MODULATORY EFFECTS OF A SINGLE NEURON ON THE ACTIVITY OF THE PYLORIC PATTERN GENERATOR IN CRUSTACEA

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The firing of a single neuron (named anterior pyloric modulator: APM) of the esophageal ganglion considerably modifies the pyloric rhythm of the rock lobster. These modifications, characterized by a long delay to onset and a long duration, include increased frequency and amplitude of oscillations of the motor neurons, changes in the efficacy of certain synapses within the network, and voltage-dependent modifications of membrane properties of some motor neurons. APM thus seems to be a true modulatory neuron. The APM-provoked changes resemble changes seen in the whole animal, making this a suitable system for an analysis of modulation on several levels.

Isolated invertebrate nervous systems have proven invaluable in characterizing behavioral sequences in terms of networks of identified neurons [5]. However, the wiring diagrams of the central pattern generators which have been elucidated generally appear too rigid to explain short- and long-term changes in the behavioral sequences they control and to account for the observed flexibility in the expression of the central pattern generators. In the present paper we show that such changes can be related to a modulatory mechanism which conforms to those recently discussed in the literature [4, 6–8].

The cellular interactions of the central pattern generator controlling the pyloric stomach of crustaceans have been well characterized. The pyloric network, located in the stomatogastric ganglion, is comprised of 14 neurons, which can produce a highly stereotyped rhythmic output when completely deafferented [9, 11]. This pyloric output can be considerably modified by the discharge of a single neuron which is located in a more rostral nervous center and which projects directly into the stomatogastric ganglion. We discuss some of these modifications and show that the mechanism by which they are induced cannot be understood in terms of conventional synaptic interactions, although the nature of the synapses formed by this neuron is still unknown. Specifically, a brief (seconds) firing of the modulatory neuron induces a long-term (tens of seconds) change, which can be related to modifications of intrinsic membrane properties of at least some neurons of the
pyloric pattern generator. Finally it is reported that some of the modifications induced by this modulatory neuron are similar to changes of the pyloric rhythm observed in electromyographic recordings of intact animals. The system described here is thus a particularly suitable system for an analysis, from the cellular to the behavioral level, of the phenomenon of modulation.

The stomatogastric nervous system, consisting of the stomatogastric ganglion (STG), the esophageal ganglion (OG) and the paired commissural ganglia (CG) (Fig. 1A), was dissected out of the stomach of the Cape lobster, Jasus lalandii, and pinned in a Sylgard-lined Petri dish filled with oxygenated artificial sea water. The ganglia were desheathed and surrounded by petroleum jelly barriers to permit

![Image](image_url)

Fig. 1. Neuron APM: morphology and long-lasting effects on the pyloric rhythm. A: diagram of the stomatogastric nervous system, showing the location of the cell body (1) and axons of APM and the positions of electrodes. CG, commissural ganglion; Ion, inferior esophageal nerve; OG, esophageal ganglion; Ion, superior esophageal nerve; STG, stomatogastric ganglion; stn, stomatogastric nerve. B and C: APM has axons which project directly into the STG via the Ion, stn and stn. In B an orthodromic spike is first recorded in the soma of APM (1), then in the ion (4) and finally in the stn (6). In C, antidromic spikes provoked by stimulation of the stn (6), ion (5) and ion (4) are recorded in the cell body. It can be seen from the latencies that the axon passes through the ion, CG and stn to reach the stn. Synaptic activity in the CGs is blocked by a 0 Ca2+ + 12 mM Co2+ saline in B and C. D and E: a discharge of APM, provoked by intracellular current injection (arrows), causes drastic changes in the pyloric rhythm. In D, the overall rhythm is recorded extracellularly on an output nerve (7). Both qualitative changes in the rhythm and an increase in frequency are evident. In E, note the changes in frequency and in amplitude of oscillation of both PD and LP (dilator and constrictor pyloric motor neurones, respectively). The long latency to onset and the long duration (about 30 sec for a discharge of APM 4 sec long in D and 6 sec long in E) of the effects is also evident. Calibrations: horizontal bars, 10 msec in B and C, 2 sec in D and E; vertical bars, 40 mV in B and C, 25 mV in D and E.)
selective perfusion with modified salines. Neuronal cell bodies were penetrated with glass microelectrodes filled with 3 M KCl (R = 15–30 MΩ). The stomatogastric neurons were identified by their activity recorded extracellularly with platinum wire electrodes in the output nerves of the stomatogastric ganglion, according to previous anatomical and physiological works [9, 10, 15].

