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# CONTROL OF A CENTRAL PATTERN GENERATOR BY AN IDENTIFIED MODULATORY INTERNEURONE IN CRUSTACEA

## II. INDUCTION AND MODIFICATION OF PLATEAU PROPERTIES IN PYLORIC NEURONES

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SUMMARY

In the isolated stomatogastric nervous system of the lobster *Jasus lalandii*, the strong modifications of the pyloric motor pattern induced by firing of the single anterior pyloric modulator neurone (APM) are due primarily to modulation by APM activity of the regenerative membrane properties which are responsible for the 'burstiness' of all the pyloric neurones and particularly of the non-pacemaker neurones (constrictor motoneurones).

This modulation has been studied under experimental conditions where the main extrinsic influences usually received by the pyloric constrictor neurones (intra-network synaptic interactions, activity of pacemaker neurones, and phasic central inputs from two premotor centres) are minimal. Under these conditions a brief discharge of neurone APM induces long plateaus of firing in all of the pyloric neurones.

The non-pacemaker neurones of the pyloric network are not simply passive follower neurones, but can produce regenerative depolarizations (plateau potentials) during which the neurones fire spikes. The ability of the pyloric constrictor neurones to produce plateau potentials (plateau properties) contributes greatly to the generation of the rhythmical pyloric motor pattern. When these neurones spontaneously express their plateau properties, firing of neurone APM amplifies these properties. When most of the central inputs usually received by the pyloric constrictor neurones are experimentally suppressed, these neurones can no longer produce plateau potentials. In such conditions, firing of the single modulatory neurone APM can reinduce plateau properties of the pyloric constrictor neurones.

In addition, firing in APM neurone slows down the active repolarization phase which terminates the plateau potentials of pyloric constrictor neurones. This effect is long-lasting and voltage-dependent.

Modulation by APM of the plateau properties of the pyloric neurones also changes the sensitivity of these neurones to synaptic inputs. This effect can explain the strong modifications that an APM discharge exerts on a current

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pyloric motor pattern. Moreover, it might render the motoneurones of the pyloric pattern generator more sensitive to inputs from a command oscillator, and contribute to switching on the pyloric motor pattern.

## INTRODUCTION

The mechanisms by which central pattern generators are controlled, in particular how they are turned on and off and how their activity can be modulated by higher centres, have not yet been clearly elucidated. This problem can be approached using the stomatogastric nervous system of crustaceans, in which it has been shown that the motor output of a central pattern generator (the pyloric network) is lastingly modified by the discharge of a single interneurone (APM; Nagy & Dickinson, 1983). In some cases, the modifications of the pyloric pattern engendered by APM resemble those which correspond to the initiation of feeding in the intact animal. We can thus analyse the cellular mechanisms which underly the control of a pattern generator by asking what cellular properties of the pyloric neurones are altered by the discharge of the interneurone APM.

The ability of central pattern generators to produce rhythmical motor outputs depends not only on the synaptic connections within the pattern generator, but also on the cellular properties, such as endogenous bursting capabilities (Koester, Mayeri, Liebeswar & Kandel, 1974; Maynard & Selverston, 1975; Thompson & Stent, 1976) and post-inhibitory rebound (Siegler, Mpitsos & Davis, 1974) of the neurones comprising the generator. The rhythmic pyloric pattern is in part dependent on the existence of endogenous oscillators (pacemaker neurones) within the pyloric network (Maynard & Selverston, 1975; Selverston, Russell, Miller & King, 1976). In addition, all the pyloric neurones, including the non-pacemaking neurones, can produce long-lasting regenerative depolarizations, the 'plateau potentials', which are responsible for the burstiness of these neurones and thus play a major role in the generation of the pyloric rhythm (Russell & Hartline, 1978).

The expression of plateau properties in pyloric neurones depends upon inputs from higher centres, stimulation of the single input nerve to the stomatogastric ganglion being able to induce or amplify these properties in all the pyloric neurones (Russell & Hartline, 1978, 1981; Moulins & Cournil, 1982). The source of these inputs has, however, been obscure.

We show in this paper that one of these inputs is the single modulatory interneurone APM, which can induce plateau properties in the non-pacemaking pyloric neurones and amplify these properties in all the pyloric neurones, including the pacemakers. The induction and amplification of plateau properties in the pyloric neurones can fully explain the quantitative and qualitative modifications of the pyloric motor output provoked by an APM discharge (see Nagy & Dickinson, 1983).

## MATERIALS AND METHODS

Experiments were performed on the isolated stomatogastric nervous system of male and female Cape lobsters, *Jasus lalandii*. With a few exceptions, the dissection, recording techniques and neurone identification were as outlined previously (Moulins

Nagy, 1981) and in the accompanying paper (Nagy & Dickinson, 1983). The isolated nervous system (Fig. 1A) was pinned on a Sylgard-lined Petri dish and maintained in oxygenated artificial sea water at 20 °C. Petroleum jelly barriers around the commissural ganglia (CG) permitted superfusion with a $0Ca^{2+}+12$ mM-$Co^{2+}$ saline (artificial sea water in which $Ca^{2+}$ was replaced with $Mg^{2+}$ and to which 12 mM-$Co^{2+}$ was added) to block synaptic activity in these ganglia. A similar barrier was built around the stomatogastric ganglion (STG) in those experiments in which this ganglion was perfused with $10^{-5}$ M-picrotoxin (gift from Fluka) or with high $K^+$ saline (three times normal $K^+$, with the increased $K^+$ replacing equivalent $Na^+$). In most experiments involving injection of hyperpolarizing currents (see Figure legends), the neurones were penetrated with two microelectrodes, one for recording and one for injecting current. Current injected was monitored with a virtual ground circuit connected to the bath.

## RESULTS

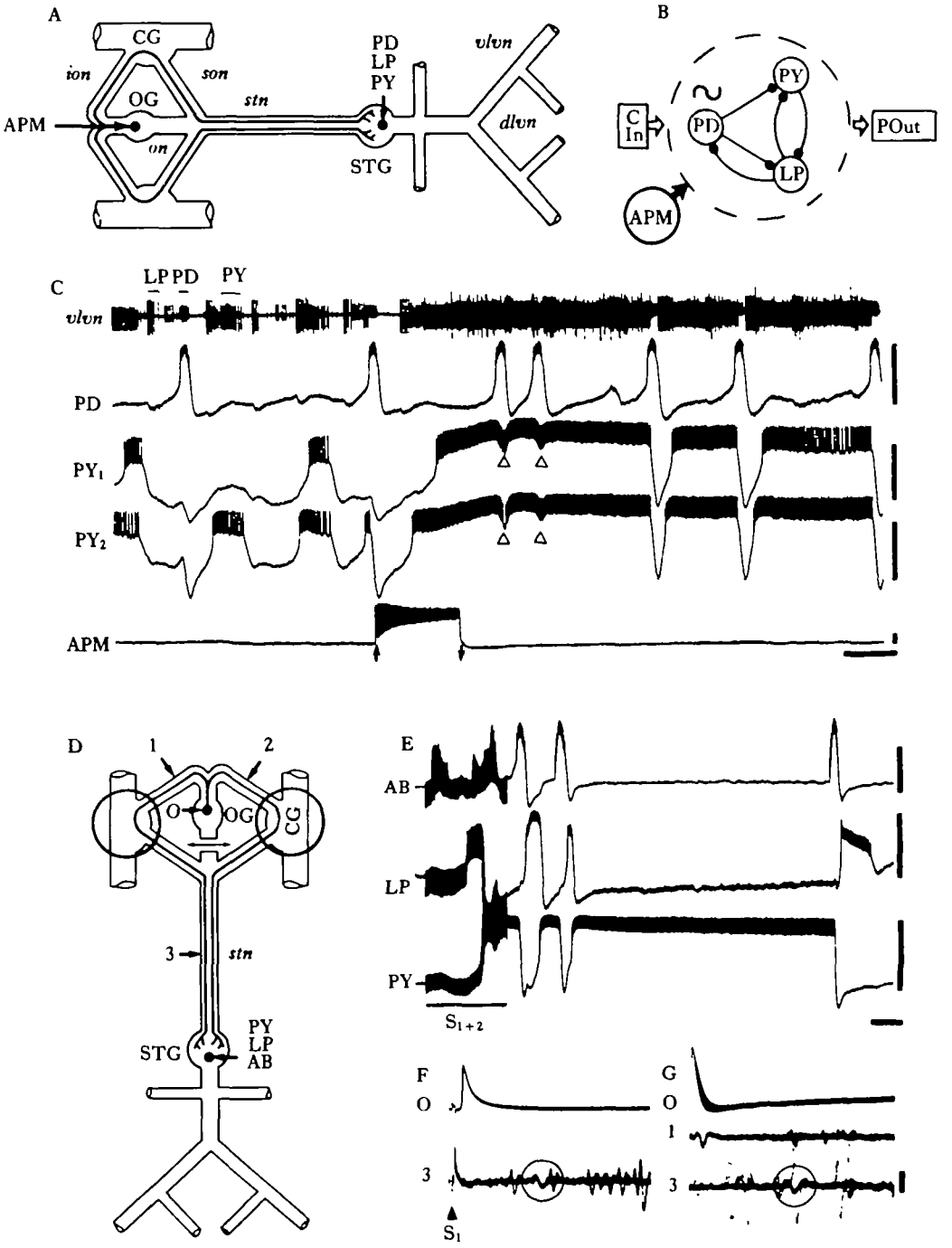
### *Background observations*

In the isolated stomatogastric nervous system (Fig. 1A) of the lobster, the pyloric motor pattern, which is responsible for producing rhythmic movements of the pyloric filter, can be readily recorded. This rhythmic pattern is produced by the 14 neurones of the pyloric network (see Maynard, 1972; Maynard & Selverston, 1975; Selverston & Miller, 1980) and consists of bursts of action potentials produced alternately in the dilator and in the constrictor motoneurones. For the present purposes, we shall consider only a subset of the pyloric neurones: a group of three electrically coupled neurones, the two pyloric dilators (PD) and the anterior burster (AB), which are the pacemakers of the pyloric rhythm and can be considered as a single functional unit, and two types of constrictor motoneurones, the single lateral pyloric (LP) and the eight electrically coupled pyloric (PY) neurones. These neurones interact via a number of inhibitory synapses, shown in Fig. 1B. The dilators (PD) inhibit both types of constrictor (LP, PY); the constrictors LP and PY are mutually inhibitory; the constrictor LP also inhibits the dilators (PD).

