 40× water-immersion objective lens, electrically isolated from the body of the microscope with a Perspex insert. The cell bodies of GI 2 and GI 3 together with the axon of LFHSN were identified visually as described elsewhere (Blagburn *et al.* 1985*a*). GIs were described as left or right, and ipsilateral or contralateral, according to the location of the cell body. Each GI has an axon and major dendritic field which are contralateral to the cell body, and a minor dendritic field on the neurite which is ipsilateral to the cell body.

For intracellular recording, glass capillary microelectrodes were filled with 1·0 mol l^{-1} KCl with 5·0 mmol l^{-1} Hepes (adjusted to pH 7·2 with KOH) or 1·0 mol l^{-1} potassium acetate (adjusted to pH 7·2 with acetic acid) giving a resistance of 30–60 MΩ. The microelectrode resistance was subtracted electronically using the 'bridge balance' technique of Engel, Barcilou & Eisenberg (1972). In the present study this technique gave good results since the microelectrode time constant was generally short in comparison to that of the neuronal membrane, particularly in the

case of LFHSN. Other parameters of the neurone, such as the reversal potential of the LFHSN spike after-potential or the spike threshold of the GIs, were used to confirm that the electrodes were accurately balanced. In cases where depolarizing pulses were applied during application of constant hyperpolarizing current, the microelectrode resistance was balanced for the hyperpolarizing current. Due to Type II nonlinearity of the electrode resistance (Purves, 1981) the depolarizing pulses showed some imbalance. Oscilloscope traces were recorded on a Racal Store 4 DS tape-recorder and permanent records were made using a Medelec storage oscilloscope.

RESULTS

Monosynaptic connection between filiform hair sensory neurones and giant interneurones 2 and 3

Resting potentials were recorded in the range of −50 to −60 mV in lateral (LFHSN) or medial (MFHSN) filiform hair sensory neurone axons and −70 to −80 mV in the cell bodies of giant interneurone 2 (GI 2) and giant interneurone 3 (GI 3). Action potentials (spikes) were generated spontaneously by the FHSN cell bodies within the cerci. The spikes recorded in the FHSN axons within the ganglion ranged in amplitude from 40 mV to 70 mV, usually peaking at a membrane potential of about −4 mV.

Synchronous recording from LFHSN and the contralateral GI 3 cell body showed that spikes in the sensory axon gave rise to depolarizing postsynaptic potentials in the interneurone (Fig. 1A). At high frequency these potentials could summate to give rise to interneurone spikes; they are therefore termed excitatory postsynaptic potentials (EPSPs). The amplitude of these EPSPs was 6·4 ± 0·3 mV (mean ± S.E., $N = 91$) when measured in the soma of GI 3, and a latency of 1·37 ± 0·06 ms (mean ± S.E., $N = 21$) was measured from the rising phase of the presynaptic spike to the onset of the EPSP.

Stimulation of LFHSN by injection of current to produce a suprathreshold depolarization (approximately 10–12 mV from rest) elicited action potentials at rates of up to 300 Hz. GI 3 EPSPs followed these spikes with a constant latency and no failure of synaptic transmission was detected (Fig. 1A).

Alteration of the Ca^{2+}:Mg^{2+} ratio by perfusion with a Ca^{2+}-free saline containing 20 mmol l^{-1} Mg^{2+} resulted in progressive abolition of EPSPs (Fig. 1B). Washing with normal saline resulted in recovery of synaptic transmission within 10 min.

The above physiological evidence supports the hypothesis that there is a monosynaptic chemical connection between LFHSN and GI 3, though it does not rule out the possibility that a non-spiking neurone is interposed between them. Considered together with the results of ultrastructural studies, in which synapses between identified FHSNs and GIs were located (Blagburn *et al.* 1984, 1985*a*), the evidence for monosynaptic chemical connections between LFHSN and GI 3 and between MFHSN and GI 2 becomes compelling.

Giant interneurone responses to filiform hair movements

FHSN spikes elicit monosynaptic EPSPs in both GI 2 and GI 3. MFHSN elicits 4–8 mV EPSPs in the contralateral GI 2 but supplies little or no synaptic input to the ipsilateral GI 3. LFHSN elicits 4–8 mV EPSPs in both ipsilateral and contralateral GI 3 and has little synaptic connection with either GI 2. The GI inputs from the FHSNs are illustrated diagrammatically in Fig. 2. No synaptic connections between

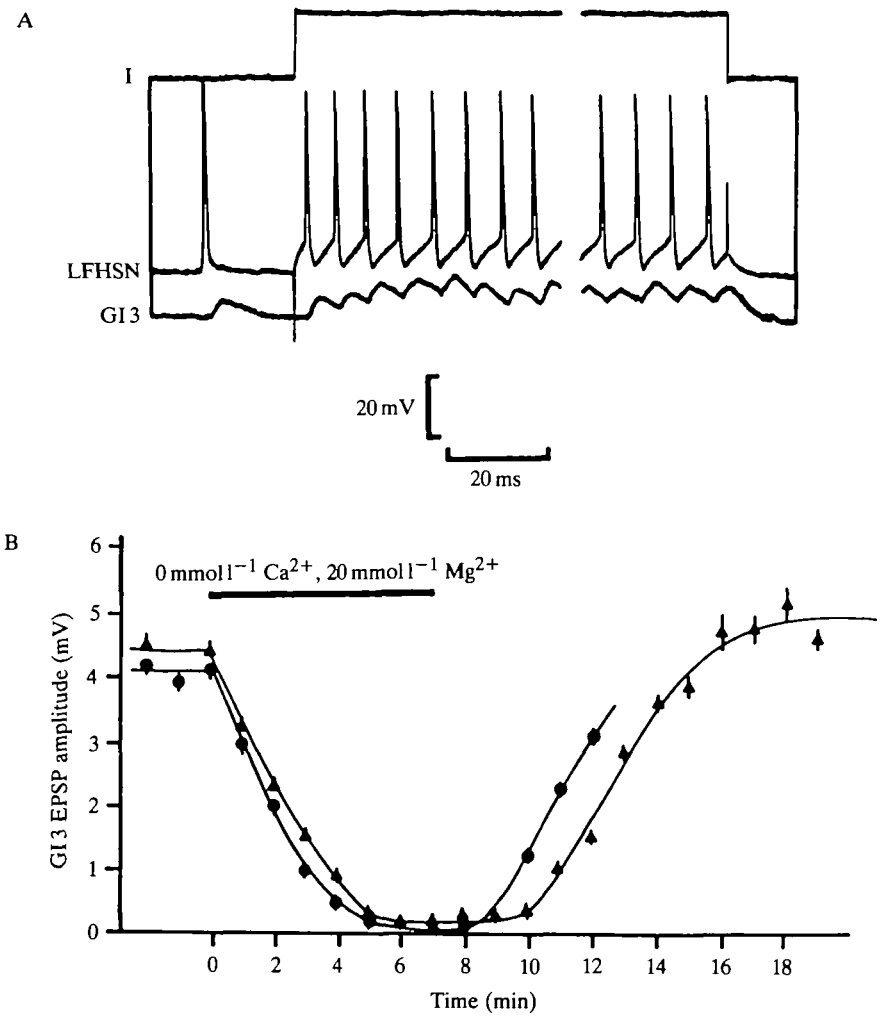

Fig. 1. Monosynaptic transmission between lateral filiform hair sensory neurone (LFHSN) and giant interneurone 3 (GI 3). (A) LFHSN spiking frequency increased by suprathreshold depolarization (200 ms duration). LFHSN spikes elicit EPSPs in GI 3 which follow the spikes with a constant latency of 1·4 ms. Top trace, current; centre trace, LFHSN potential; bottom trace, GI 3 potential. (B) Gradual and reversible block of LFHSN–GI 3 synaptic transmission by replacement of Ca^{2+} with Mg^{2+} (bar) and subsequent recovery on washing in normal saline. Results from two preparations are shown.