The 14 neurons of the pyloric pattern generator can be divided into two sets of antagonistic motor neurons, the activity of which regularly alternates. The dilator motor neurons are endogenous burster cells which produce the pyloric rhythm and control the constrictor motor neurons via inhibitory chemical synapses. The rhythmical pyloric sequence is considerably altered by the firing of a single neuron that we have named anterior pyloric modulator (APM) (Fig. 1D). Its cell body (diameter 10–15 μm) is located in the esophageal ganglion. Conventional electrophysiological tests (Fig. 1B, C) demonstrate that APM has an axon in each inferior oesophageal nerve (ion) and that these axons pass through the commissural ganglia into the superior oesophageal nerves (son) and the stomatogastric nerve (stn). Blocking synaptic activity in the commissural ganglia with a 0 Ca2+ + 12 mM Co2+ saline [12] does not alter the result of such tests, indicating that the activity related to APM firing which is recorded in the stn is not due to a post-synaptic neuron activated by APM in the commissural ganglia. In other words, APM has two 4-cm long axons which project directly to the stomatogastric ganglion. APM is the only one of the 12 neurons located in the esophageal ganglion which has such an axonal geometry and can modulate the pyloric sequence. It can thus be easily identified by means of electrophysiological tests.

We will consider here, for the pyloric sequence, the behavior of only two neurons: the pyloric dilator (PD) and a constrictor, the lateral pyloric (LP), which are representative of the two sets of pyloric motor neurons. A discharge of APM, induced by intracellular current injection, strongly modifies the activity of these neurons (Fig. 1E). The amplitude of the oscillations of both PD and LP is significantly increased (Fig. 2B), each cell passing alternately from a more hyperpolarized to a more depolarized state than before the APM discharge (see Fig. 2D). In addition, the frequency of the oscillations is increased; this change is sometimes less obvious than the change in amplitude because it depends upon the pyloric frequency before the firing of APM. The spontaneous pyloric rhythm takes one of two general forms, one characterized by a slow and rather variable frequency, the other by a very regular frequency of approximately 1 Hz. In the first case, an APM discharge tremendously increases the pyloric frequency (Fig. 2C, circles), whereas in the second case the same discharge provokes no change in frequency, (Fig. 2C, squares). In cases of intermediate spontaneous frequency, an APM discharge transiently enhances the frequency to a level corresponding to the spontaneous fast rhythm.

More interesting than these quantitative changes provoked by the firing of APM is a qualitative alteration of the pyloric rhythm, seen as a modification of the
Fig. 2. Quantitative and qualitative modifications of the pyloric rhythm induced by APM discharge. A: diagram showing the synaptic relations of the neurons recorded — PD inhibits LP; the efficacy of this inhibition is modified by APM. B and C: quantitative effects of APM. Each curve shows the averages and standard deviations of 2 trials in a single experiment, with points averaged in 2 sec intervals. In B, the amplitudes of oscillation of PD and LP, expressed as % of the control amplitude, are increased by an APM discharge (bar on graph) and return to control (100%) only after about 45 sec. C shows APM effects on frequency for a fast rhythm (squares), in which there is no change, and for a slow rhythm (circles), in which there is a large increase. Note that the slow frequency is increased to roughly the same frequency as the rapid rhythm; it then slowly returns to normal, remaining elevated even after 50 sec. D and E: APM affects the inhibition of PD onto LP. The activity of PD is shown in an extracellular recording (PD), the inhibition it produces is measured as angle α, and the resulting pause between firing of PD and LP as latency t. D1 is a recording before APM firing, D2 just after a 6 sec discharge of APM. Note the decrease in angle α and the virtual disappearance of latency t (arrow). E shows graphically (averages of 3 trials in a single experiment) the changes in angle α and latency t after an APM discharge (horizontal bar on graph). Again the slow onset and long duration are visible. Calibration: horizontal bar, 1 sec.

...efficacy of certain synapses of the network, leading to a change in the phase relationships of the discharge of the neurons involved. It has been shown that, of the several synapse types in the pyloric network, the inhibitory chemical synapses of the dilator neurons, such as PD, onto the constrictor neurons (such as LP) are the most important with respect to generation of the pyloric rhythm [15]. The inhibition from PD onto LP, which can be represented by the angle between vertical and the slope of the hyperpolarization produced in LP (angle α, Fig. 2D1), causes a silent period t between the end of a PD burst and the beginning of LP firing. An APM discharge markedly decreases the efficacy of this synapse, as shown by the decrease of angle α (Fig. 2D2, E). At the peak of the effect, the inhibition no longer produces
a hyperpolarization in LP, instead merely diminishing its rate of depolarization. This causes the suppression of the silent period between the firing of PD and LP and leads to a change in their phase relationships (Fig. 2E).