The pyloric motor output (see Fig. 1B) is normally determined by the pacemaker activity of the dilator neurones (PD) and by the synaptic connections within the network (Maynard, 1972; Selverston *et al.* 1976). In addition, the pyloric rhythm is influenced by phasic inputs from the commisural ganglia (CG, premotor centres for the pyloric network) (Selverston & Miller, 1980; Robertson & Moulins, 1981). Because the goal of the present paper is to examine the action of APM's discharge on the cellular properties of the pyloric neurones, particularly the non-pacemaking neurones (LP and PY), we attempted to study the pyloric network under conditions in which the influences mentioned above (activity of pacemaker neurones, phasic central inputs, intra-network synaptic interactions) were minimal. This can be realized in three ways: firstly by choosing preparations in which the background activity of PD neurones is minimal; secondly by experimentally blocking synaptic activity in the commisural ganglia, which suppresses phasic inputs to the pyloric network from these ganglia and greatly diminishes the activity of the pyloric pacemakers (PD); and thirdly by pharmacologically decreasing the influence of synapses within the network.

*APM causes the pyloric neurones to fire in plateaus*

When some of the influences that pyloric neurones usually receive are weakened (see above), a discharge of APM causes these neurones to fire in plateaus (Figs 1C, E, 2).



This happens when the pyloric pacemaker neurone PD is spontaneously minimally active. As was shown in the preceding paper (Nagy & Dickinson, 1983), spiking in APM when the pyloric rhythm is spontaneously weak strongly activates all the pyloric neurones, particularly the constrictors (LP, PY). In some cases this activation is accompanied by an extensive increase in the pacemaker activity of the dilators (PD) and hence an increase in the frequency of the rhythm; in other cases pacemaker activity is not activated. It is in cases of the latter category that the influence of PD is minimal. In such a case (Fig. 1C), the major effect of an APM discharge is to provoke long plateaus of firing in the constrictor neurones (PY shown here). During these plateaus, spike frequency is elevated and corresponds to the long, intense bursts which can be recorded extracellularly (Fig. 1C, *vlvn*). At the peak of the effect, the inhibition by PD is considerably less effective than usual and cannot interrupt plateaus of the constrictor neurones (Fig. 1C, open triangles).

Such plateauing of pyloric neurones under APM influence is also obtained when synapses are blocked in commissural ganglia with $0Ca^{2+}+Co^{2+}$ saline (Fig. 1D, E). This experimental situation not only minimizes phasic inputs from these ganglia on the pyloric network but offers an added technical advantage: under these conditions, and with the oesophageal nerve cut (*on*, Fig. 1A, D), extracellularly stimulating the inferior oesophageal nerves (Fig. 1A, *ion*; Fig. 1D, 1 and 2) activates APM axons without activating other inputs to the stomatogastric ganglion (Fig. 1F, G; for further

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Fig. 1. When the pyloric rhythm is spontaneously slow or experimentally weakened, the constrictor neurones fire in long plateaus after an APM discharge. (A) The isolated stomatogastric nervous system used in all experiments. (B) Three mechanisms cooperate to produce the pyloric motor pattern (P Out): the oscillatory activity of the pacemaker neurones (PD, ∿), the inhibitory synaptic relations (filled circles) within the network, the synaptic inputs coming from higher nervous centres (C In). (C) Simultaneous intracellular recordings of the activity of a pacemaker (PD) and two constrictor ($PY_1$, $PY_2$) neurones. When the pyloric rhythm is spontaneously slow and irregular a brief discharge of the neurone APM only slightly accelerates the frequency of oscillations in PD, but provokes long plateaus of firing in the PY neurones. Long intense bursts of action potentials are also recorded in the motor nerve *vlvn*. The first two bursts in the pacemakers after the APM discharge were unable to bring about the repolarization of the PY neurones (open triangles; compare with the effects of later pacemaker bursts). Activity in APM was induced by intracellular current injection. (D) Diagram of the preparation in (E), (F) and (G). The commissural ganglia (encircled) are bathed in $0Ca^{2+}+Co^{2+}$ saline, which blocks synaptic activity. The *on* is cut (↔). 1, 2, extracellular electrodes used to record or stimulate the *ions*; 3, extracellular recording electrode on the *stn*; O, intracellular electrode in the soma of APM. (E) In the experimental conditions shown in (D), electrical stimulation of the two *ions* ($S_{1+2}$, 5 s, 35 Hz) provokes long plateaus in the three pyloric neurones recorded, a pacemaker (AB) and two constrictor (LP, PY) neurones. (F) In same conditions as in (D), stimulation of the left *ion* ($S_1$) generates an antidromic action potential in the soma of APM (O) and a single extracellularly recorded action potential in the *stn* (3, circle). (G) When APM is depolarized by current injection, somatic action potentials which correspond to the extracellularly recorded potentials on the *ion* (1) and the *stn* (3) are generated. The potential recorded on the *stn* is identical to that provoked by the stimulation $S_1$ in (F); the delay between the action potential in the *ion* and that in the *stn* is equal to that separating the stimulation $S_1$ from the potential provoked in the *stn* in (F). Under these conditions the axons of APM are the only inputs to the pyloric network which are activated by extracellular stimulation of the *ions*. In (F), the superimposed oscilloscope sweeps were triggered by the extracellular stimulation ($S_1$); in G they were triggered by the somatic action potential of APM. Calibrations: horizontal bars, 2 s in (C), (E); 20 ms in (F), (G); vertical bars, 20 mV. AB, anterior burster neurone; APM, anterior pyloric modulator neurone; CG, commissural ganglion; C In, central inputs; *dlvn*, dorsal lateral ventricular nerve; *ion*, inferior oesophageal nerve; LP, lateral pyloric neurone; OG, oesophageal ganglion; *on*, oesophageal nerve; PD, pyloric dilator neurone; P Out, pyloric output; PY, pyloric neurone; *son*, superior oesophageal nerve; *stn*, stomatogastric nerve; STG, stomatogastric ganglion; *vlvn*, ventral lateral ventricular nerve.

details see Fig. 1 legend). This is experimentally useful because the cell body of API is often difficult and sometimes impossible to penetrate with a microelectrode (10–15 μm diameter, surrounded by a thick individual sheath).