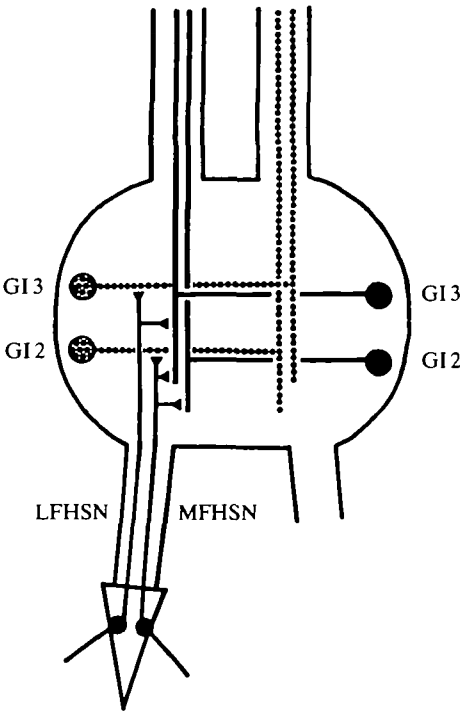

Fig. 2. 'Wiring diagram' for the lateral and medial filiform hair sensory neurones (L and MFHSN) and giant interneurones GI2 and GI3, constructed from the results of microelectrode recordings from the interneurone cell bodies and the FHSN axons (Blagburn, Beadle & Sattelle, 1986). Left and right GI2 and GI3 are shown; each cell has a contralateral homologue. Only the two FHSNs from the left cercus are represented. LFHSN forms an excitatory synaptic connection (denoted by a filled triangle) onto the neurite of the left (ipsilateral) GI3 and the main dendrites of the right (contralateral) GI3. Neither GI2 receives excitatory synaptic input from LFHSN. MFHSN forms excitatory synaptic connections onto the neurite of the ipsilateral GI2, the main dendrites of the contralateral GI2 and the main dendrites of the contralateral GI3. MFHSN forms no synapses with the neurite of the ipsilateral GI3.

contralateral GIs have been observed. These results have been described in more detail previously (Blagburn *et al.* 1986).

Action potentials were produced spontaneously by the FHSNs, but the pattern of spiking changed according to the mobility of the filiform hairs. If the filiform hair innervated by the FHSN was immobilized, action potentials occurred with an approximately constant frequency of 30–60 Hz. However, if the filiform hair was allowed to remain mobile, movements of the saline resulted in 3–4 Hz oscillations of the hair in a plane of movement oriented obliquely to the long axis of the cercus. Intracellular recording from FHSN axons innervating mobile filiform hairs showed that 3–4 Hz oscillations of the hair resulted in bursts of spikes in the FHSNs during which spikes occurred at a frequency of up to 300 Hz (Fig. 3A). FHSN spike bursts elicited summating bursts of EPSPs in the postsynaptic GI which sometimes reached the threshold for GI spikes (about 15 mV as recorded in the soma).

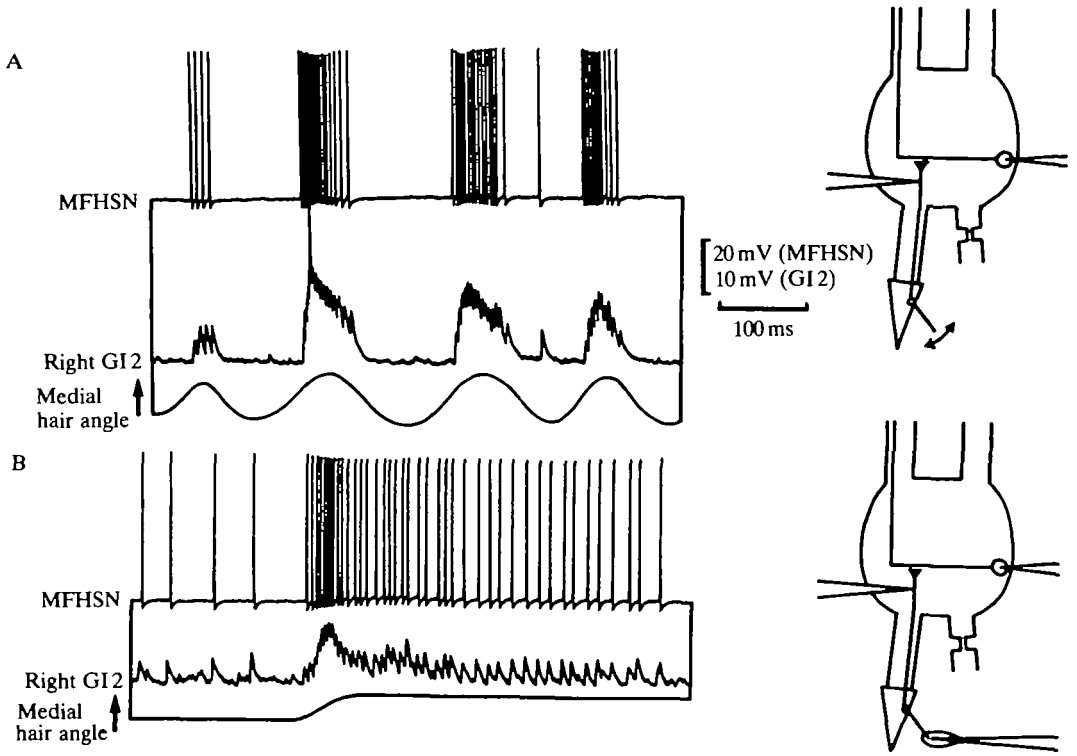

Fig. 3. Microelectrode recordings from the axon of the medial filiform hair sensory neurone (MFHSN, upper trace) and from the cell body of the right (contralateral) giant interneurone 2 (GI 2, second trace). The right cercal nerve was crushed and the left lateral filiform hair immobilized, leaving only the medial hair free to move in the saline. Movements of the filiform hair are represented by a qualitative record of changes in the angle of the hair subtended with the cercus (lower trace). The resting angle of the hair is about 45°, and the maximum deflection about ±30°. The arrow indicates the direction of movement towards the base of the cercus. (A) Oscillations of the hair produce bursts of spikes in MFHSN. Spikes in MFHSN evoke EPSPs in GI 2 which temporally summate, sometimes reaching the spike threshold of the interneurone. (B) Initial one-third of record: the medial filiform hair adhered to a micropipette coated with petroleum jelly, producing spikes of regular frequency in MFHSN. Final two-thirds of record: sudden movement of the hair towards the base of the cercus produced a burst of spikes in MFHSN, which gradually declined in frequency. MFHSN spikes evoke EPSPs in GI 2.

It is known from extracellular recording from the cercal nerve in the first instar that a FHSN responds to movement in one direction by producing a high-frequency burst of spikes and responds to movement in the other direction by an absence of spikes (Dagan & Volman, 1982). LFHSN is excited by movement of the hair towards the tip of the cercus, while MFHSN is excited by movement towards the base of the cercus.

To confirm that the oscillatory stimulus received by the FHSN was due only to filiform hair movements, a third microelectrode coated with petroleum jelly was moved so as to contact the hair. Immobilization of the hair in this way abolished the

spike bursts and resulted in regular spiking. Movement of the hair in the excitatory direction produced a long burst of FHSN spikes and GI EPSPs which declined in frequency over a period of 450 ms (Fig. 3B). Repetitive EPSP bursts in GIs were never observed when all four filiform hairs were immobilized. With a single mobile filiform hair it was assumed that repetitive bursts of EPSPs in a GI were caused by spike bursts in the FHSN innervating that hair.

Inhibition of EPSPs in giant interneurone 3 during medial filiform hair sensory neurone spike bursts

With the left cercal nerve intact and only the medial filiform hair mobile, simultaneous recordings were made from the left GI 3 cell body and the right GI 2 cell body. The left GI 3 receives synaptic input from the regularly spiking LFHSN innervating the fixed lateral hair, but not from the bursting MFHSN. The occurrence of MFHSN spike bursts was inferred by recording the resultant EPSP bursts in the right GI 2, which receives no synaptic input from LFHSN. This recording protocol prevented any possible impalement trauma from affecting the behaviour of the FHSNs.

