Activation of a Lobster Motor Rhythm-Generating Network by Disinhibition of Permissive Modulatory Inputs

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Faumont, Serge, John Simmers, and Pierre Meyrand. Activation of a lobster motor rhythm-generating network by disinhibition of permissive modulatory inputs. J. Neurophysiol. 80: 2776–2780, 1998. Rhythm generation by the gastric motor network in the stomatogastric ganglion (STG) of the lobster Homarus gammarus is controlled by modulatory projection neurons from rostral commissural ganglia (CoGs); blocking action potential conduction in these inputs to the STG of a stomatogastric nervous system in vitro rapidly renders the gastric network silent. However, exposure of the CoGs to low Ca^{2+} saline to block chemical synapses activates a spontaneously silent gastric network or enhances an ongoing gastric rhythm. A similar permissive effect was observed when picrotoxin was also superfused on these ganglia. We conclude that in the CoGs continuous synaptic inhibition is exerted on modulatory projection neuron(s) and that release from this inhibition allows strong activation of the gastric network.

INTRODUCTION

Motor networks, and central pattern generators (CPGs) in particular, are controlled by modulatory projection neurons that initiate or modify network activity according to changing behavioral demands (Marder and Calabrese 1996). This in turn requires that these inputs are themselves appropriately regulated to ensure proper target CPG function. Such higher level control may be exerted by sensory influences (e.g., Simmers and Moulins 1988), by other central modulatory interneurons (e.g., Blitz and Nusbaum 1997; Meyrand et al. 1994), and even by feedback from the network(s) they control (e.g., Cardi and Nagy 1994). Although to date most studies focused on the way motor networks can be activated by exciting activatory neurons or centers, the role of inhibitory influences on input pathways is still poorly understood.

A suitable preparation for addressing this issue is the crustacean stomatogastric nervous system (STNS). The 16-neuron gastric network in the STG of the STNS, which is well known in terms of its cellular and synaptic properties (Harris-Warrick et al. 1992), is strongly dependent on descending modulatory inputs from the anterior commissural ganglia (CoGs). Moreover the spatial arrangement of these ganglia and their axonal connections to the STG via a single nerve provide an excellent opportunity for selective pharmacological manipulation of the modulatory centers without directly affecting the STG. We demonstrate that projection neurons located in the CoGs and that activate the gastric network are themselves under continuous inhibitory control. Release from this inhibition results in the initiation or reinforcement of gastric network activity.

METHODS

Experiments (n = 44) were performed on adult lobster, Homarus gammarus, that were cold anesthetized and then dissected by using standard procedures (Meyrand et al. 1994). The combined STNS (STG, CoGs, OG; Fig. 1A) was continuously superfused with chilled (12–14°C) physiological saline composed of the following (in mM): 479 NaCl, 12.7 KCl, 13.7 CaCl2, 10 MgSO4, 3.9 Na2SO4, 5 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES), pH 7.45. We used a low Ca^{2+} saline (to which Mn^{2+} was added) to block chemical synapses in the CoGs, as previously reported by Nagy et al. (1994). This saline consisted of (in mM) 479 NaCl, 12.7 KCl, 3 CaCl2, 10 MgCl2, 10 MnCl2, and 5 HEPES. Picrotoxin (SIGMA) was used at 10–5 M in normal saline. Vaseline wells (volume ~4 ml) were built around the desheathed CoGs for selective superfusion (2 ml/min) of these modified salines (Fig. 1A). In some preparations, inputs from the CoGs to the STG were reversibly blocked by superfusing a desheathed portion of the stomatogastric nerve (stn) with isotonic sucrose (750 mM). Intracellular recordings were made with sharp microelectrodes [20–30 MΩ] and Axoclamp 2A amplifiers. Extracellular axonal recordings were performed with platinum wire electrodes isolated with Vaseline. Signals were displayed on a Tektronix 5113 oscilloscope, transposed on a Gould TA11 chart recorder, and stored for off-line analysis on videotape coupled to a Neurocorder DR990. Burst pattern analysis was performed with Spike2 and Sigmaplot softwares. For each neuron, mean discharge frequency was calculated in successive 0.1-phase bins of the gastric cycle, defined as the period of MG neuron bursts. These frequencies were averaged over eight cycles from three different preparations (i.e., 24 cycles per graph) and plotted against the corresponding phase of the gastric cycle.

