Gating of Afferent Input by a Central Pattern Generator

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INTRODUCTION

Centrally patterned motor behavior in the absence of sensory input is a ubiquitous feature of the organization of rhythmic motor systems (Delcomyn 1980). However, sensory input to central pattern generator (CPG) networks is often required for the production of behaviorally appropriate motor patterns (Pearson 1993). In addition, modulation of sensory input to CPGs is observed in a wide variety of motor systems either directly or by acting on interneurons in the system (Pearson 1993; Prochazka 1989). Direct modulation of primary afferents in vertebrate and invertebrate systems is usually associated with primary afferent depolarization (PAD), which modulates transmitter release by the shunting conductance activated by the presynaptic input (Rudomin et al. 1993). In many systems PAD is correlated with the production of a rhythmic motor pattern to modulate reflexes during ongoing motor activity (El Manira et al. 1991; Paul 1989; Sillar and Skorupski 1986). This study describes the complete gating of primary afferent input by direct cyclic inhibition by the ventilatory CPG in the crab.

Gill ventilation in decapod crustacea is produced by the rhythmic dorso–ventral movements of the scaphognathite (SG) or gill bailer of the second maxilla. Rhythmic movement of the SG pumps water through the branchial chamber in either of two directions (forward or reverse ventilation) determined by the recruitment sequence of levator and depressor motor neurons (DiCaprio 1989).

The only proprioceptor in the SG is the oval organ, which is located adjacent to the SG flexion axis (Pasztor 1969). The oval organ is innervated by three afferent neurons with cell bodies located in the thoracic ganglion. In the lobster these afferents employ a dual mode of information transmission, that is, they generate action potentials but also decrementally propagate membrane potential changes to the CNS because of their large length constant of ~1 cm (Pasztor and Bush 1982). In the shore crab, however, the oval organ afferents do not generate action potentials and therefore signal only by decremental conduction to the thoracic ganglion. The SG is also extensively invested with innervated hairs and hooked sensilla. Extracellular stimulation of this population of afferents has been shown to reset, entrain, and, in the case of tonic stimulation, to increase the rate of the ventilatory rhythm to near physiological maximum rates (Wilken 1994; Wilken and DiCaprio 1994).

METHODS

Male and female green shore crabs, C. maenas, were used in all experiments. The isolated ganglion preparation used in this study was described in detail elsewhere (DiCaprio and Fourtner 1984; Simmers and Bush 1983). The walking legs and chelea were autotomized, and the dorsal carapace, viscera, and brain were removed. The sternal artery was immediately cannulated, and the thoracic ganglion was perfused with chilled oxygenated saline. The nerves to both SGs were dissected from the SG musculature, all remaining nerves from the thoracic ganglion were severed, and the thoracic ganglion was removed and pinned to a sloping Sylgard base. The ganglionic sheath above the ventilatory neuropil was removed with fine forceps to permit intracellular recording.

Intracellular recordings of the ventilatory motor pattern from SG levator and depressor motor nerves were made with polyethylene suction electrodes. Recordings from the levator nerve were usually made proximal to the branch of this nerve, which innervates depressor muscle D2a, thereby allowing the combined recording of activity in axons innervating muscle D2a along with levator motor neuron activity. Intracellular recordings from ventilatory neurons were made in the neuropil with Lucifer yellow–filled microelectrodes amplified with a bridge electrometer (WPI 767). All signals were recorded on an eight-channel instrumentation tape recorder (HP model 3968A) for later reproduction and analysis.

Oval organ afferents were penetrated in the ventilatory neuropil and were identified by their distinctive morphology and physiology. Oval organ afferents were distinguished from motor neurons based on the following criteria: initial penetration of a cell did not evoke action potentials because of injury on penetration or with the injection of depolarizing current, on release from hyperpolarization, when depolarized after an initial period of hyperpolarization or when the SG motor nerves were stimulated extracellularly. In contrast, intracellular recordings from motor neurons produce action potentials in phase with the ventilatory rhythm, with the injection of small (0.2–0.5 nA) depolarizing currents or when stimulated antidromically from the appropriate motor nerve. The Lucifer yellow fills of the afferents revealed a central cell body located on the dorsolateral surface of the
When the ventilatory motor output transiently switched from forward to reverse ventilation, the oscillation in the membrane potential of the afferent continued in phase with the motor output and remained coincident with the D1 depressor motor neuron burst (Fig. 1B). There was a small depolarizing DC shift in the oscillation of 2–3 mV, and the peak-to-peak amplitude of the membrane potential oscillation initially increased relative to the magnitude of the oscillation observed during forward ventilation and then steadily decreased to the initial level during the reverse interval. In some recordings discrete PSPs could be resolved on the depolarizing phase of the oscillation during reversed ventilation. The origin of these PSPs is not known, but they were not correlated with any motor neuron spikes occurring during the depressor burst and may arise from the interneuron (RSi) that mediates the switch from forward to reverse ventilation (DiCaprio 1990).