In nine out of nine preparations it was noted that during EPSP bursts in GI 2 caused by MFHSN spike bursts, the LFHSN-induced EPSPs in GI 3 were usually reduced in amplitude or totally abolished (Fig. 4). In three of these preparations it was noted that there were periods of variable duration when this inhibition did not take place. Statistical comparison of the amplitude of GI 3 EPSPs during intervals between GI 2 bursts with the amplitude of those occurring during bursts showed that there was a marked change in the distribution of EPSP amplitudes (Fig. 5). The interburst EPSPs were distributed normally about a mean of 5·8 mV (S.E. ±0·1, $N = 131$) whereas the EPSP occurring during the bursts had a significantly different mean of 2·0 mV (S.E. ±0·2, $N = 131$) (Student's t-test: $P < 0{\cdot}001$).

Presynaptic inhibition of lateral filiform hair sensory neurone–giant interneurone 3 synapses during medial filiform hair sensory neurone spike bursts

Simultaneous recordings were made from the left GI 3 cell body, the right GI 2 cell body and the synaptic terminal region of the intact left LFHSN. It was not possible to impale both LFHSN and the ipsilateral MFHSN, so EPSP bursts in GI 2 were used as an indicator of MFHSN spike bursts. The appearance of small spike artefacts 1·4 ms prior to GI 3 was considered to be attributable to capacitative coupling between the electrodes impaling GI 3 and LFHSN.

EPSP bursts in GI 2 coincided with reductions in GI 3 EPSP amplitude, as noted previously (Fig. 4). EPSP bursts in GI 2, presumably caused by spike bursts in MFHSN, also coincided with depolarizations of LFHSN which were accompanied by a reduction in LFHSN spike amplitude (Fig. 6). These results indicated that there was a synaptic connection (mono- or polysynaptic) between the bursting MFHSN and LFHSN, producing depolarizations in the latter. These depolarizations were coincident with, and may have been the cause of, a reduction in

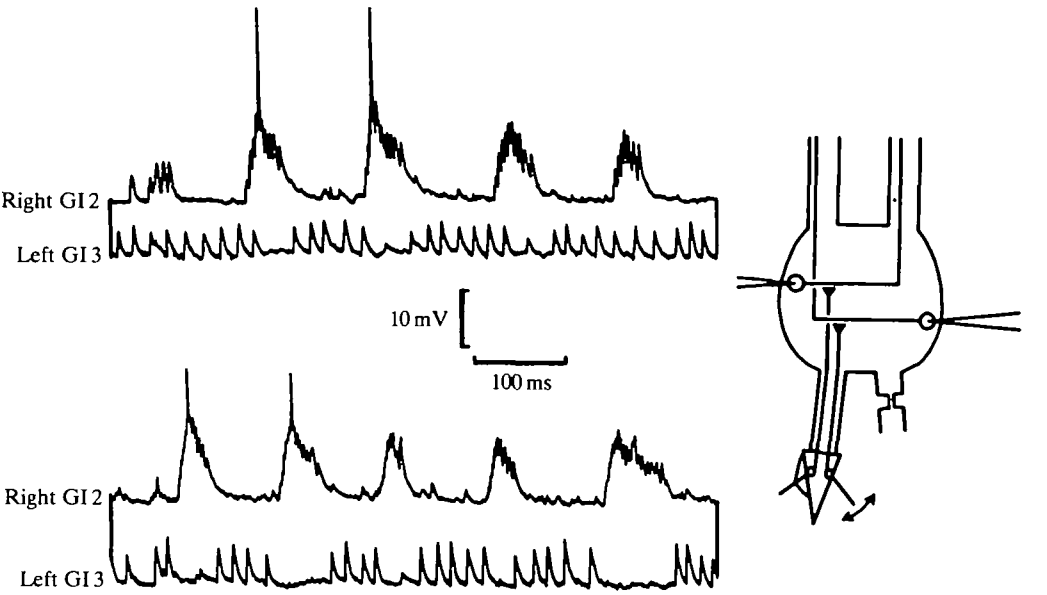

Fig. 4. Microelectrode recordings from the cell bodies of the right giant interneurone 2 (GI 2) and the left GI 3. Two preparations are illustrated. The cell body of GI 2 is contralateral to the cercus bearing the mobile filiform hair, and from it are recorded bursts of EPSPs corresponding to bursts of spikes in MFHSN. The cell body of GI 3 is ipsilateral to the cercus bearing the mobile hair. GI 3 receives no direct synaptic input from MFHSN, only from the LFHSN innervating the immobilized hair. The regularly spaced EPSPs evoked by LFHSN spikes are reduced or abolished during bursts of EPSPs in GI 2.

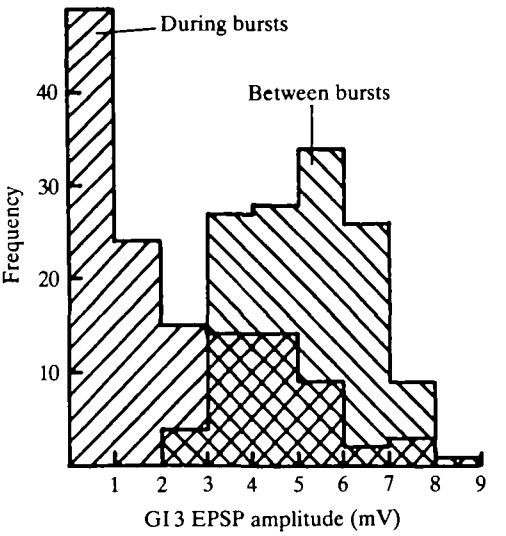

Fig. 5. Frequency–amplitude histograms of the giant interneurone 3 (GI 3) EPSPs during and in between EPSP bursts in GI 2.