Both the quantitative and the qualitative modifications of the pyloric network caused by APM have temporal characteristics which cannot be explained by a conventional synaptic mechanism. Firstly, the onset of these modifications is slow; they generally start 2 sec and peak 4 sec after the first spike recorded in APM cell body. This cannot be due to a polysynaptic pathway since the APM effects on the pyloric network are preserved when synaptic activity in the commissural ganglia is blocked, and no element presynaptic to the pyloric neurons is known to exist in the stomatogastric ganglion, indicating that APM acts directly on the pyloric neurons. Nor can the slow onset of the APM effects be explained by a slow conduction velocity of APM spikes. Travelling at approximately 1 m/sec, APM spikes reach the stomatogastric ganglion in about 60 msec. Secondly, the effects of an APM discharge continue for 8–10 times the duration of the discharge; as seen in Fig. 2B, E, 6 sec of firing in APM provokes effects which last 45–60 sec. The non-conventional nature of the APM effects is also suggested by the fact that no post synaptic potentials related to APM spikes have yet been detected in any pyloric neuron. These characteristics suggest that APM acts as a modulator. To conform to the concept of modulation [1, 3, 4], however, APM must also be able to act on the pyloric neurons in a voltage-dependent manner. It should be noted here that the pyloric neurons have complex membrane properties; they are ‘plateau cells’, characterized by two ranges of stable membrane potential and capable of passing actively from one to the other [14]. We chose to examine, in LP, the repolarizing phase, which normally terminates each burst of spikes (see Fig. 1E) and which can be experimentally induced by injecting hyperpolarizing current into the soma (Fig. 3). To eliminate possible interference from inhibitory synaptic inputs, we used preparations perfused with 10⁻⁵ M picrotoxin, which is known to decrease significantly these inhibitory inputs [2]. Fig. 3A shows the normal response of LP to hyperpolarizing pulses of increasing intensity. When the same pulses are given during a discharge of APM, the apparent time constant of the hyperpolarizing response is greatly increased (Fig. 3B). More precisely, the initial time constant (passive response) remains unchanged (arrow), but a secondary hyperpolarizing response (active response) develops, and is considerably slower than the control. It is thus the active repolarizing phase of the LP oscillation that is delayed by APM, resulting in an increased stability of its higher level of membrane potential; this may explain the diminished efficacy of the synapse from PD during the firing of APM. For the present purposes, however, the most important characteristic of this phenomenon is that it seems to be voltage dependent. In Fig. 3B, the lower the membrane potential of LP after the initial passive response is, the less delayed the next active response is. The voltage dependence of APM effect also appears in Fig. 3C. If a hyperpolarizing pulse is given during an APM discharge when the LP
Fig. 3. Voltage-dependent effects of an APM discharge on the active repolarization of LP. Current (trace 2) was injected into LP with one electrode; changes in membrane potential (trace 1) were recorded with a second. A and B: responses of LP to increasing current pulses (1 sec in duration before (A) and after (B) a discharge of APM. In B, the initial fast descent (passive), marked with an arrow, is equivalent to that in A. The active descent which follows is distinctly retarded. C: this effect is voltage-dependent. Two successive sweeps are triggered by identical hyperpolarizing pulses injected into LP just after an APM discharge. If LP is sufficiently depolarized (upper trace), the active repolarization develops slowly after the initial fast passive response (arrow). If, however, the LP potential is below a threshold value (lower trace), its repolarization is not delayed. Calibrations: horizontal bars, 0.5 sec; vertical bars, 5 nA in A and B, 2.5 nA in C.

Potential is above a threshold value (depending upon the preparation, but – 55 mV in Fig. 3C), the active mechanism develops slowly (Fig. 3C, upper trace, arrow) after the initial fast passive response. If, however, the LP membrane potential is below the threshold value (Fig. 3C, lower trace), the hyperpolarizing response is not delayed. To summarize, the effects of APM on the pyloric network have a slow onset and long duration and are, at least in some aspects, voltage dependent. The name ‘anterior pyloric modulator’ is thus suitable for this neuron.

Because the roles of the pyloric neurons in the functioning of the pyloric stomach are well established, the functional implications of the modulation caused by APM can be easily determined. By modifying the discharge of the motor neurons, APM likely enhances the strength and frequency of the cyclic muscular contractions and modifies the phase relationships of antagonistic muscles. Interestingly, an APM discharge can induce the pyloric network to pass from a slow and irregular to a fast and regular rhythm. A similar duality of rhythm is seen in electromyographic recordings from the pyloric stomach of intact lobsters, the change from the slow to the fast rhythm occurring at feeding [13]. It is thus possible that APM is involved in the control of the pyloric stomach during feeding.

To conclude, the system presented here is highly suitable for an analysis of neuronal modulation on several levels. For the cellular level of analysis it provides a pattern generator comprised of a small number of neurons, the connections of which are known. Moreover, these neurons present interesting endogenous properties (oscillation, plateau properties) which are altered by a single identified modulatory neuron. Using chronic myographic recordings on intact animals it is possible to extend the analysis to the behavioral level, allowing examination of a neuronal modulation in its biological context.
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