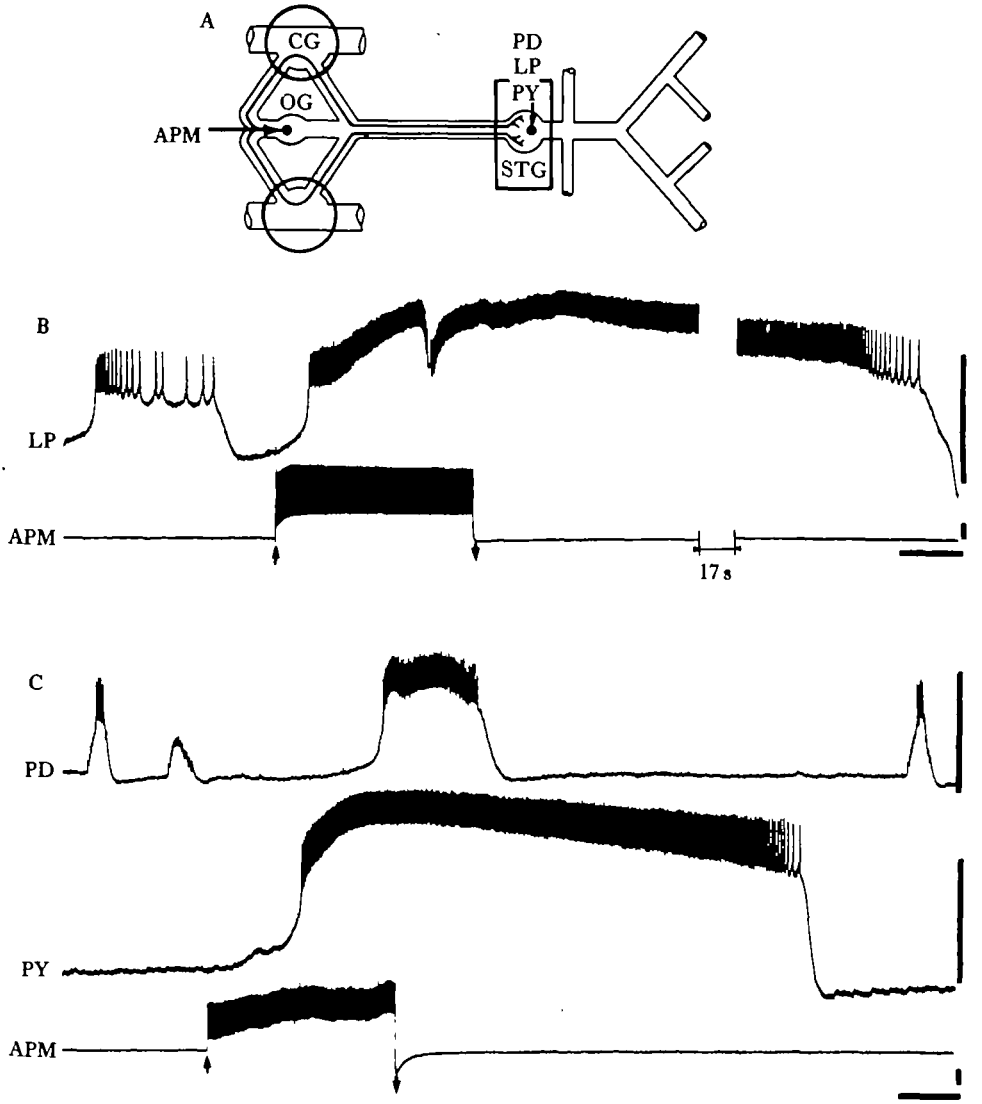


Fig. 2. When synaptic activity in the commissural ganglia is blocked and that in the stomatogastric ganglion is weakened, an APM discharge provokes plateaus of firing in all the pyloric neurones. (A) Diagram of the preparation. Circles indicate perfusion of the CG by $0Ca^{2+}+Co^{2+}$ saline to block synaptic activity; the rectangle indicates perfusion of the STG with $10^{-5}$ M-picrotoxin, which weakens the inhibitions within the pyloric network. Arrows indicate intracellular electrodes. (B) A 6 s discharge of APM provokes a long (35 s) plateau of firing in the LP neurone (17 s have been cut from the recording). The slight hyperpolarization seen in LP near the beginning of its plateau is the result of inhibition by a burst in the pacemaker neurones. (C) Under the same conditions as in (B), but in a separate experiment, a 6 s discharge of APM provokes a 16 s plateau in the PY neurone. This plateau is unaffected by the discharge of the pacemaker neurone PD. The 3 s plateau simultaneously provoked in PD is about ten times the length of PD's normal bursts (see Fig. 1C). In (B) and (C), the APM discharge was induced by intracellular current injection (arrows). Calibration: horizontal bars, 2 s; vertical bars, 20 mV. For abbreviations see legend to Fig. 1.

In the experimental conditions of Fig. 1E, duration of the plateau potentials induced in the pyloric neurones by APM firing is variable because it is largely determined by the inhibitory synapses within the pyloric network (see Fig. 1B). Therefore to observe more easily the effects of APM on individual pyloric neurones it is necessary, in addition to weakening descending inputs to the pyloric network, to weaken the synaptic interactions within the network itself. By bathing the stomatogastric ganglion in picrotoxin (Fig. 2A, rectangle), it is possible to block or partially block inhibition from the AB, LP and PY neurones (Marder & Paupardin-Tritsch, 1978; Bidaut, 1980). Under these conditions, plateaus considerably longer than usual are generated in both LP (35 s plateau, Fig. 2B) and PY (16 s plateau, Fig. 2C) when APM fires (6 s discharge in these examples). The plateaus generated in PD under the influence of APM are still much shorter than those in the constrictors, but are nevertheless about ten times longer than usual (3 s in Fig. 2C). In picrotoxin, the duration of the plateaus is no longer limited by inhibitory synaptic influences within the network, as can be seen, for example, in Fig. 2C, in which the PY plateau is not terminated by the burst in the PD-AB group (the AB to PY synapse is picrotoxin sensitive). Therefore, when the pyloric neurones are nearly isolated from external influences, the final effect of an APM discharge is the induction of very long plateau potentials, which in turn lead to the firing of long and intense bursts of spikes.

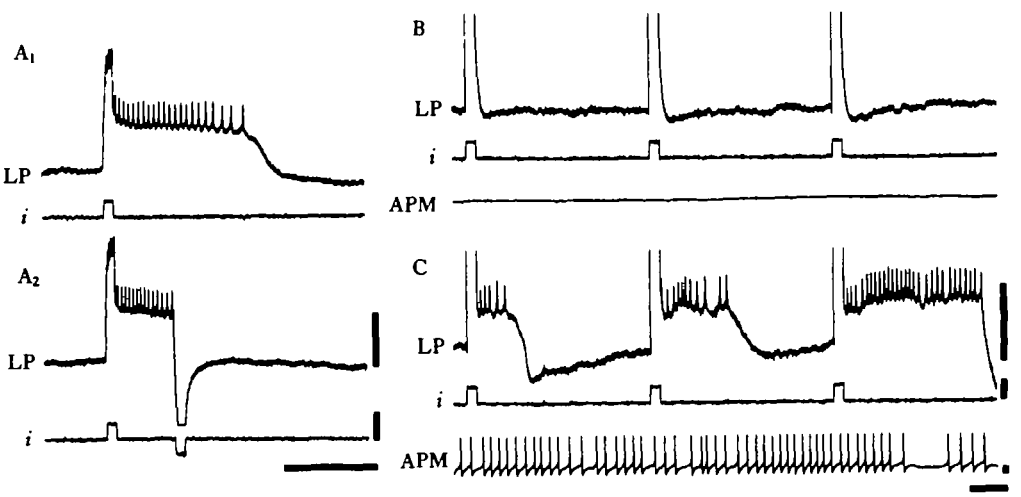


Fig. 3. Activity in APM induces 'plateau properties' in the constrictor neurone LP. (A) Under normal conditions, LP is spontaneously capable of generating plateau potentials. The injection of a brief pulse of depolarizing current (*i*) provokes a long-lasting plateau potential ($A_1$) which can be prematurely terminated by a pulse of hyperpolarizing current ($A_2$). (Separate current and voltage electrodes were used.) (B) Under the same experimental conditions shown in Fig. 2, LP lost its plateau properties; it responds to brief, depolarizing current pulses (*i*) by equally brief, passive depolarizations. (C) When, under the same conditions, APM is induced to fire tonically at a low frequency, the same depolarizing currents injected into LP produce plateau potentials. Current injected through recording electrode in B and C; tops of current pulses are distorted by the bridge circuit and clipped by the recording apparatus in (B) and (C). Calibrations: horizontal bars, 1 s, vertical bars, 10 mV, 5 nA in (A), 10 mV, 2 nA in (B) and (C). For abbreviations see legend to Fig. 1.

*APM induces plateau properties in the pyloric neurones*

All the neurones of the pyloric network are capable of producing regenerative depolarizations or plateau potentials (Russell & Hartline, 1978), which appear to be of considerable importance in the generation of the pyloric rhythm. These neurones are characterized by a bistable membrane potential that can only transiently cross between two narrow ranges of membrane potential stability. As seen in Fig. 2C (for instance in PY), the plateau potential is comprised of an active transition from the lower level of stability to the depolarized stable level, then a period of time during which the neurone remains depolarized and finally an active repolarization which terminates the plateau. The depolarized stable level in the pyloric neurones is above the spike threshold, so that a burst of spikes, whose duration is determined by the length of time the cell remains at the upper level, is fired during each plateau potential. The transition between the two levels is an active process, but can be triggered by an external event (synaptic input or injected pulse of current). It is thus possible to test for the ability of a neurone to generate plateau potentials by the injection of brief pulses of current in its soma (Russell & Hartline, 1978; see Fig. 3A). A brief pulse (200 ms) of depolarizing current triggers a longer (several seconds), regenerative depolarization (plateau) in the neurone (Fig. $3A_1$). Injection of a brief pulse (200 ms) of hyperpolarizing current can prematurely trigger repolarization and terminate the plateau (Fig. $3A_2$).

Using this method of testing for plateau properties we have shown that when inputs to the pyloric neurones are minimized (commissural ganglia synapses blocked and synapses in the stomatogastric ganglion weakened), neither LP nor PY exhibits plateau properties (Figs 3B and 4A, respectively). Simple depolarization or hyperpolarization of constrictor neurones does not restore their ability to generate plateaus. Thus these neurones have lost their plateau properties. The influence of APM alone is, however, able to restore them. When APM fires tonically at a low frequency, the same 200 ms depolarizing pulse injected into LP (Fig. 3C) or PY (Fig. 4B, C) produces a long-lasting plateau. This plateau potential can be prematurely terminated by a brief pulse (200 ms) of hyperpolarizing current (Fig. 4C), the membrane potential of the neurone then remains at its lower level of stability until the next depolarizing pulse. APM induced plateau properties in these neurones.

Fig. 5 further demonstrates that APM's discharge does not simply provoke a depolarization of the pyloric neurones, but rather induces bistability, which is a major criterion for plateau properties. In this case, tonic firing of APM provokes long spontaneous plateaus in the neurone PY (Fig. 5A). Brief (100 ms) pulses of hyperpolarizing current injected into the cell can then repolarize the neurone and terminate the plateaus; the cell remains silent at its lower level of stability for approximately 1·2 s (Fig. 5B). In contrast, the same neurone behaves differently when (APM being silent) it is simply depolarized with current injection until it reaches a spike frequency similar to that recorded during the plateau provoked by APM (Fig. 5C); pulses of hyperpolarizing current identical to those used in Fig. 5B interrupt the neurone's discharge only for the duration of each pulse, indicating that under these conditions (without APM firing), PY is no longer a bistable neurone.