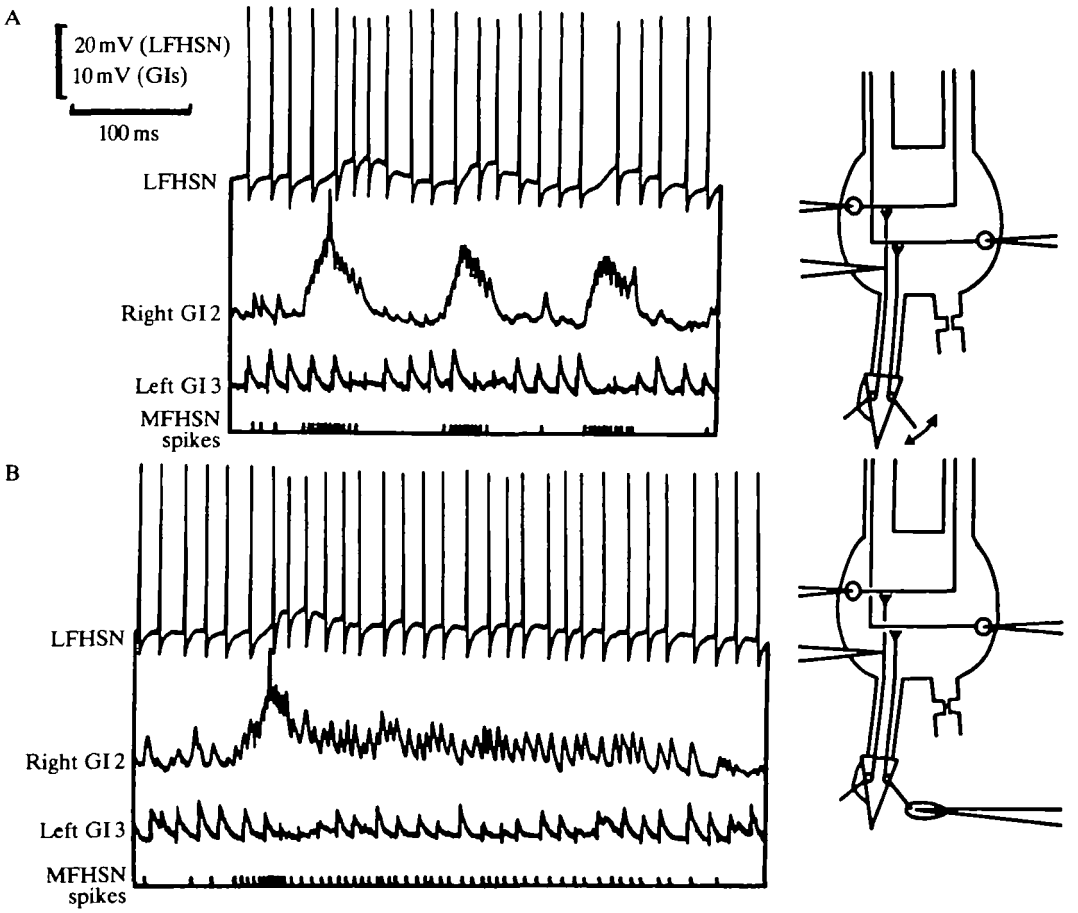

Fig. 6. Microelectrode recordings from the lateral filiform hair sensory neurone (LFHSN, upper trace), right (contralateral) giant interneurone 2 (GI 2, second trace) and left (ipsilateral) GI 3 (third trace). The occurrence of MFHSN spikes was inferred from the positions of EPSPs in GI 2 (bottom trace). (A) GI 3 receives input only from the regularly spiking LFHSN. GI 2 receives input only from the bursting MFHSN. During MFHSN bursts (monitored by recording bursts of EPSPs in GI 2), LFHSN is depolarized. These depolarizations reduce the spike amplitude and GI 3 EPSPs are suppressed. (B) The medial filiform hair is held motionless, then suddenly moved towards the base of the cercus, producing a prolonged burst of spikes in MFHSN (compare Fig. 3B) and a prolonged burst of EPSPs on GI 2. During this burst there is a depolarization of LFHSN and inhibition of LFHSN–GI 3 synaptic transmission.

LFHSN spike amplitude. The reduction in spike amplitude presumably reduced the synaptic Ca^{2+} current. This, in turn, would reduce the amount of acetylcholine released, thus inhibiting the EPSP in GI 3. This phenomenon was termed presynaptic inhibition since, although LFHSN was depolarized, an inhibition of synaptic transmission occurred.

In two preparations the lateral filiform hair was left mobile, while the medial hair was fixed. Recording from the cell bodies of the left GI 3, the right GI 2 and the axon

of MFHSN showed that LFHSN spike bursts mediate presynaptic inhibition of MFHSN–GI 2 synaptic transmission.

Evidence for a delayed rectifier potassium conductance in the axon of the lateral filiform hair sensory neurone

Injection of depolarizing current into LFHSN was accompanied by a decrease in resistance (Fig. 7). The onset of this decrease in resistance (equivalent to a conductance increase) was delayed, resulting in an initial transient depolarization which reached a peak after approximately 2–3 ms (Fig. 8). The delayed conductance increase was only evident when the membrane potential became more depolarized than approximately −50 mV. The conductance increase was blocked by the presence of 0·1 mmol l^{-1} 4-aminopyridine, which has been shown to block inward potassium currents responsible for delayed rectification in cockroach giant axons (Pelhate & Pichon, 1974; Pelhate & Sattelle, 1982). This evidence suggests that the membrane of LFHSN exhibits delayed rectification, a prerequisite for the transmission of fast axonal spikes. Depolarization to potentials above approximately −50 mV results in the opening of voltage-gated K^+ channels and an efflux of K^+.

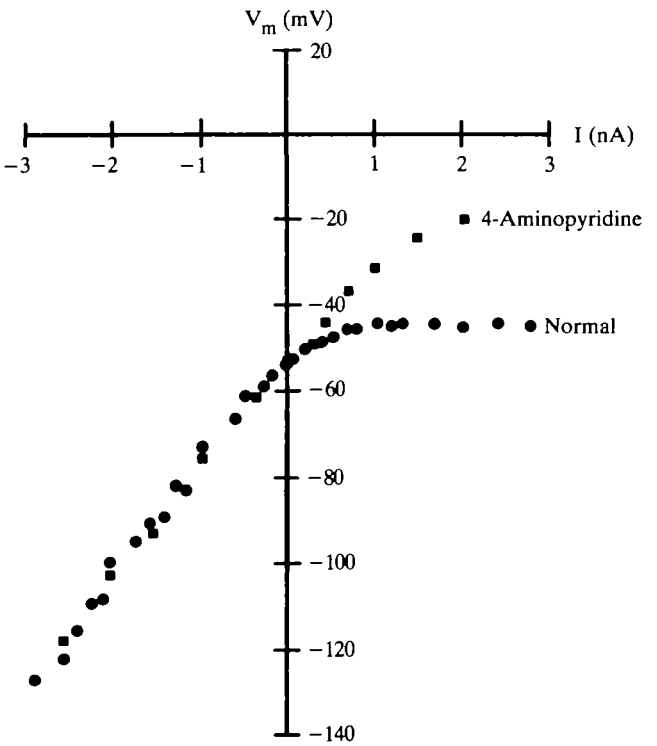

Fig. 7. Current/voltage relationship for the lateral filiform hair sensory neurone (LFHSN), recorded from its axon within the terminal ganglion. The amplitude of the change in potential was measured 20 ms after the onset of the pulse. Rectification is normally present in the depolarizing direction (circles). This is significantly reduced by application of 0·1 mmol l^{-1} 4-aminopyridine (squares).

Depolarization of the lateral filiform hair sensory neurone produces presynaptic inhibition

Depolarizing pulses which were below the spike threshold of LFHSN (about 10 mV positive to rest), and thus failed to increase the firing rate, were normally above the level at which rectification became significant. Spikes occurring spontaneously during the initial period of the depolarizing pulse, prior to the onset of delayed rectification, were not reduced in height as much as those occurring later in the pulse when the K^+ conductance was increased (Fig. 8). Presumably it is the increase in ionic conductance which is important rather than the level of depolarization itself.

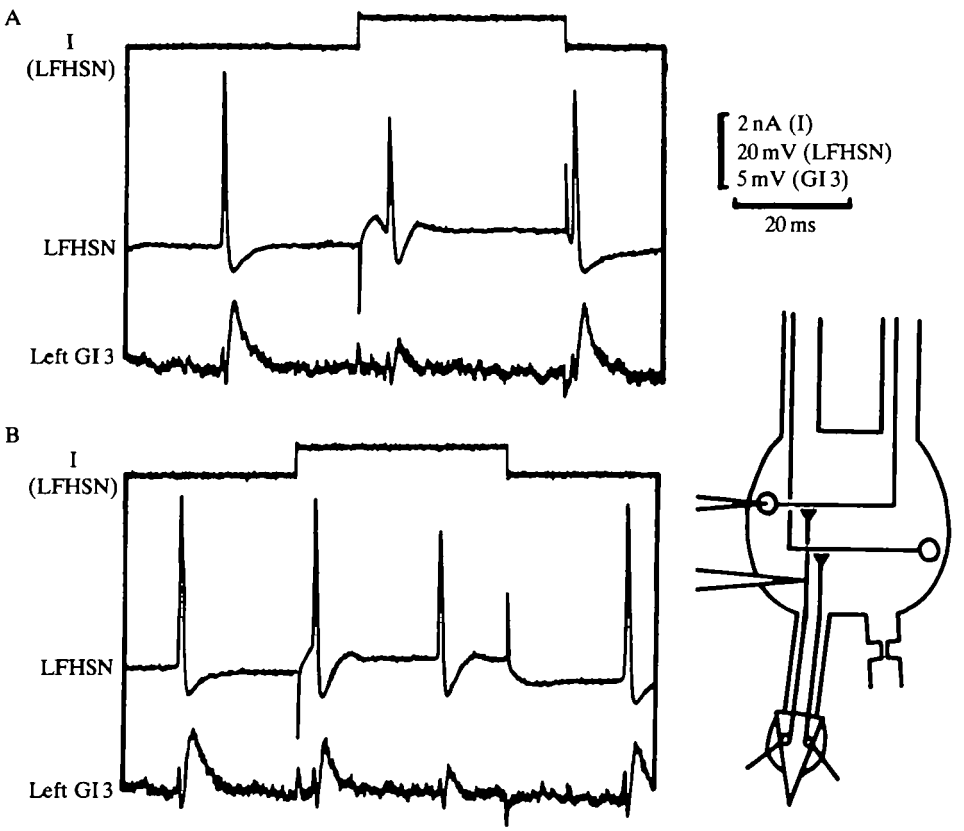

Fig. 8. Delayed rectification in the lateral filiform hair sensory neurone (LFHSN) axon affects spike amplitude. Injection of positive current pulses (top trace) depolarizes the LFHSN (second trace). (A) The initial value of depolarization is higher than the steady-state value (after 5 ms), indicating that delayed rectification takes place. LFHSN spikes are spontaneously produced at regular intervals; those coinciding with depolarizations are reduced in amplitude. (B) Spikes coinciding with the initial part of the pulse, before the onset of delayed rectification, are not greatly reduced. Reduction in spike height reduces EPSPs in giant interneurone 3 (GI 3, lower trace). Small spike artefacts in the GI 3 trace are due to capacitative coupling between the microelectrodes.