RESULTS

Under control saline conditions in vitro, the gastric network (Fig. 1B) in a combined STNS (Fig. 1A) generally expresses spontaneous rhythmicity. As illustrated in Fig. 1C, top panel, this activity consists of repetitive spike bursts in GM-type gastric motoneurons (see d-lvn trace) in phase with LG neuron bursts, but in antiphase with LPG neuron bursts. The strict dependence of this activity on continuous activatory control from the rostral ganglia is evident when impulse traffic in descending inputs is blocked in the single connecting stomatogastric nerve (stn; see Fig. 1A). Within several minutes the gastric network falls silent (Fig. 1C, middle

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panel), but rhythmicity returns soon after washout of the stn blockade with normal saline (Fig. 1C, bottom panel).

Because gastric motor output reflects descending input activity, we used this output to monitor projection neuron responses to pharmacological manipulation of the CoGs. Specifically, we were interested in whether the projection neurons are themselves subject to continuous synaptic control. In a first step therefore we deprived projection neurons of all chemical synaptic input by selectively superfusing the CoGs with low Ca\textsuperscript{2+} saline. As seen in Fig. 2A, this manipulation strongly reinforces an ongoing gastric rhythm (10/11 preparations) or activates a spontaneously silent network (9/11 preparations; not shown). The time course of this activating influence is shown in the pooled measurement of Fig. 2B. In these four experiments a 20-min exposure (i.e., the time found necessary to reach maximal stable effect) of the CoGs to low Ca\textsuperscript{2+} saline increased the gastric cycle frequency from 0.055 ± 0.011 Hz to a peak of 0.198 ± 0.008 Hz (means ± SE). After washout, control frequencies gradually returned over the ensuing 40 min. That these modifications are indeed correlated with changes in chemical synaptic transmission is demonstrated in Fig. 2B, inset, where a direct intracellular monitor from an identified CoG neuron (CG interneuron) (Simmers and Moulins 1988) showed excitatory postsynaptic potentials that were reversibly abolished by the low Ca\textsuperscript{2+} saline over a time course similar to the changes in gastric rhythmicity.

In addition to this global enhancement of gastric cycling by CoG synaptic blockade, clear-cut alterations occurred in both the discharge patterns of individual neurons and the overall structure of the gastric rhythm. As seen in the pooled data (n = 3) of Fig. 2C, most notable changes include a dramatic increase in the firing rates of all motoneurons and a sharpening of their duty cycles. Specifically, we were interested in whether the projection neurons are themselves subject to continuous synaptic control. In a first step therefore we deprived projection neurons of all chemical synaptic input by selectively superfusing the CoGs with low Ca\textsuperscript{2+} saline. As seen in Fig. 2A, this manipulation strongly reinforces an ongoing gastric rhythm (10/11 preparations) or activates a spontaneously silent network (9/11 preparations; not shown). The time course of this activating influence is shown in the pooled measurement of Fig. 2B. In these four experiments a 20-min exposure (i.e., the time found necessary to reach maximal stable effect) of the CoGs to low Ca\textsuperscript{2+} saline increased the gastric cycle frequency from 0.055 ± 0.011 Hz to a peak of 0.198 ± 0.008 Hz (means ± SE). After washout, control frequencies gradually returned over the ensuing 40 min. That these modifications are indeed correlated with changes in chemical synaptic transmission is demonstrated in Fig. 2B, inset, where a direct intracellular monitor from an identified CoG neuron (CG interneuron) (Simmers and Moulins 1988) showed excitatory postsynaptic potentials that were reversibly abolished by the low Ca\textsuperscript{2+} saline over a time course similar to the changes in gastric rhythmicity.