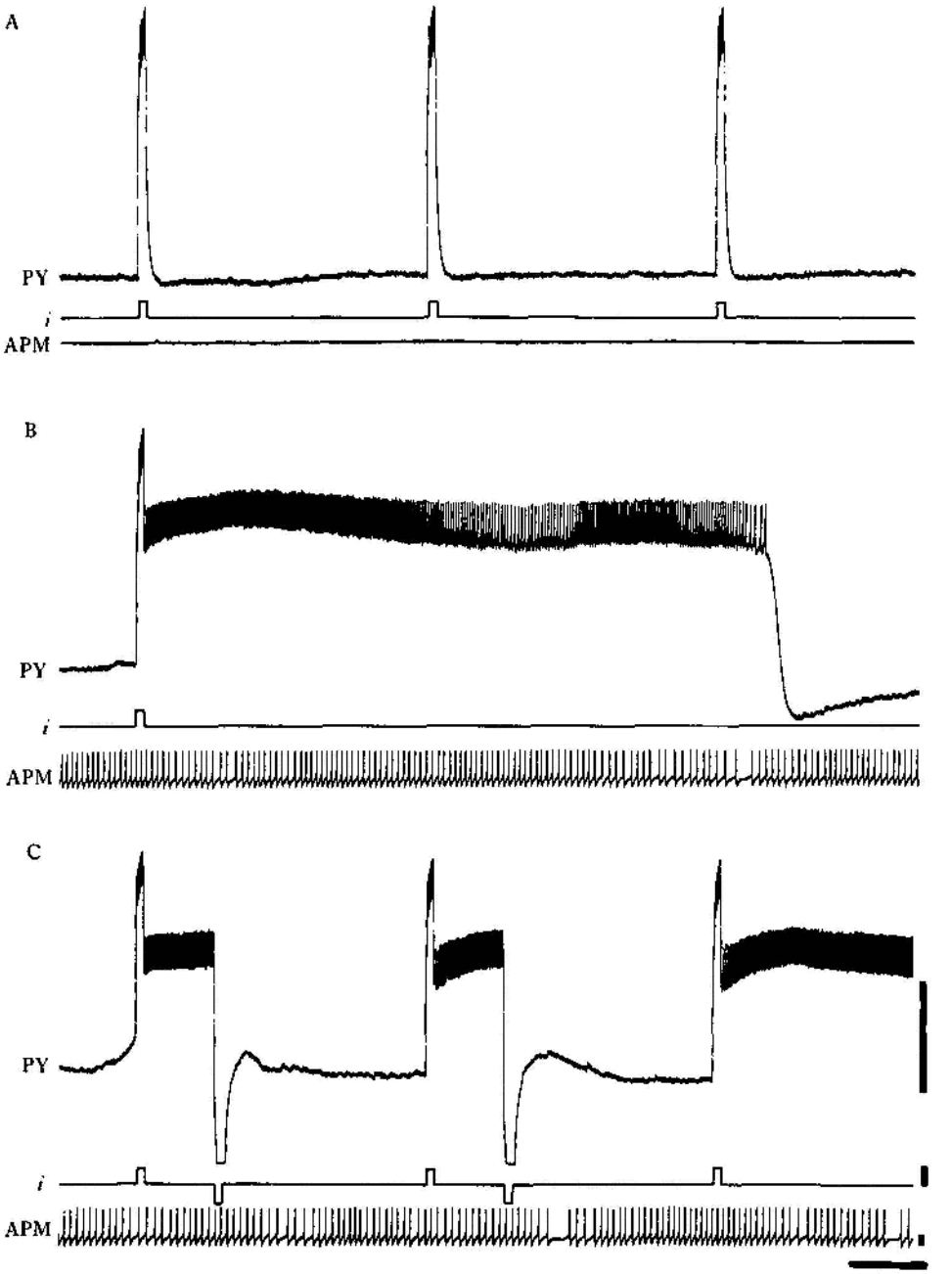


Fig. 4. Activity in APM induces plateau properties in the PY constrictor neurones. (A) In the same experimental conditions used in Fig. 2A (CG synapses blocked; STG synapses weakened), the PY neurone has lost its plateau properties. It responds to depolarizing current pulses (*i*) injected into its cell body with passive depolarizations of short duration. (B) When, under the same experimental conditions, APM fires tonically at low frequency, an identical current pulse provokes a 16 s plateau in PY. (C) In the same conditions as in (B), the plateaus in PY (provoked by brief depolarizing current pulses), can be terminated by the injection of brief pulses of hyperpolarizing current (1·5 nA), indicating that the bistability of PY's membrane has been increased by the activity of APM. Calibration: horizontal bar, 2 s; vertical bars, 20 mV, 1·5 nA. For abbreviations see legend to Fig. 1.

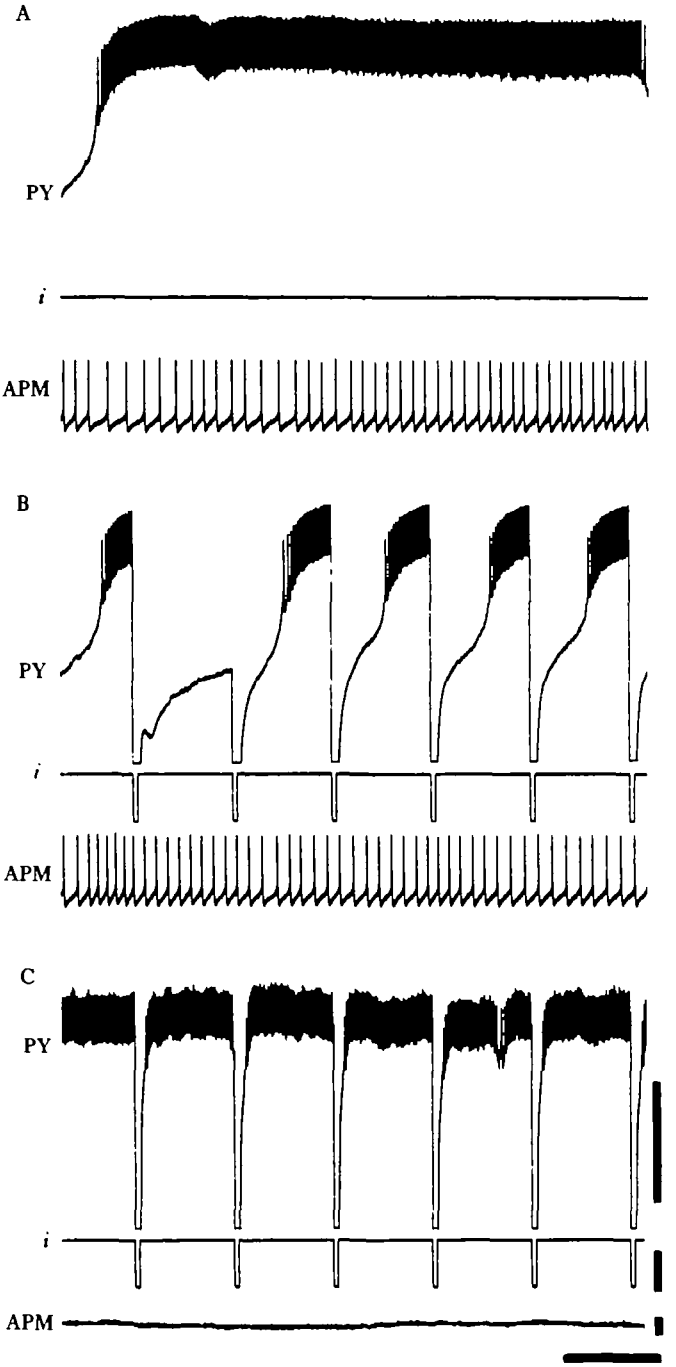


Fig. 5. The activity of APM increased the bistability of the PY constrictor neurones. (A) In the experimental conditions of Fig. 2A (CG synapses blocked by $0Ca^{2+}+Co^{2+}$; synapses within the network weakened with picrotoxin), a tonic discharge of APM causes a PY neurone to fire in long plateaus. (B) The plateau provoked by the APM activity is repeatedly interrupted for at least 1·2 s by brief pulses of hyperpolarizing current (*i*, 2·3 nA, 100 ms). (C) When the neurone APM is silent, and the PY neurone is experimentally depolarized to fire at the same frequency as that recorded during the plateaus in (A) and (B), the same hyperpolarizing pulses injected into PY lower its membrane potential and stop its firing only for the duration of the pulse. Calibration: horizontal bar, 2 s; vertical bars, 20 mV, 2 nA. For abbreviations see legend to Fig. 1.

### *APM amplifies plateau properties in the pyloric neurones*

Under the conditions described above, the pyloric constrictor neurones lose their plateau properties. However, they are capable of generating plateau potentials to some extent, in most unmanipulated preparations. In such cases (Fig. 6), activity in APM amplifies rather than induces the plateau properties of these neurones.