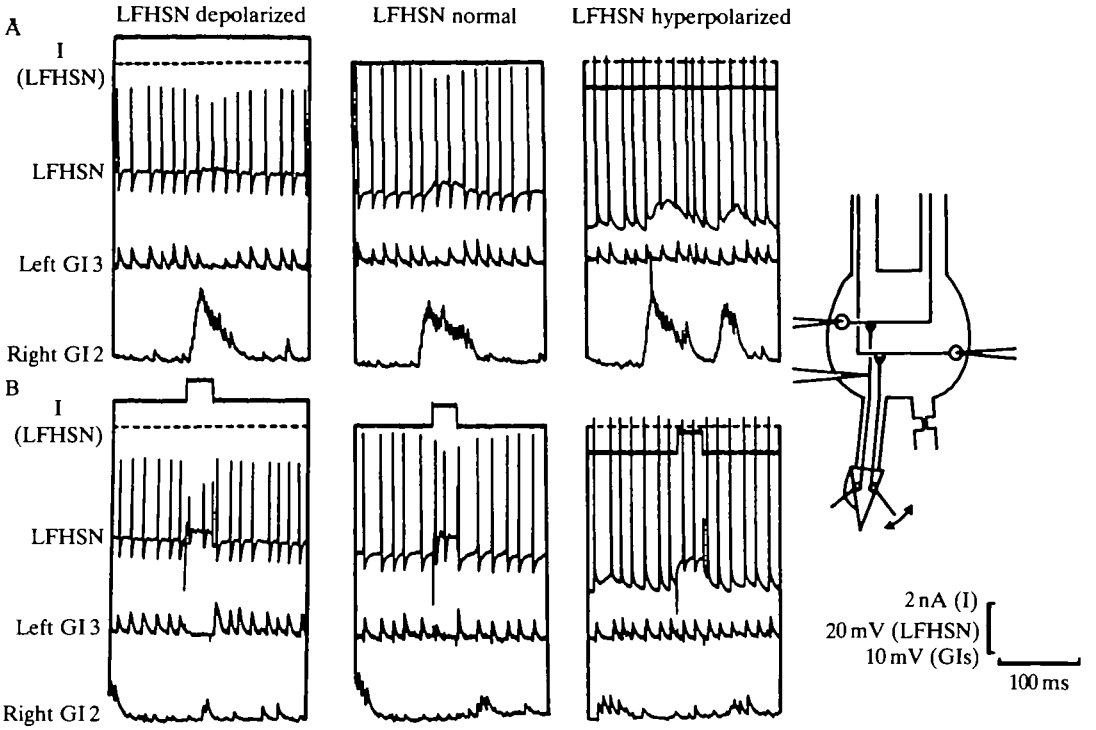

Fig. 9. Simultaneous microelectrode recording from the lateral filiform hair sensory neurone (LFHSN, second trace), left giant interneurone 3 (GI 3, third trace) and right GI 2 (bottom trace). Upper trace: current monitor. LFHSN is shown at depolarized (left), normal (centre) and hyperpolarized (right) resting potentials. (A) At a depolarized resting potential (−41 mV) the degree of presynaptic inhibition of LFHSN–GI 3 synaptic transmission during GI 2 EPSP bursts is increased. At hyperpolarized resting potential (−66 mV) the degree of presynaptic inhibition is reduced. (B) Depolarizing current pulses were applied to LFHSN *via* the microelectrode. The degree of membrane rectification present at different potentials is reflected in the discrepancy between the initial transient depolarization and the steady-state depolarization. Delayed rectification is also present following the spike hyperpolarizing after-potentials. At a depolarized resting potential the degree of delayed rectification shown by LFHSN in response to depolarizing current injection is increased, as is presynaptic inhibition of LFHSN–GI 3 synaptic transmission. At a hyperpolarized resting potential no rectification occurs, there is a small increase in LFHSN spike height, and the amplitude of the GI 3 EPSPs is not changed. The LFHSN electrode is balanced for negative current injection.

Alteration of LFHSN resting potential by injection of depolarizing or hyperpolarizing current showed that the degree of spike reduction was dependent on the degree of delayed rectification (Fig. 9). Reduction of the height of the spike peak reduced the postsynaptic EPSP. However, increasing the height of the spike by hyperpolarization had no effect on the size of the EPSP. Neither depolarization nor hyperpolarization affected the duration of the presynaptic spikes.

The depolarizing potentials in LFHSN were reduced in amplitude when the membrane was depolarized to potentials more positive than −50 mV, and were not

detected at membrane potentials above −35 to −40 mV. Their reversal potential could not, therefore, be measured, but is likely to be more positive than −35 mV.

The medial filiform hair sensory neurone is not connected monosynaptically to the lateral filiform hair sensory neurone

LFHSN was hyperpolarized to increase the amplitude of the depolarizations which were compared with the bursts of EPSPs recorded from GI 2 (Fig. 10). There was a latency of up to 8 ms between the first GI 2 EPSP and the onset of the LFHSN depolarization, whereas if MFHSN were to form monosynaptic connections with both GI 2 and LFHSN, the EPSPs in both would be expected to be synchronous.

It was not possible to distinguish individual PSPs within the LFHSN depolarizations but they were too irregular in shape to represent single depolarizing PSPs.

Giant interneurone 2 does not synapse directly onto the lateral filiform hair sensory neurone

Stimulation of GI 2 by injection of large current pulses into the cell body had no effect in inhibiting GI 3 EPSPs (Fig. 11A). The depolarizing pulses elicited spikes with the same amplitude and time-course as those normally evoked by summating EPSP bursts. Similarly, hyperpolarization of GI 2 to prevent spiking or otherwise to alter its synaptic transmission had no effect in suppressing presynaptic inhibition of GI 3 EPSPs (Fig. 11B). The assumption that the membrane potential of the GI 2 dendrites is affected by injection of current into the cell body was supported by the

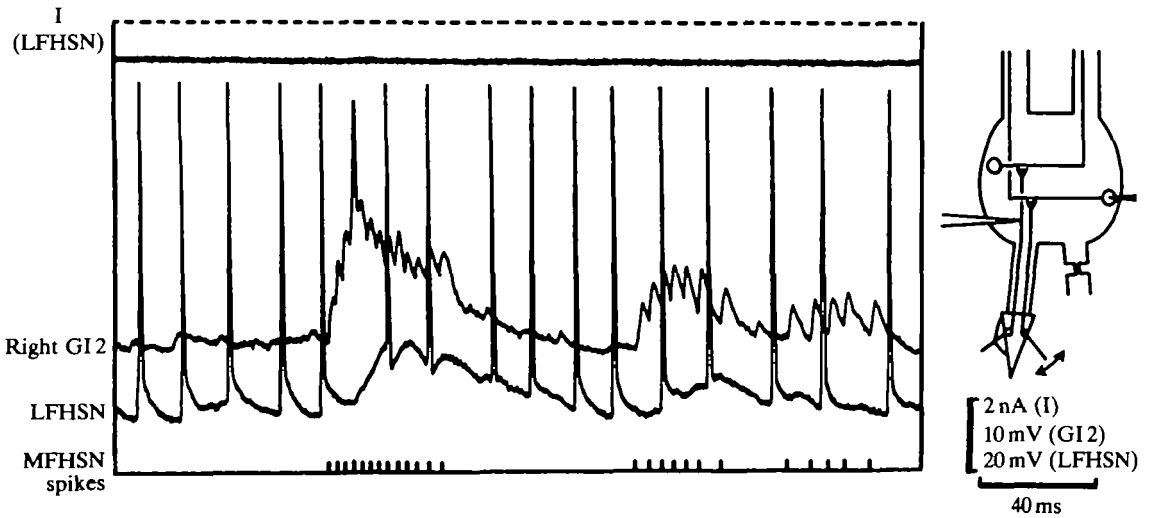

Fig. 10. Depolarizations in the lateral filiform hair sensory neurone (LFHSN) axon do not coincide precisely with EPSP bursts in the right giant interneurone 2 (GI 2). LFHSN was hyperpolarized to enlarge the depolarizations (current monitor, top trace). The EPSPs in GI 2 (second trace) indicate the occurrence of spikes in MFHSN (bottom trace). There is a latency of 5–8 ms between the onset of the MFHSN spike burst and the onset of the LFHSN depolarization (third trace), suggesting that MFHSN is not connected monosynaptically to LFHSN.