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FIG. 2. Blockade of chemical synapses in anterior ganglia initiates and enhances gastric network expression. A: weak gastric rhythm is enhanced by superfusion of low Ca\textsuperscript{2+} saline on the CoGs. Control levels of activity partially return after 45-min washout. B: time course of gastric network activation after CoG synaptic blockade. Cycle frequency is plotted as a function of time before, during, and after 20-min superfusion of low Ca\textsuperscript{2+} saline on CoGs. Data pooled from 4 preparations. Inset: time course of blockade of spontaneous excitatory postsynaptic potentials (EPSPs) in an identified commissural gastric (CG) interneuron. Shown are 5 superimposed oscilloscope sweeps triggered by the presynaptic action potential (first event on each top trace) recorded extracellularly on a CoG input nerve. The presynaptic spike remains during EPSP blockade, consistent with a direct effect of low Ca\textsuperscript{2+} on synaptic transmission. Recovery of synaptic transmission required 25-min washout in this typical experiment. C: changes in gastric motor pattern. Mean firing frequencies of all 5 gastric motoneuron types plotted against the phase of the gastric cycle, defined by MG neuron bursts (see METHODS). Data pooled from 3 preparations. Note that oscillations in MG discharge (see also Fig. 2A) are due to an influence from the faster pyloric rhythm. Horizontal bars: 5 s (A), 10 ms (B, inset). Vertical bars: 10 mV (A), 5 mV (B, inset), 10 Hz (C).
FIG. 3. Blockade of inhibitory synapses in CoGs initiates/enhances gastric network expression. A: reversible induction of rhythmicity in 3 recorded gastric motoneurons by superfusing picrotoxin ($10^{-5}$ M) on CoGs. B: time course of rhythm activation by picrotoxin. Data pooled from 4 preparations. Inset: inhibitory postsynaptic potentials evoked by electrical stimulation of a CoG input nerve monitored in commissural L cell are abolished by picrotoxin, but recover after $\sim 1$ h wash in normal saline. C: picrotoxin-induced changes in gastric pattern. Data pooled from 3 preparations and treated in the same manner as in Fig. 2C. Pyloric-timed perturbation of MG and LPG neuron bursts are again evident. Horizontal bars: 5 s ($A$), 10 ms ($B$, inset). Vertical bars: 10 mV ($A$), 2 mV ($B$, inset), 10 Hz ($C$).
qualitatively similar to that seen with low Ca\(^{2+}\) saline, with the different rates of recovery presumably reflecting differing washout abilities of the two salines (cf. Figs. 3B and 2B).

**DISCUSSION**

This study demonstrates that, although continuous extrinsic input is necessary for expression of the gastric network, the permissive projection neurons are themselves under continuous inhibitory control. Release from this higher-order inhibition, either by blocking chemical synapses or more specifically, inhibitory synapses, results in initiation or enhancement of gastric network activity.

Numerous studies emphasized the way motor networks can be activated via excitation of descending input neurons or centers (see Marder and Calabrese 1996). Nevertheless inhibitory control of command elements also occurs in motor systems (e.g., Hikosaka 1991; Krasne and Teshiba 1995) and disinhibition is implicated in the initiation of a range of motor acts (e.g., Hikosaka, 1991; Wang and Bieger 1991). For instance, locomotion in cat is activated both by blocking GABAergic inhibition in supraspinal centers as well as by injection of excitatory transmitters (Garcia-Rill et al. 1985; Noga et al. 1988), implying dual mechanisms of control. In our study, however, the effects of general chemical synaptic blockade in higher centers were surprisingly similar to the responses to specific suppression of inhibitory synapses. This suggests that, under basal in vitro conditions (i.e., without either movement-related sensory feedback or brain and neurohemal influences), control of projecting STG neurons is predominantly inhibitory rather than arising from a balance between continuous synaptic excitation and inhibition. Thus levels of gastric network activity would more directly reflect the extent to which permissive inputs are released from inhibition than the extent to which they are actively excited. It could be that, whereas sustained inhibitory influences are responsible for the selection of projection neuron ensembles, synaptic excitation mediates transient adaptive changes, such as in response to sensory input. In any case, the stomatogastric preparation is well suited to explore these notions because not only is the gastric motor network well described but also several of its modulatory inputs (Harris-Warrick et al. 1992), their synaptic interactions (e.g., Blitz and Nusbaum 1997; Meyrand et al. 1994), and sensory inputs (e.g., Simmers and Moulins 1988) are now identified.

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