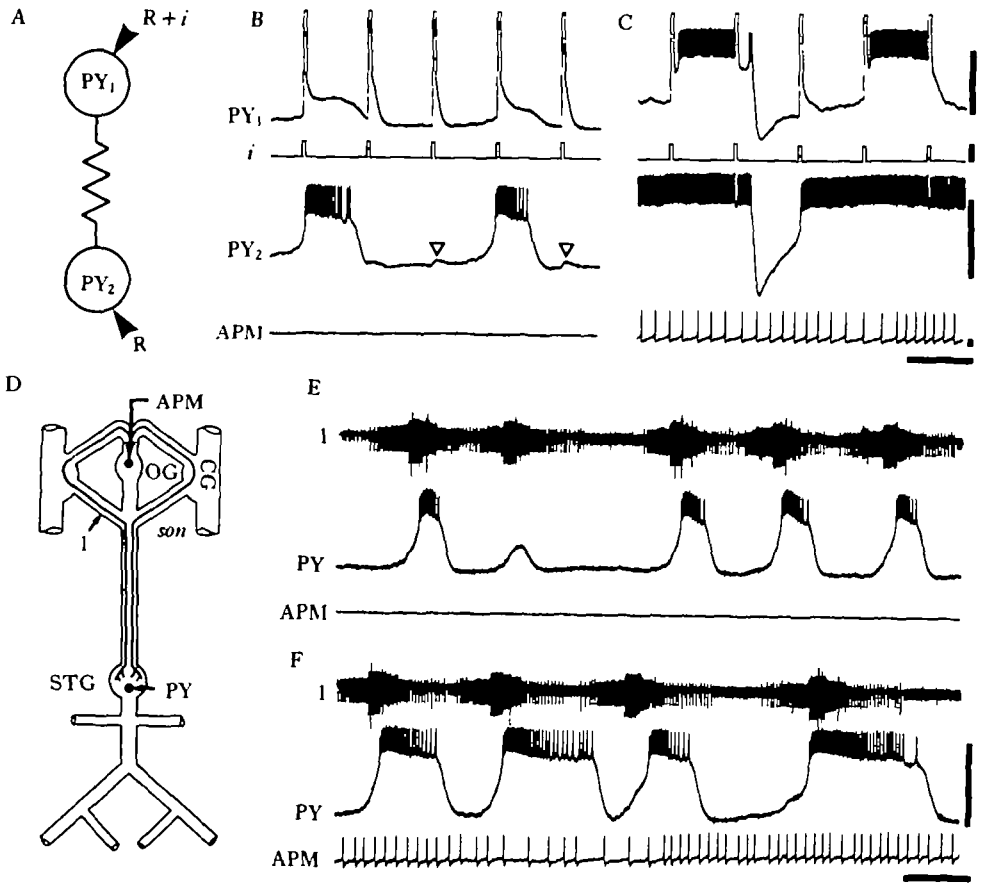


Fig. 6. Activity in APM amplifies the plateau properties of the constrictor neurones when they are spontaneously present. (A) Diagram of the experimental situation in recordings (B) and (C). R+*i*, intracellular electrode used for recording and for current injection; R, recording electrode; resistance (⁓), electrical synapse between the two PY neurones being recorded. (B) When the pyloric rhythm is spontaneously slow, one PY neurone ($PY_1$) has very weak plateau properties; in response to injected depolarizing current pulses (*i*, 2 nA), it sometimes produces prolonged depolarizations, but never fires action potentials. A second PY neurone ($PY_2$) shows stronger plateau properties; in two cases, the current transmitted to it *via* its electrical synapse with $PY_1$ provokes plateaus in which the neurone fires a train of action potentials. (C) In the same conditions as in (B), a tonic discharge of APM amplifies the plateau properties of both PY neurones. $PY_1$ produces full plateaus with action potentials and the plateaus in $PY_2$ are longer and more intense. (D) Diagram of the preparation corresponding to the recordings in (E) and (F); 1, extracellular recording electrode on the *son*. (E) A PY neurone spontaneously produces plateau potentials correlated with bursts of activity in the oesophageal network, recorded on the *son* (1). (F) Tonic activity in APM amplifies these spontaneous plateaus. Calibrations: horizontal bars, 2 s; vertical bars, 20 mV and 2 nA in (B) and (C), 20 mV in (E) and (F). For abbreviations see legend to Fig. 1.

In some cases, as in Fig. 6B, the plateau properties of the several PY neurones are developed to differing extents. Here, repetitive depolarizing pulses (100 ms) provoke only partial plateaus in one constrictor neurone ($PY_1$), whose plateau properties are very weak. $PY_1$ is electrically coupled (Fig. 6A) to another PY neurone ($PY_2$) and the pulses of current injected into the soma of $PY_1$ produce depolarizing electrotonic potentials in $PY_2$ (Fig. 6B, open triangles). In this case, the plateau properties are stronger in $PY_2$ than in $PY_1$, and the electrotonic potentials transmitted from $PY_1$ provoke fully developed plateaus in $PY_2$. When APM is induced to fire tonically at a low frequency (Fig. 6C), $PY_1$ produces true plateaus in response to the same pulses, and the plateaus in $PY_2$ are stronger than before APM firing: they last longer, spike frequency within the plateau is elevated, and the amplitude of the plateau potential is increased.

Fig. 6E, F illustrates a situation in which APM amplifies naturally occurring plateaus. In this case, plateaus in a PY neurone were spontaneously produced more or less in synchrony with bursts in the oesophageal network, another rhythmic network of the stomatogastric nervous system (Moulins & Nagy, 1981) whose activity is recorded extracellularly from a *son*. During a low frequency discharge of APM (Fig. 6F), these plateaus are both longer and of greater amplitude than they are when APM is silent. Thus, APM can either induce or amplify plateau properties in the constrictor motoneurones (PY and LP).

### *APM modifies the repolarization phase of plateaus in the pyloric neurones*

#### *The repolarization phase is slowed down*

We have shown that APM can amplify the plateau properties of the constrictor motoneurones and that one manifestation of this amplification is an increase in the duration of plateaus, such that the spontaneous repolarization phase, which usually terminates a plateau potential, occurs later. In addition, when the plateau properties are so amplified, this repolarization phase in constrictor neurones is less easily triggered by inhibitory synaptic inputs from the pacemaker neurones. In some cases, these inhibitory inputs are unable to interrupt a plateau potential when APM is active (see for example Fig. 1C, open triangles). Further, as demonstrated in the preceding paper (Nagy & Dickinson, 1983), APM decreases the efficacy of these same synapses when the pyloric rhythm is active. This suggests that APM may specifically modify the repolarization phase responsible for the termination of the plateaus. Modifications of this repolarization phase of plateaus in the constrictor neurones may be of particular importance functionally, for the duration of each burst of spikes in these neurones depends on the duration of the depolarized phase of the plateaus. We therefore considered the effects of APM's discharge on the repolarization phase of the pyloric neurones, after triggering these repolarizations by injecting pulses of hyperpolarizing current into the neuronal somata.

Fig. 7 shows that, when APM fires, the repolarization from plateaus in the constrictors is distinctly slowed down. Repolarizations of LP in response to 1 s hyperpolarizing current pulses of increasing intensity injected during spontaneous plateaus are shown in Fig. 7B. In this instance LP's plateau properties were well developed. The stomatogastric ganglion was bathed in $10^{-5}$ M-picrotoxin to minimize synaptic

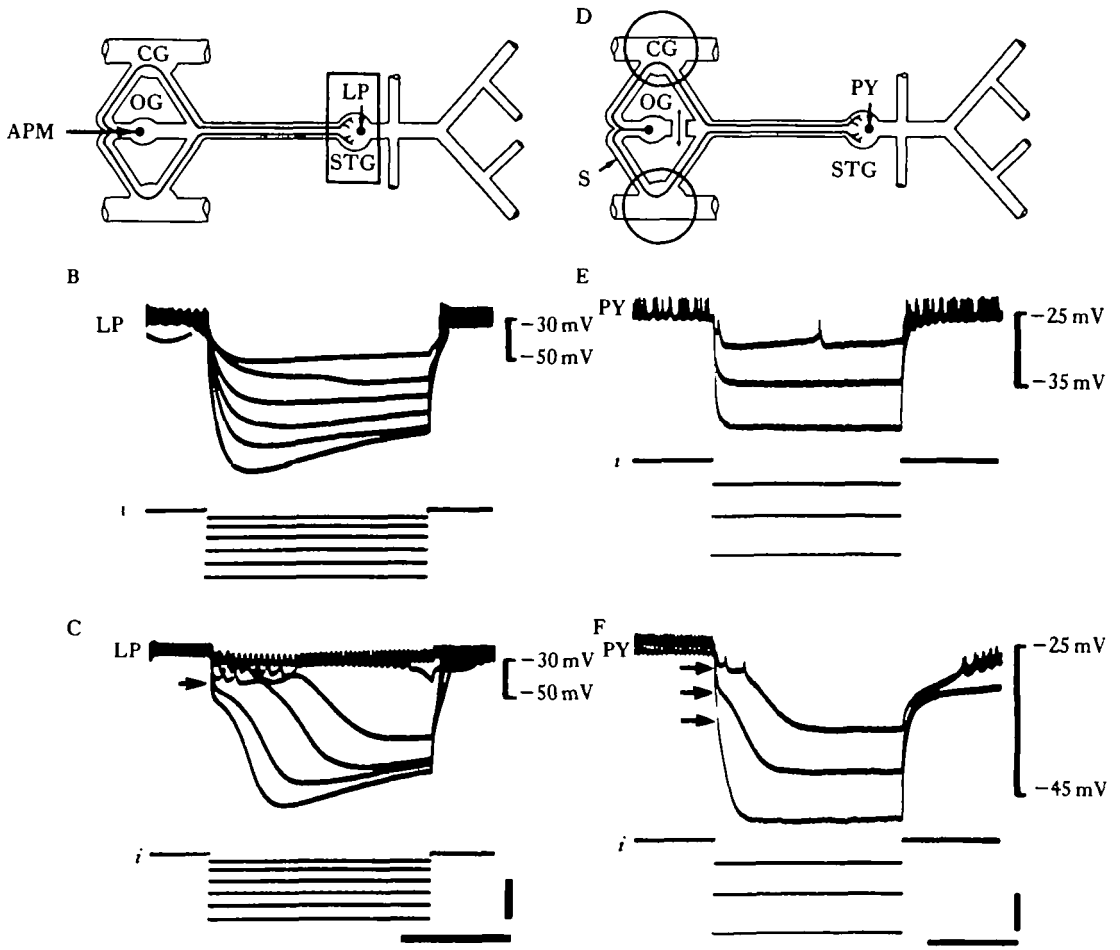


Fig. 7. Activity in APM retards the development of the repolarization phase of plateaus in the constrictor neurones. (A) Diagram of the preparation used in (B) and (C); the rectangle indicates the area perfused with $10^{-5}$ M-picrotoxin to weaken synaptic inhibitions within the network. (B) The LP neurone is spontaneously capable of generating plateau potentials. Successive 1 s pulses of hyperpolarizing current of increasing intensity were injected into the cell body through one electrode (*i*); the voltage responses of the neurone were recorded with a second electrode (LP). (C) During an APM discharge (induced by current injection), the response of LP to the same intensity current pulses is slower. Specifically, the initial passive response (arrow) remains rapid, but the active phase which follows is distinctly retarded. The amplitude of the responses in (B) and (C) is equivalent. (D) Diagram of the preparation used in (E) and (F); synaptic activity in the commissural ganglia (CG) is blocked with $0Ca^{2+}+Co^{2+}$ saline (circles) and the *on* is cut (↔). (E) A PY neurone has lost its plateau properties. When depolarized by current injection, it fires tonically; it responds only passively to injected pulses of hyperpolarizing current of increasing intensity (*i*). One electrode was used for current injection, a second for recording. (F) During an APM discharge provoked by electrical stimulation of its axons (35 Hz), the same hyperpolarizing currents injected into PY provoke responses of greater amplitude, the development of which is retarded. The initial rapid descents (arrows) correspond roughly to the passive responses in (E). Calibrations: horizontal bars, 0·5 s; vertical bars 5 nA in (B) and (C), 2·5 nA in (E) and (F). For abbreviations see legend to Fig. 1.