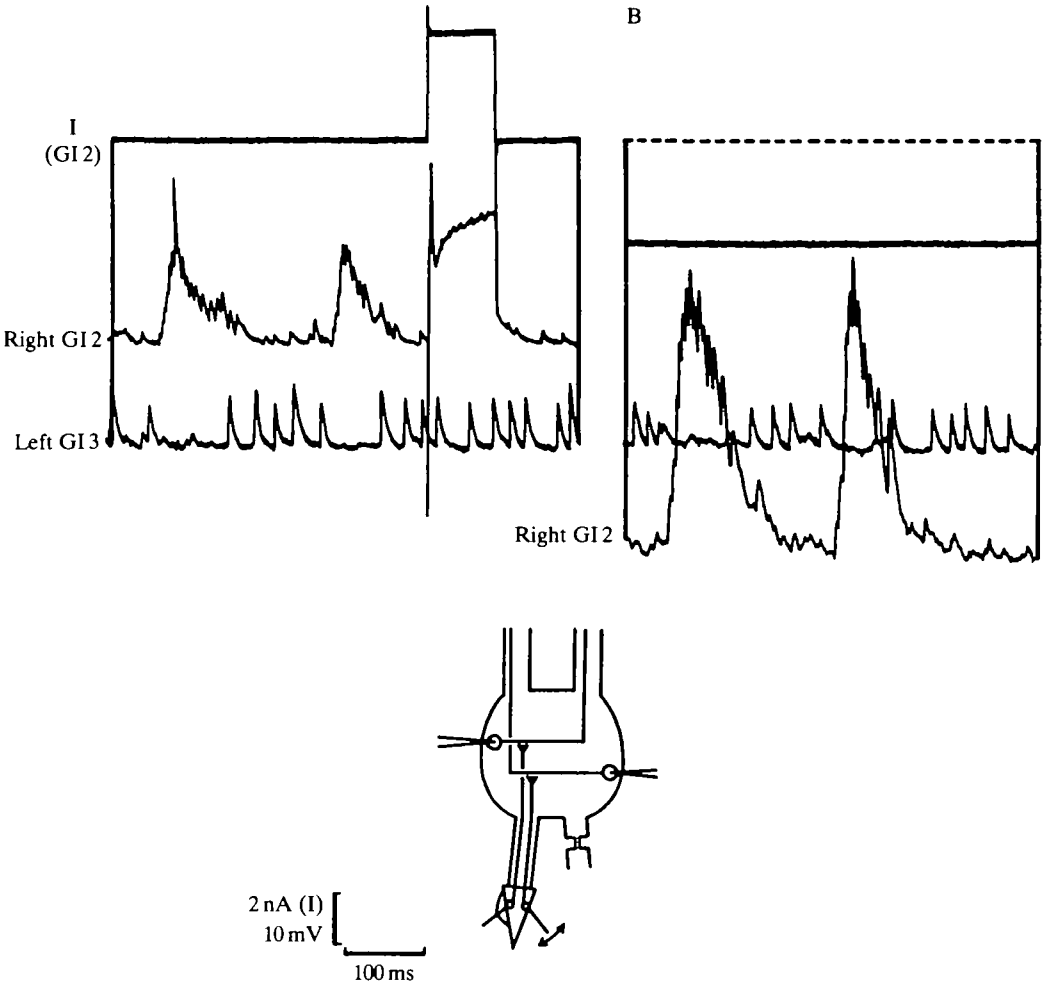

Fig. 11. Giant interneurone 2 (GI 2) does not mediate presynaptic inhibition. Presynaptic inhibition of GI 3 EPSPs (lower trace) occurs during GI 2 EPSP bursts (second trace). GI 2 current monitor: top trace. The microelectrode was balanced for passing hyperpolarizing current. (A) Depolarization of GI 2 to above spike threshold and for the same duration as an EPSP burst does not produce presynaptic inhibition. The microelectrode resistance increases during the pulse. (B) Hyperpolarization of GI 2 enlarges the EPSP bursts but does not abolish presynaptic inhibition.

observation that the amplitude of the EPSPs in GI 2 was increased by hyperpolarization. In addition, GI 2 has similar dimensions and cell body membrane properties to GI 3, in which it has been observed that potential changes in the cell body are attenuated to 0·5–0·7 times that value when recorded with a microelectrode inserted into the primary dendrite (J. M. Blagburn & D. B. Sattelle, unpublished observations). The above evidence supports the hypothesis that presynaptic inhibition of LFHSN–GI 3 transmission by MFHSN is not mediated by GI 2–LFHSN synapses.

Effects of prolonged hyperpolarization of the lateral filiform hair sensory neurone upon presynaptic inhibition

Injection of hyperpolarizing current into LFHSN for long periods (e.g. hyperpolarization by 5 mV for 3 min, 10 mV for 1 min or 30 mV for 30 s) was observed to change temporarily the spike threshold of LFHSN. The change was prolonged after the return to the original resting potential. An initially subthreshold depolarizing pulse was applied to LFHSN throughout the experiment; initially this did not increase the firing rate, but delayed rectification during the pulse decreased the amplitude of coincident spikes and caused presynaptic inhibition of the LFHSN–GI 3 synapse. During the period of hyperpolarization, rectification and presynaptic inhibition were abolished. After return to the normal resting potential, LFHSN was maximally excited by what had previously proved to be subthreshold pulses (Fig. 12A,B). In addition, the EPSPs evoked in GI 3 by LFHSN spikes were increased in amplitude. The increased excitability of LFHSN gradually declined, until after 5 min at resting potential the test pulses were again subthreshold (Fig. 12D).

No change in the current/voltage relationship of LFHSN was observed at any stage of the experiment; it is apparent that the spike threshold of the axon was temporarily lowered by about 3 mV. The lowered spike threshold enabled the pulse to elicit spikes, thereby preventing the potential from attaining the level at which rectification was able to reduce the spike amplitude enough to reduce synaptic transmission. Depolarizations induced by MFHSN spike bursts were similarly ineffective in reducing transmission during this period (Fig. 12C).

Hyperpolarizations of LFHSN by 30 mV for 1 min are unlikely to be of physiological significance, but this extreme case illustrates clearly the immediate effects of returning to normal resting potential (Fig. 12A). With more physiological hyperpolarizations (e.g. 5 mV for 2 min), a temporary lowering of the LFHSN spike threshold was still observed.

