Multimodal Convergence of Presynaptic Afferent Inhibition in Insect Proprioceptors

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INTRODUCTION

In vertebrates and invertebrates proprioceptive sensory information about posture and movement is often tailored by presynaptic inhibition even before it reaches the first layer of interneurons or motoneurons. In vertebrates, presynaptic inhibition of a given proprioceptor was shown to result from its own activity, that of several other sense organs and from centrally generated activity (reviews, Nusbaum et al. 1997; Rudomin 1990). In invertebrates, however, despite detailed knowledge of presynaptic interactions between afferents of the same proprioceptor (review, Clarac and Cattaert 1996), little is known about interactions between different sense organs. Interactions between different proprioceptors have not been described yet.

In this study, we provide for the first time direct evidence for the existence of an interaction of different leg proprioceptors at this first possible stage of information processing in insects. We recorded intracellularly from afferents of the femoral chordotonal organ (fCO) of the middle leg of stick insects, while specifically stimulating two other proprioceptive sense organs, either the ventral coxal hairplate (cxHPv) or the trochantero-femoral campaniform sensilla (CS). We tested for similarities with primary afferent depolarizations (PADs) known to result from the action of other fCO afferents (Sauer et al. 1997). Furthermore, we investigated interactions between the cxHPv and CS.

METHODS

Female stick insects, Carausius morosus were mounted ventral side up on a foam platform and were opened ventrally. To prevent leg movements, the coxa of the left middle leg was fixed with dental cement (Protemp, ESPE), leaving the cxHPv exposed. Cuticular stress of the trochantero-femur, known to specifically activate trochantero-femoral CS groups (Delcomyn 1991), was applied by means of a stimulus clamp that was attached to the tip of the femur and moved the distal part of the femur relative to the immobilized base (see Schmitz 1993). To activate the cxHPv exclusively, a second stimulus clamp was attached to a small flap that was cut out of the thoraco-coxal joint membrane. This flap was moved over the hairs of the cxHPv and mimicked a forward movement of the leg (see Büschges and Schmitz 1991). Both stimulus clamps were controlled by servo motors (G300PD, General Scanning). FCO afferents were identified by their well-known response characteristics to various movements of the femur-tibia joint (Sauer et al. 1997). As a second method to activate the cxHPv afferents, the Nervus lateralis 3 (nl3) was electrically stimulated using a bipolar stimulation electrode placed at the entrance of the cxHPv branch.

The activity of single sensory neurons of the fCO, the CS, or the cxHPv was recorded intracellularly from their axons close to their entrance into the mesothoracic ganglion (see Sauer et al. 1997) using glass microelectrodes, and a single-electrode current-clamp amplifier (SEC10L, NPI) either in bridge or in discontinuous current clamp (DCC) mode. To prove the effectiveness of cxHPv and CS stimulation, the reflex response of the retractor coxae motor neurons was monitored.

RESULTS

Synaptic potentials in afferents of the femoral chordotonal organ

The axons of the fCO that senses position and movement of the middle leg femur-tibia joint (Bässler 1983) project to the mesothoracic ganglion through the Nervus cruris. Intracellular recordings from these afferent axons revealed that the afferents responded with an increase of activity either during flexion or during extension of the tibia (Fig. 1A). Most of the fCO afferents also received PADs arising from other fCO afferents in this situation (see also Sauer et al. 1997).

In a small fraction of >30 recordings, fCO afferents also received a PAD during the stimulation of other proprioceptors. Six afferents received a PAD during stimulation of the CS, four of them also during stimulation of the cxHPv (Fig. 1, B and C) and one exclusively during stimulation of the cxHPv. The PADs were not due to fCO afferent activity, because stimulation of the CS and the cxHPv did not change fCO spike activity.

The campaniform sensilla fields contain ~50 unipolar re-
ceptors, the axons of which also project through the Nervus cruris into the ganglion. The CS afferents respond to cuticular stress applied to the trochantero-femur by increasing their activity, depending on the stimulus direction and stimulus velocity. This activity induced a PAD in fCO afferents, the amplitude of which typically increased with increasing CS stimulus velocity.

The cxHPv consists of ~35 unipolar sensory hairs, the axons of which reach the ganglion through the Nervus lateralis 3 and are activated during forward movements of the leg. Such a movement in the thoraco-coxal joint was mimicked by moving a small flap of the joint membrane over the hairplate and caused a PAD in fCO afferents.

**Properties of synaptic input**

The PAD in fCO afferents during both exclusive stimulation of CS or cxHPv, depended on stimulus direction and varied between different afferents. In most of the recordings at resting potentials of −63.4 ± 4.2 mV (mean ± SD; n = 10), a PAD with rather small amplitude was observed. Hyperpolarizing the afferent by current injection during both stimulus situations increased the amplitude of the PADs, whereas depolarizing currents between 0.5 and 4 nA (n = 6) reversed the sign of the PAD (Fig. 1, B and C). From DCC recordings, we estimated the reversal potential at −59 mV (Fig. 1D). This potential is close to the reversal potential of PADs in fCO afferents resulting from their own activity (Sauer et al. 1997).