interference from neurones within the pyloric network. During an APM discharge, the repolarizations caused by identical hyperpolarizing current pulses are drastically modified (Fig. 7C), the apparent time constant of the repolarizations being increased.

Careful examination, however, indicates that the initial, passive descent is unchanged (arrow), but that the active phase which completes the repolarization is considerably slowed.

That APM slows the active repolarization of the constrictor neurones is confirmed by the experiment illustrated in Fig. 7D, E, F. In this case, a PY neurone had completely lost its plateau properties (synapses blocked in commissural ganglia, Fig. 7D). PY was induced to fire tonically by injection of depolarizing current, and was then injected with pulses of hyperpolarizing current. The purely passive responses to 1 s hyperpolarizations of several intensities (Fig. 7E) can be compared with the responses to identical pulses during plateau potentials in PY induced by APM firing (Fig. 7F). When APM fires, the repolarizations of PY are increased in amplitude, and are slowed down in a manner analogous to that seen in LP in Fig. 7C. The initial rapid descent (Fig. 7F, arrows) corresponds roughly to the passive response in Fig. 7E. The retarded descent (starting at the arrows in Fig. 7F), which increases the amplitude of the response, probably corresponds to the active repolarization phase of a plateau, which is induced and slowed down by APM's discharge.

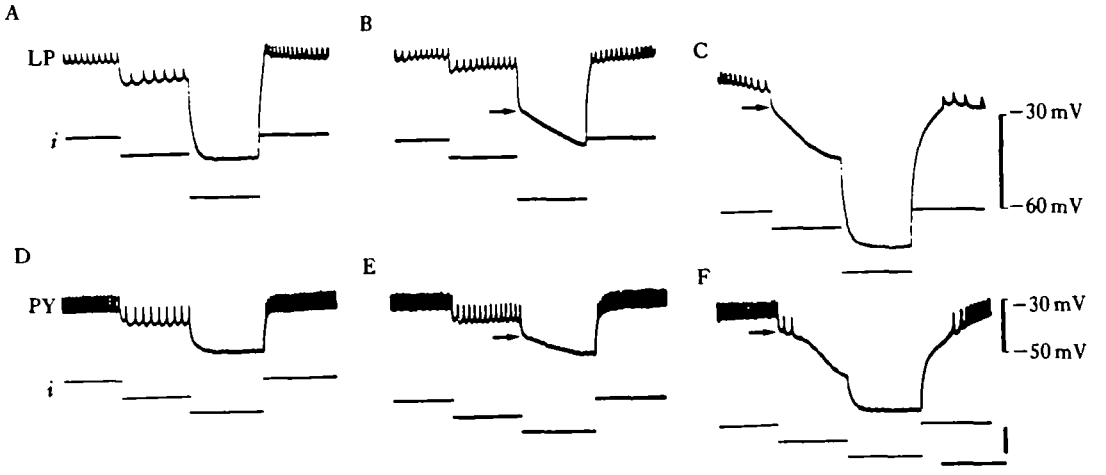


Fig. 8. The activity of APM induces and specifically retards the active phase of repolarization in the constrictor neurones. (A), (B) and (C) Experimental conditions are as in Fig. 7D (CG synapses blocked, *on* cut), with the STG bathed in picrotoxin to weaken synapses within the network. LP was subjected to a two step hyperpolarization by the injection of a double pulse of current (*i*); a second electrode was used to monitor the responses of the LP neurone. (A) When APM is silent, the LP neurone does not exhibit plateau properties, responding passively to pulses of hyperpolarizing current. (B) During an APM discharge (provoked by stimulating its axons), the LP neurone responds passively to the first hyperpolarizing step [amplitude and time course identical to those seen in (A)]. In response to the second hyperpolarizing step, it shows an initial rapid and passive descent, but beyond a certain threshold voltage (−57 mV, arrow), a retarded response. (C) Again during an APM discharge, the LP neurone was hyperpolarized before the double hyperpolarizing pulse was delivered; the first hyperpolarizing step thus brought the LP membrane potential to the level reached during the second pulse in (B) (arrow). Beyond this potential, a retarded hyperpolarization, which increases the amplitude of the response to the first pulse [compare with (A) and (B)] is seen. The retarded hyperpolarization which occurs during one of the two current pulses in (B) and (C) reflects the active response of LP, induced and modified by the APM discharge. (D), (E) and (F) Analogous experiments conducted with a PY neurone. (D) APM is silent and PY responds passively to the two current pulses. (E) APM is firing; PY's response to the second current pulse is retarded. (F) APM is firing; PY, which was hyperpolarized before the current pulses were injected, responds to the first current pulse with an active, retarded descent, which is initiated at the same threshold potential as the active response in (E) (−42 mV, arrow). Calibration: horizontal bar, 0·5 s; vertical bar, 2·5 nA. For abbreviations see legend to Fig. 1.

Further evidence that APM acts specifically on the active phase of the repolarization from a plateau is provided by the injection of double steps of hyperpolarizing current (Fig. 8). It is known (Russell & Hartline, 1978; Tazaki & Cooke, 1979*b*) that the repolarization phase of a plateau potential is an active process with an all-or-none character; once set in motion, it continues until repolarization is complete. Thus, if a neurone is subjected to a hyperpolarization in two steps (of sufficient duration) during a plateau, the active response occurs during only one of the two hyperpolarizing steps, the one in which the membrane potential reaches the threshold for active repolarization. Fig. 8 shows that when such two-step hyperpolarizing currents are injected into LP or PY, the retardation of repolarization is likewise seen during only one of the two steps. Fig. 8A–F illustrates two-step hyperpolarizations of LP and PY, respectively, the two current steps in PY being of equal intensity, the second step in LP being stronger. Neither LP nor PY showed plateau properties in the absence of APM firing and responded passively to the double hyperpolarizations (Fig. 8A, D).

Fig. 8B and E shows the responses of the same neurones just after stimulation of the *ion* axons of APM. In both cases, only the response to the second hyperpolarizing step is slowed down. The experimental conditions in Fig. 8C and F are identical to those in B and E, respectively, except that, in addition to the two hyperpolarizing steps, a constant hyperpolarizing current was injected into each neurone, so that the first step in C and F brought the membrane to the same potential reached during the second step in B and E (compare arrows in B, C and in E, F). In these cases only the first hyperpolarizing response was slowed. Thus, the modifications provoked by APM's discharge are visible during only one of the two steps, which supports the hypothesis that APM modifies active membrane properties and is able to induce these properties in pyloric neurones which do not spontaneously exhibit them (as is the case in Fig. 8A and D).

The induction of these active properties can also be seen as an increase in the amplitude of the response to a given hyperpolarizing step. In Fig. 8C and F (with APM's discharge), the responses to the first steps are much larger than those in A and D (control), respectively, whereas the responses to the second hyperpolarizations are nearly equal in amplitude in A and C and in D and F. Similarly, the response to the second hyperpolarizing step in E (with APM's discharge) is larger than the response to the second step in D (control), whereas the response to the first steps are similar. That the response to the second hyperpolarization in B (with APM's discharge) is smaller, not larger, than that in A (control) can be explained by the fact that the slowed active phase in B did not have sufficient time to reach maximum hyperpolarization before the hyperpolarization was terminated and the cell was depolarized.