Fig. 12. Long-term hyperpolarization temporarily abolishes presynaptic inhibition. Lateral filiform hair sensory neurone (LFHSN, second trace) was hyperpolarized by approx. 30 mV for 1 min (current monitor, top trace). Because the lateral hair was immobilized, the LFHSN produces spikes at a regular frequency. (A) On return to normal resting potential, previously subthreshold positive pulses are above spike threshold, eliciting prolonged spike bursts in LFHSN. EPSPs in left giant interneurone 3 (GI 3, third trace) are enlarged after prolonged hyperpolarization. (B) 30 s after return to normal resting potential, a previously subthreshold test pulse now elicits a burst of spikes in LFHSN which, in turn, evokes a burst of EPSPs in GI 3. (C) 30 s after return to resting potential movement of the medial hair towards the base of the cercus (shown in bottom trace by an increase in the angle of the hair relative to the cercus) causes an MFHSN spike burst which results in the depolarization of LFHSN. During this depolarization the firing rate of LFHSN is increased and only a small reduction in spike amplitude takes place, thus GI 3 EPSPs are not greatly reduced in amplitude. (D) 5 min after return to resting potential the same test pulse is once again subthreshold for LFHSN spikes, and so does not increase the firing rate. Coincident LFHSN spikes are reduced in amplitude by delayed rectification during the pulse, producing presynaptic inhibition of GI 3 EPSPs. In these records the microelectrode was balanced for passing hyperpolarizing current; due to non-linearities in its current-passing capability positive pulses are poorly balanced.

Other modulatory inputs to filiform hair sensory neurones

In addition to the MFHSN-induced depolarizations in LFHSN, other depolarizations, in the form of single, large postsynaptic potentials, were seen when both filiform hairs were immobilized (Fig. 13A). These also had a marked inhibitory effect upon LFHSN–GI 3 synaptic transmission.

Infrequently hyperpolarizing PSPs of generally slower time-course were observed in the FHSN axons (Fig. 13B). In one preparation the regular occurrence of these

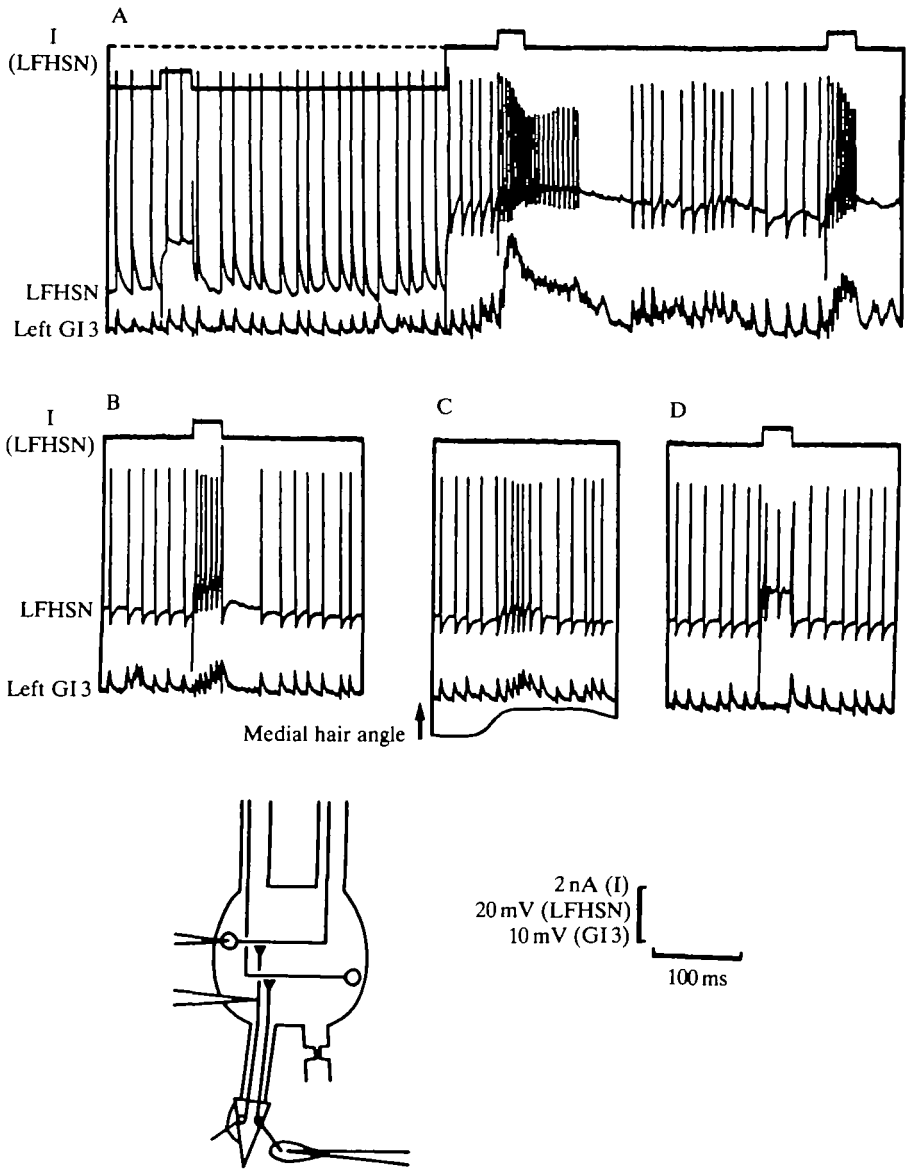

Fig. 12

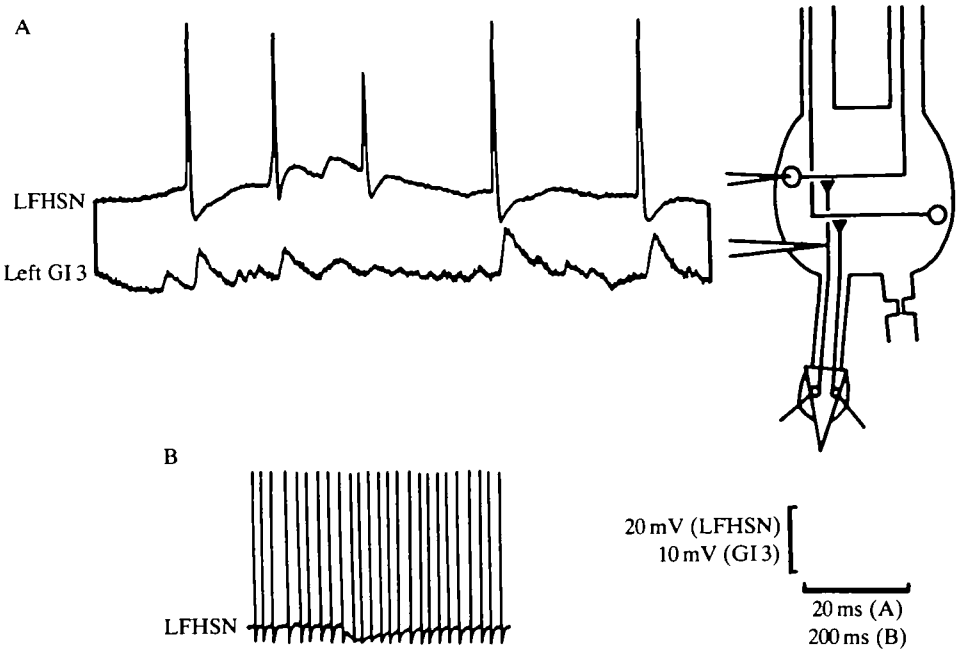

Fig. 13. Unidentified synaptic inputs onto the lateral filiform hair sensory neurone (LFHSN). All four filiform hairs immobilized. (A) Large depolarizing PSPs in LFHSN (upper trace) mediate presynaptic inhibition of giant interneurone 3 (GI 3) EPSPs (lower trace) by reducing the LFHSN spike amplitude. (B) Long-lasting hyperpolarizing PSP in LFHSN.

hyperpolarizing PSPs at frequencies of 1–2 Hz led to the gradual hyperpolarization of the axon by 5–10 mV over a period of 2 min.

DISCUSSION

Evidence has accumulated for a monosynaptic, cholinergic connection between cercal mechanosensory neurones and identified giant interneurones in the cockroach (Callec, 1974; Sattelle, 1985). In the present study we have made direct intracellular recordings from an identified filiform hair sensory neurone (LFHSN) and a giant interneurone (GI 3), and show that the synapse between these cells exhibits physiological properties which fulfil the criteria for a monosynaptic chemical connection (reviewed by Berry & Pentreath, 1976). These are as follows: (1) postsynaptic EPSPs follow every presynaptic spike, with a constant latency of 1·4 ms, which is too long to be due to electrical transmission; (2) at high spike frequencies an EPSP follows each spike with a constant latency; (3) EPSPs are progressively and reversibly reduced when Mg^{2+} is substituted for Ca^{2+} in the bathing medium. Electron microscopy has also enabled confirmation that there are morphological synapses between LFHSN and GI 3, and between MFHSN and GI 2 (Blagburn *et al.* 1984, 1985*a*).