The ability of input from an oval organ afferent to perturb the ventilatory rhythm was assessed by compiling a phase response curve that describes the phase shift in the motor output pattern caused by the injection of brief current pulses into the afferent at different phases of the ventilatory cycle (Fig. 2). Depolarizing or hyperpolarizing pulses at amplitudes of up to 6 nA applied during the portion of the cycle when the neuron was hyperpolarized (phase range of 0.5–0.95) had no effect on the timing of the motor pattern. Depolarizing pulses (open triangles) injected early in the cycle (beginning of the depressor burst) caused a phase advance that decreased in magnitude with increasing stimulus phase until an increasing phase delay was evoked at a stimulus phase greater than 0.25. Hyperpolarizing pulses (Fig. 2, filled circles) caused a large phase delay when applied early in the cycle, which abruptly switched to a large

\[ \text{Phase Shift} = \frac{t_b - t_s}{t_b}, \]

where \( t_b \) is the mean duration of the ventilatory cycle before the stimulus pulse (3 ≤ n ≤ 5) and \( t_s \) is the duration of the ventilatory cycle when the stimulus pulse was applied. Positive values of phase shift denote a phase advance, and negative values denote a phase delay of the ventilatory motor pattern. Phase shift data were only calculated when the variation of the mean cycle period before and after the stimulus pulse was <5%. Open triangles indicate depolarizing pulses, and filled circles are hyperpolarizing pulses.
phase advance at a stimulus phase of 0.15, which decreased in magnitude as stimulus phase was increased. The ability of hyperpolarizing pulses to reset the rhythm and the relatively depolarized value of their resting membrane potential indicate that these afferents are probably continuously releasing transmitter. Maintained depolarization of a single afferent stopped the motor pattern, although tonic activity persisted in some depressor motor neurons (Fig. 3A), which mimics the effect of restraining the SG in a maximally elevated or depressed position (not shown). Injection of sustained hyperpolarizing current resulted in a slight increase in cycle frequency accompanied by a decrease in the burst duration of some D1 group motor neurons (Fig. 3B).

DISCUSSION

Sensory input from the oval organ in the crab ventilatory system is likely to be blocked (gated) for ~50% of the ventilatory cycle by a phasic hyperpolarizing inhibitory input to the oval organ afferents derived from the ventilatory CPG. In contrast, presynaptic input to primary afferents (PAD) in vertebrate and invertebrate systems is usually modulatory in nature, whereas in rhythmic systems PAD is correlated with the production of a rhythmic motor pattern to modulate reflexes during ongoing motor activity (El Manira et al. 1991; Paul 1989; Sillar and Skorupski 1986; Wolf and Burrows 1995).

The nature of the oval organ input to the ventilatory CPG and its role in the production of the motor pattern are as yet unclear. It proved to be extremely difficult to obtain intracellular recordings from these cells during ventilatory movements, and extracellular recordings are ineffective as these neurons are nonspiking in the crab. In addition, it was not possible to record from any ventilatory neurons in a preparation that maintains the integrity of the pumping chamber and permits the normal development of a pressure differential across the branchial chamber.

The oval organ consists of a network of connective tissue strands and associated nerve endings located within the hemocoel of the second maxilla, spanning a narrow gap between the E2 endite and the arthrodial membrane in the region of maximum flexion and extension at the base of the SG. The oval organ is likely to be excited by SG movement and/or by the stress produced at the base of the SG during the development of negative pressure pulses by the ventilatory pump (Pasztor 1969). Excitation of oval organ afferents may occur twice during the ventilatory cycle as imposed movement of the SG in the lobster elicits action potentials from the afferents during levation and depression of the SG (Pasztor 1969). Recordings of the pressure in the branchial chambers of an intact crab show that there are two negative pressure pulses present during each ventilatory cycle (Hughes et al. 1969). Although the precise position of these pulses with respect to the motor output is unknown, correlation of these recordings with the data on blade movement and motor pattern (Young 1975) indicates that only one pressure pulse would fall within the period of the cycle when afferent input would be effective. The cyclic inhibitory input to the oval organ afferents restricts effective sensory input to one phase of the motor pattern and may thus transform an otherwise ambiguous twice per cycle sensory input to a signal linked to the motor pattern produced by the ventilatory CPG.

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REFERENCES