A final line of evidence that APM slows the active repolarizing phase of plateaus is shown in Fig. 9, in which APM no longer modifies the repolarization when the active phase is experimentally blocked. Studies of the ionic mechanisms producing plateau potentials suggest that the active repolarization is dependent on $K^+$ currents (Tazaki & Cooke, 1979*c*; Gola & Selverston, 1981). Such $K^+$ currents can be decreased by increasing the external $K^+$ concentration. In such conditions ($3 \times K^+$, Fig. 9B), two-step pulses of hyperpolarizing current injected into a PY neurone just after stimulation of APM's axons in the *ion*s, engender a hyperpolarization whose amplitude is considerably smaller than that produced when the stomatogastric ganglion is bathed

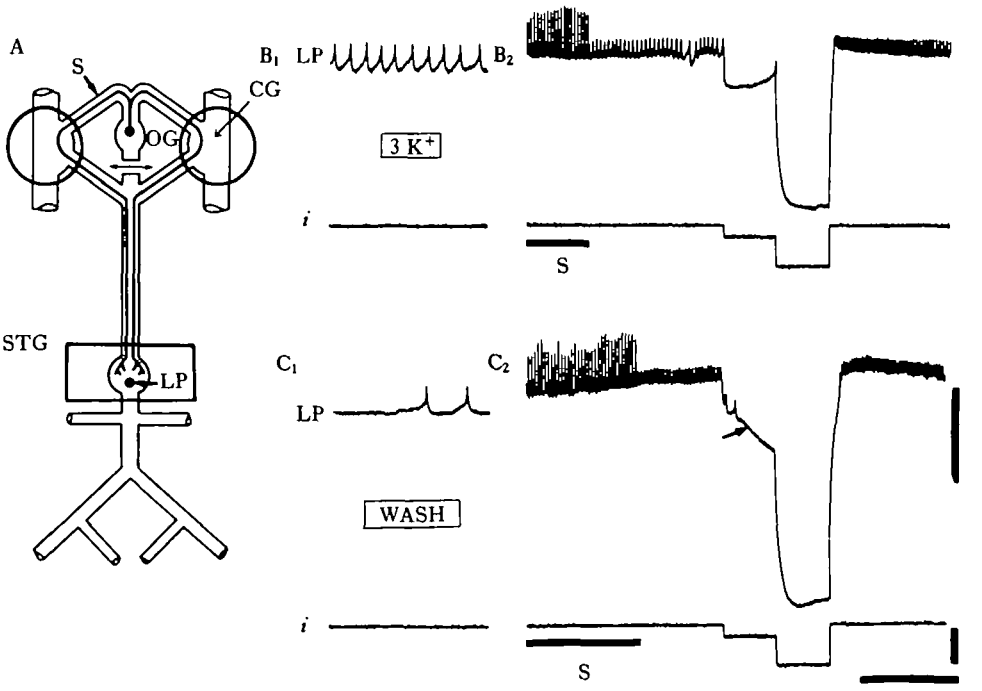


Fig. 9. When the active phase of the repolarization of the constrictor neurones is blocked (with high potassium saline), APM does not influence the responses of these neurones to hyperpolarizing current pulses. (A) Diagram of the preparation; synaptic activity in the commissural ganglia is blocked by $0Ca^{2+}+Co^{2+}$ saline (circles); the *on* is cut (↔); the rectangle indicates the perfusion of the stomatogastric ganglion with high $K^+$ saline ($3 \times K^+$) in (B), and with normal saline in (C). (B) In $3 \times K^+$ saline, which blocks the active repolarization of the pyloric neurones (see text), the LP neurone, activated by an APM discharge (stimulation of APM's axon, S), responds passively ($B_2$) to a double pulse of hyperpolarizing current injected into the cell body. ($B_1$) Control activity of LP before the firing of APM. (C) In normal saline, the same current pulses injected into LP during an APM discharge (stimulation of APM's axon, S) results in an active response ($C_2$) to the first current pulse. This response is slow (arrow) and increases the amplitude of the response (compare the total amplitude of the responses in (B) and (C). ($C_1$) Control activity of LP before the APM discharge. In both (B) and (C), two electrodes were used, one for current injection (*i*), the other for recording (LP). Calibration: horizontal bar, 1 s; vertical bars, 20 mV and 5 nA. For abbreviations see legend to Fig. 1.

in normal saline (31 mV instead of 48 mV, Fig. 9C). In addition, the slowing which normally accompanies the active repolarization induced by APM's discharge is not present in high external $K^+$ (compare the response to the first hyperpolarizing step in B with that in C).

It is not unlikely that APM also modifies the depolarizing phase of plateau potentials. However, except for the induction and amplification of plateau properties, which involve this phase, it was not studied in detail here.

*The effects of APM are voltage-dependent*

The two stage hyperpolarizations in Fig. 8 point to another fundamental characteristic of the action of APM: it is voltage-dependent. APM slows down the repolarization of pyloric neurones only within a certain range of voltages, which varies somewhat from neurone to neurone, but is quite stable within a given cell during a single experiment (−55 to −70 mV in LP, −40 to −60 mV in PY in the example of

Fig. 8). The membrane potential of the constrictor neurones (LP, PY) considered in Fig. 8 is brought into this range by the first hyperpolarizing step in C and F and by the second step in B and E.

The voltage dependence of APM's effects is further illustrated in Fig. 10A. Currents of increasing intensity injected into the soma of PY, just after stimulation of APM's axons, first produce passive hyperpolarizations of the PY neurone to different levels (indicated by arrows; −32 mV, upper trace; −39 mV, middle trace; −43 mV, lower trace). These passive hyperpolarizations then trigger active repolarizations which are slowed down by the activity of APM. But it is clear that the more negative

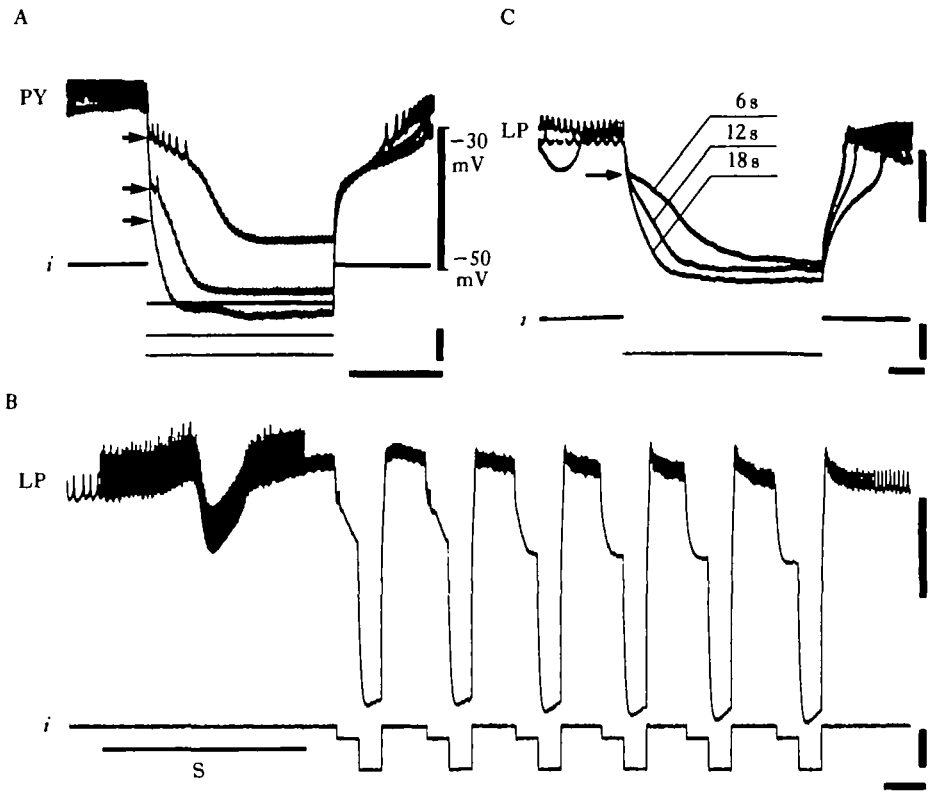


Fig. 10. The modifications of the repolarization phase which an APM discharge provokes in the constrictor neurones are voltage dependent and are long lasting. The CG were perfused with $0Ca^{2+}$ $+Co^{2+}$ saline and the *on* was cut in all cases; in addition, the STG was bathed in picrotoxin in (B) and (C). (A) Just after spiking in APM (stimulation of its axon), hyperpolarizing pulses of increasing intensity were injected into PY. With larger currents, the membrane potential reached passively is more negative (arrows), and the active response which is then triggered is less retarded. (B) Stimulation of the axon of APM (S) provokes long plateaus of firing in the LP neurone. Double pulses of hyperpolarizing current were then repetitively injected into LP. The response to the first step of the double pulse is slow; this retardation gradually decreases but is still visible 10 s after the end of the APM discharge. (C) In the same conditions, single hyperpolarizing current pulses were injected into LP at several intervals (6, 12, 18 s) after the end of stimulation of the APM axon; the three oscilloscope traces are superimposed. The retardation of the repolarization which is due to the APM discharge progressively decreases, but is still noticeable 18 s after the discharge. Note that the retarded active responses all begin at the same level of membrane potential (arrow). In all cases, two electrodes were placed in the cell, one for current injection (*i*), the other for recording (PY, LP). Calibrations: horizontal bars, 0·5 s in (A), 1 s in (B), 0·2 s in (C); vertical bars: 20 mV, 5 nA in (B) and 2·5 nA in (A) and (C). For abbreviations see legend to Fig. 1.

the membrane potential of the pyloric neurone is when the active response starts (i.e. the stronger the initial passive hyperpolarization is), the less this active response is slowed by the influence of APM. Thus, the extent to which an active repolarization of a pyloric neurone is slowed as a result of an APM discharge is voltage-dependent. This characteristic of APM's effects has important functional consequences for the expression of the pyloric motor pattern (see below).

*APM's effects are long lasting*

One of the several striking features of APM's effects on the pyloric network is its temporal characteristics: the global effects of an APM discharge continue well after the end of that discharge (see Nagy & Dickinson, 1983). Thus, the temporal evolution of APM's effects on the active repolarization of pyloric neurones is of interest. Such changes with time can be seen in Fig. 10B, during a series of two-step hyperpolarizations of LP after stimulation of APM's axons in the *ion*s. The slowing of the repolarization, which occurs during the first of the two hyperpolarizing steps in this case, gradually decreases, but is still present 10 s after a 5 s APM stimulation. The persistence of the effects of APM is also shown in Fig. 10C, in which the response of LP to pulses of hyperpolarizing current injected at several times after stimulation of APM's axons in *ion*s are superimposed. Here again a gradual decrease in the extent to which the active response is slowed can be seen (compare traces at 6, 12 and 18 s after stimulation). It should also be noted that these active responses all start at the same level of membrane potential (arrow).