We have used intracellular recording from the two pairs of presynaptic (LFHSN and MFHSN) and postsynaptic (GI 2 and GI 3) neurones to investigate whether and how presynaptic inhibition takes place. LFHSN forms excitatory cholinergic synapses with the ipsilateral GI 3 (cell body ipsilateral to LFHSN), and MFHSN forms excitatory cholinergic synapses with the contralateral GI 2. There is no convincing evidence to date for a direct synaptic connection between LFHSN and contralateral GI 2 and between MFHSN and ipsilateral GI 3.

We have shown that during bursts of spikes in MFHSN, produced by oscillations of its mobile filiform hair, the EPSPs elicited in GI 3 by regular LFHSN spikes (produced when its hair is immobilized) are either reduced in amplitude or abolished. This reduction is not due to a synaptic connection between MFHSN and GI 3, nor is it due to synapses between GI 2 and GI 3.

The results obtained in this study support the idea that depolarizing synaptic inputs onto LFHSN reduce the amplitude of LFHSN-evoked EPSPs in GI 3, i.e. LFHSN output is presynaptically inhibited. Presynaptic inhibition of LFHSN occurred during MFHSN spike bursts and it is likely that there is an indirect synaptic connection between MFHSN and LFHSN. The identity of the intermediate neurone or neurones is not known, but it is unlikely that GI 2 itself is part of the pathway. It has also been observed that spike bursts in LFHSN mediate presynaptic inhibition of synaptic transmission between the ipsilateral MFHSN and the contralateral GI 2.

LFHSN depolarizations evoked by MFHSN spike bursts result in a reduction of LFHSN spike amplitude, but not of spike duration, suggesting that this may be the mechanism by which synaptic transmission is reduced. Artificial depolarization of LFHSN by injection of current results in a comparable reduction in spike height. Neither type of depolarization has any effect on spike height when the axon is hyperpolarized to potentials at which delayed rectification does not take place. At such potentials the spike height is increased but the EPSPs are not increased in amplitude, possibly because there is little change in the potential at which the spikes peak.

Thus, depolarization brought about by current injection or by the activation of synaptic receptors will cause an increase in voltage-gated K^+ conductance, driving the membrane potential towards the potassium equilibrium potential and reducing the amplitude of coincident spikes. Spike reduction reduces the increase in voltage-gated Ca^{2+} conductance, so reducing the amount of transmitter released. These results contrast with those obtained in *Aplysia*, where depolarization has been found to increase, and hyperpolarization to decrease, the amplitude and duration of the presynaptic spike due to changes in transient K^+ conductances (Klein, Shapiro & Kandel, 1980).

In studies on neuronal synapses of adult locusts and cockroaches it has been shown that the direction of the synaptically induced change in potential is not significant since reversal of the sign of the PSPs by injection of current into the presynaptic neurone does not affect presynaptic inhibition. In these cases it is postulated that it is the increase in conductance which reduces the amplitude of the presynaptic spike

(Pearson & Goodman, 1981; Hue & Callec, 1983) and a GABA-mediated increase in Cl^- conductance is implied.

The ionic basis of the MFHSN-induced depolarizations in LFHSN in the first instar cockroach is not known. It is not possible to reverse the depolarization in LFHSN by altering the resting potential because of the strong rectifying properties of the membrane. The reversal potential of the depolarizations is therefore not known, but it is likely to be more positive than −35 mV, suggesting that Cl^- and K^+ are unlikely to be major current carriers. In addition, IPSPs have been observed in FHSN axons and these have no depressing effect on either spike height or synaptic transmission.

Presynaptic inhibition of FHSN–GI synaptic transmission by spike bursts in the ipsilateral FHSN would have an obvious functional significance. In the first instar nymph, wind from certain directions, e.g. 90° lateral to the animal, could cause spiking in both LFHSN and MFHSN (Dagan & Volman, 1982). If this took place, mutual presynaptic inhibition would reduce the spike amplitudes, thus reducing GI EPSPs and lowering the probability of GI spiking. Presynaptic inhibition would thus sharpen the boundaries of the GI's directional sensitivity.

In the cricket terminal ganglion the giant interneurones receive excitatory and inhibitory inputs from the cerci and it has been shown that some sensory afferents are presynaptically inhibited by other afferents of different directional sensitivity or by afferents from the contralateral cercus (Levine & Murphey, 1980). The inhibitory inputs to the GIs may arise from GABA-containing neurones which have been localized in the cricket terminal ganglion (Jacobs, Redfern & Miller, 1985). In addition to sharpening of the boundaries of directional sensitivity of the giant interneurones, Levine & Murphey (1980) suggest that presynaptic inhibition protects cercal axon–interneurone synapses from synaptic depression during movement of the animal and sustained background stimulation. However, we have observed no tendency for FHSN–GI synaptic transmission to be less effective with prolonged activity. It may be that this function of presynaptic inhibition is less important in the first instar cockroach, with its very simple cercal filiform hair sensory system.

Presynaptic inhibition of FHSN synaptic transmission is not mediated only by firing of the ipsilateral FHSN, but also by other, unidentified synaptic inputs. Large EPSPs, which cannot be correlated with spikes in other FHSNs, have been frequently observed in LFHSN axons. These unidentified synaptic inputs onto the FHSNs remain after nicotinic cholinergic inputs are blocked with mecamylamine or tetraethylammonium (TEA) (J. M. Blagburn & D. B. Sattelle, unpublished observation).

GABAergic inhibitory synaptic input onto the cercal afferent axons is present in the adult cockroach (Hue & Callec, 1983). The results of the above study are not directly comparable with those of the present work because in the adult cockroach each cercus bears 220 filiform hairs and many bristle hairs and campaniform sensilla, all of which could supply synaptic inputs to the giant interneurones *via* the cercal nerve. At present it is not known if GABAergic synaptic inputs onto FHSN axons are present in the first instar. However, in a few instances, hyperpolarizing PSPs

have been observed in LFHSN, but these do not suppress LFHSN–GI 3 synaptic transmission.

Prolonged hyperpolarization of LFHSN by current injection temporarily lowered the spike threshold and enhanced synaptic transmission upon the return to resting potential. Both of these changes could be due to an enhancement of the steady-state Ca^{2+} current, perhaps by removal of Ca^{2+} channel inactivation. It has been shown that LFHSN spikes have a small Ca^{2+}-dependent component (J. M. Blagburn & D. B. Sattelle, unpublished observations) and an increase in the steady-state Ca^{2+} current produced by depolarization has been shown to enhance synaptic transmission in *Aplysia* (Shapiro *et al.* 1980*a*).

Hyperpolarization of a FHSN by hyperpolarizing synaptic potentials could have a similar effect to that produced by injection of hyperpolarizing current, resulting in a temporarily increased spike frequency, removal of presynaptic inhibition and an increase in transmitter release when the axon is returned to a more depolarized potential.

We can conclude that, although the FHSN axon/terminal may exhibit some modulation of steady-state Ca^{2+} current in a similar manner to neurones in *Aplysia*, the principal effect of depolarizing synaptic inputs onto the terminal is to reduce spike amplitude. This effect results from the high degree of rectification shown by the FHSN axonal membrane. This, in turn, reduces the amount of Ca^{2+} entering the terminal, thus reducing synaptic transmission.

Primary afferent depolarization has frequently been associated with presynaptic inhibition, with the supposed mechanism being a conductance increase reducing the spike height and thus the amount of transmitter released. We have shown that this is the case at the first instar cockroach FHSN–GI synapse, but that the conductance increase is not a direct result of the opening of transmitter-gated ionic channels, but of the opening of the voltage-gated K^+ channels which mediate delayed rectification. The change in membrane potential is a cause of presynaptic inhibition, rather than being a consequence of it.

It is becoming increasingly clear that presynaptic inhibition is simply one facet of the more complex phenomenon of synaptic modulation, and that there are probably several means by which this is achieved.

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