The PAD was associated with a significant decrease of input resistance as revealed from DCC recordings and injection of hyperpolarizing current pulses (−1 nA) through the recording electrode (Fig. 2, A and B). The effect of such a decrease of input resistance onto afferent spike amplitude was determined by comparing the amplitudes of spontaneously occurring spikes in the fCO afferents before and during CS stimulation. The amplitudes of spikes were reduced by 7.0 ± 2.7% (evaluated for n = 18 PADs in n = 3 afferents) if they occurred at the same time as the PAD, suggesting that the PAD acted as presynaptic inhibition (Fig. 2C). For example, spontaneously occurring afferent action potentials in one afferent had an amplitude of 55.2 ± 0.7 mV (n = 10) at the recording site within the ganglion. If the spikes were coincident with the

![FIG. 1. A: intracellular recording of femoral chordotonal organ (fCO) afferent (hyperpolarized, −2 nA) during movement of the tibia. ↑, tibia flexion. B and C: primary afferent depolarization (PAD) in a fCO afferent (solid arrowheads mark the beginnings of PADs) during stimulation of campaniform sensilla (CS; B, ↓, increase of cuticular stress) and ventral coxal hairplate (cxHPv; C, ↓, mimicked leg movement to anterior) at 3 different membrane potentials. D: reversal potential of PAD. E: schematics of interactions between different proprioceptors. Scale bars: 1 s; 3 mV.](http://jn.physiology.org/)

![FIG. 2. A: intracellular recording of fCO afferent while injecting current pulses (−1 nA). Discontinuous current clamp (DCC) recording. ↑, increase of cuticular stress. B: input resistance significantly decreased during PAD occurrence [† P < 0.001, modified t-test after Dixon and Massey (1969)]. C: decrease of spike amplitude during PAD occurrence (black bars). D: intracellular recording of CS afferent during electrical stimulation of nerve nL3 (bottom trace), sub- and suprathreshold stimuli (* stimulus artifact). Arrow indicates PAD after suprathreshold stimulus. E: schematics of interactions between cxHPv and CS. Scale bars: A, 1 s; 1 mV; C, 0.2 s; 2 mV; D, 10 ms; 1 mV.](http://jn.physiology.org/)
PAD elicited by stimulation of the campaniform sensilla, they were reduced in amplitude by 1.9 ± 0.8 mV. Because the PAD had an amplitude of 2.1 ± 0.6 mV (n = 6), the overall reduction reached on average 4 mV (7.2%). The reduction of the spike amplitude appeared to depend on applied current and on the recording site. The more hyperpolarizing the afferents was applied, and the more peripherally the afferents were penetrated, the smaller was the reduction recorded.

Depolarizing postsynaptic potentials in campaniform sensilla afferents

To determine latencies between the activity in one afferent and the occurrence of the induced PAD in another afferent and to determine whether also cxHPv and CS interact, we stimulated extracellularly nl3, which contains the axons of cxHPv afferents, while we recorded intracellularly from single CS afferents. After the stimulus threshold for the cxHPv afferents was reached, a PAD was reliably induced in a CS afferent with a constant central latency of 2 ms (Fig. 2D).

DISCUSSION

Whereas short latency presynaptic inhibition between proprioceptive afferents is well known in vertebrates, our results for the first time also establish this way of tailoring the sensory information inflow for insect sensorimotor systems. This was shown here 1) for a position-proprioceptor of a proximal leg joint that modifies the information delivered by another position-proprioceptor of a more distal joint (Fig. 1E), 2) for a load-proprioceptor that modifies the information of a position-proprioceptor (Fig. 1E), and 3) for a position-proprioceptor that modifies the information of a load-proprioceptor (Fig. 2D). All tested proprioceptors are known to underlie reflexes that play important parts in the control of posture and movement (Bässler 1983; Schmitz 1993). They thus play a similar role in invertebrate sensorimotor system as do muscle proprioceptors in vertebrates, in particular the Ia and Ib afferents.

Several functions are attributed to presynaptic afferent depolarization. For example PAD could act as an automatic gain control of the afferent effectiveness of one sense organ (Burrows 1996). An inhibitory influence of afferents of one sense organ onto afferents of the same sense organ prevents saturation of the responses of the postsynaptic neurons and extends their dynamic range. Here, presynaptic inhibition of the afferents of the fCO due to the activity of other proprioceptive sense organs may also modify sensory feedback within the femur-tibia joint control loop. Hence the interaction of load-sensitive and joint posture measuring proprioceptors might be well suited for strain-reducing feedback loops in the insect leg (Schmitz 1993). During voluntary movements, a centrally generated PAD might represent an efference copy, allowing the CNS to recognize mainly the deviation between programmed and actual movement (summary, e.g., Clarac and Cattaert 1996). A similar feed-forward type of control mechanism might be represented by influence of the cxHPv onto fCO and CS afferents.

Detailed quantitative studies of extend and function of PAD in the context of insect behaviors are yet still pending. Our recent results, however, clearly demonstrate that PAD mechanisms of arthropod proprioceptors might be as complex as in vertebrates.

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