*Functional consequences*

The various modifications of membrane properties engendered by APM's discharge are interesting not only in their own right, but also in terms of their effects on the pyloric rhythm. APM affects all the pyloric neurones (see Nagy & Dickinson, 1983), but its effects are particularly pronounced on the constrictor neurones, LP and PY. We have shown here that APM is capable of inducing and amplifying the plateau properties of the constrictor neurones. Moreover, APM slows the repolarization phase of the plateau potentials, especially in response to weak hyperpolarizing inputs. Because the repolarizations in response to stronger hyperpolarizing inputs are less retarded and those to weaker inputs are more retarded (see Fig. 10A), an ultimate effect of the slowing of the repolarizing phase of plateaus is a decreased sensitivity of the pyloric neurones to weak inhibitions and consequently a tendency to stabilize the neurone at its depolarized level. However, because the overall plateau properties of the pyloric neurone are amplified (or induced in certain cases), the response of the neurone to inputs which are strong enough to provoke the transition from one stable level to the other will be larger in amplitude than it would be in the absence of APM firing. APM is thus ultimately able to decrease the sensitivity of the constrictor neurones to weak inputs and to increase their response to strong inputs.

The cellular modifications provoked by APM enable us to understand the responses of the pyloric network to APM's discharge not only when the pyloric rhythm is weak, but also when it is active. We showed in the preceding paper (Nagy & Dickinson, 1983) that, when the pyloric rhythm is active, a discharge of APM results in a strong

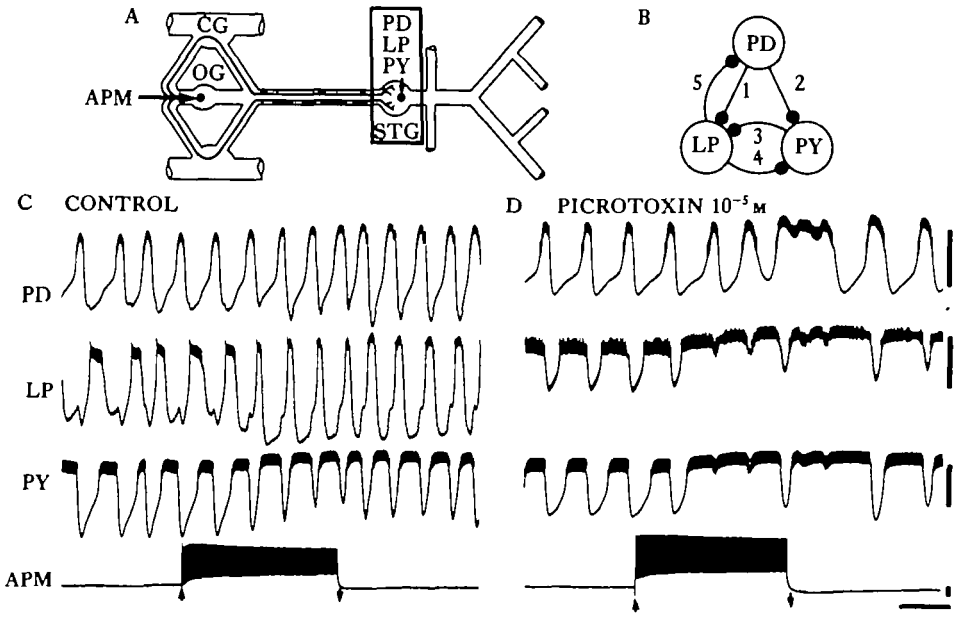


Fig. 11. The amplification and modification of the plateau properties of the constrictor neurones by an APM discharge can explain the modifications of the pyloric pattern which are provoked by such a discharge. (A) Diagram of the preparation; the rectangle indicates the perfusion of the STG by normal saline in (B) and by $10^{-5}$ M-picrotoxin, which weakens the inhibitions within the pyloric network, in (C). (B) Diagram of the inhibitory synaptic relationships between PD, LP and PY. (C) Modifications of the activity of the pyloric neurones by a 6 s APM discharge (35 Hz) when the pyloric rhythm is active (for more details, see text). (D) The picrotoxin blocks the synapses labelled 3, 4 and 5, and weakens those labelled 1 and 2; the discharges of the constrictor neurones LP and PY thus become synchronized. In response to an APM discharge, the amplitude of oscillations in the constrictor neurones decreases, and these neurones fire in long plateaus which the pacemaker bursts are not always able to terminate. The pacemaker neurone PD also tends to fire in long plateaus. In (C) and (D) the APM discharge was induced by current injection (arrows). Calibration: horizontal bar, 2 s; vertical bars, 20 mV. For abbreviations see legend to Fig. 1.

activation of all the neurones in the network, a decrease in the efficacy of the inhibitory synapses of the pacemakers (PD) onto the constrictor neurones (synapses 1 and 2, Fig. 11B) and an increase in the efficacy of PY's inhibitory synapses onto LP (synapse 3, Fig. 11B). There is thus an increase in the amplitude of oscillations in LP and PD and a decrease in the amplitude of oscillations in PY (see Fig. 11C). This would be explained if the bursts in the dilator neurones are insufficient in intensity and/or duration to cause full repolarization in the constrictors, whereas the bursts in the constrictor neurone PY are long and intense enough to repolarize LP, and the inhibitions from LP onto PD are able to provoke the repolarization of the dilator neurone PD. We thus suspect that the modifications of apparent synaptic efficacies which APM provokes are due both to differences in intensity of inhibitions and to modifications of the sensitivity of postsynaptic neurones to such inhibitions. To confirm this, we weakened the reciprocal inhibitory synapses of the constrictor neurones (synapses 3 and 4, Fig. 11B) and the inhibitory synapse of LP onto PD (synapse 5, Fig. 11B) with picrotoxin (Bidaut, 1980). After an APM discharge in these conditions (Fig. 11D), LP behaves like PY, with bursts of long duration and

decreased amplitude, the inhibition due to PY no longer being sufficient to bring LP to its lower level of stable membrane potential. Similarly the bursts in the pacemaker PD tend to be longer, suggesting that APM exerts effects on PD that are similar to, although weaker than, those it exerts on the constrictor neurones. In picrotoxin, the inhibitions from LP are no longer sufficient to repolarize PD after an APM discharge.

Thus, it is largely through the intermediary of the induction, amplification and modification of the plateau properties of the pyloric neurones that the interneurone APM is able to activate the pyloric rhythm and to modulate the expression of the motor pattern produced by the pyloric network.

## DISCUSSION

### *APM induces and amplifies the bursting ability of the pyloric neurones*

Perhaps the most notable characteristic of the interneurone APM is the fact that it acts on the plateau properties of the pyloric neurones. These properties play an important role in the generation of the pyloric rhythm by allowing the pyloric neurones to develop the depolarizations necessary for the production of bursts of action potentials. They are therefore responsible for the 'burstiness' of the neurones which make up the pyloric network (Russell & Hartline, 1978). Plateau properties are characteristic of both the pacemaker (dilator) and the follower (constrictor) neurones (Russell & Hartline, 1978, 1981) of the pyloric network. The major feature distinguishing these two categories of neurone is the ability of the pacemakers to develop 'pacemaker potentials' (Gola & Selverston, 1981), the spontaneous slow depolarizations which bring the membrane potential to the threshold at which plateau potentials are triggered. Thus, the dilator neurones spontaneously and rhythmically produce plateaus and function as endogenous oscillators. In contrast, plateau potentials in the constrictor neurones must be triggered by synaptic inputs. An analogous situation in the cardiac ganglion of the crab, *Portunus*, has been studied in considerable detail (Tazaki & Cooke, 1979*a,b,c*). In fact, plateau-like electrical activity appears to characterize a number of types of excitable cells, including both nerve cells (Ekerot & Oscarsson, 1981; Legendre, Cooke & Vincent, 1981) and non-nervous cells such as the cells of the endocrine pancreas (Cook, Crill & Porte, 1980) and cardiac muscle fibres in vertebrates (Vassale, 1979).

The expression of the plateau properties of the pyloric neurones depends on inputs of central origin. If spike conduction in the afferent nerve to the stomatogastric ganglion (the stomatogastric nerve) is blocked, the plateau properties of the constrictor neurones vanish (Russell & Hartline, 1978) and the plateau properties of the pacemaker neurones are generally weakened (Russell, 1979) or abolished (Moulins & Cournil, 1982). Electrical stimulation of the stomatogastric nerve can temporarily restore the plateau properties in these neurones; however, the nature of the inputs inducing them in the pyloric neurones, and particularly in the constrictor (non-oscillating) neurones, was heretofore unknown. We have shown that activity (even at moderate frequency) in the neurone APM can induce plateau properties in the non-pacemaker neurones of the network. Thus, a discharge of APM alone can confer on the non-pacemaker neurones of the pyloric network the ability to fire in bursts of action potentials, a mechanism which plays a fundamental role in the control of th

motor pattern's generation. This phenomenon, the induction of non-linear membrane properties that can alter the bursting capabilities of neurones, is not a well known phenomenon, and has previously been demonstrated only for neurone 11 of the visceral ganglion of the snail *Otala*. When this animal aestivates, neurone 11 loses its ability to fire in bursts; this ability can be reinduced by perfusion or iontophoresis with vasopressin or related peptides (Barker, Ifshin & Gainer, 1975; Ifshin, Gainer & Barker, 1975). The induction of plateau properties in the pyloric neurones by the activity of APM can likewise be compared to that of the electrical activity of the $\beta$ cells of the endocrine pancreas, which can produce plateau potentials only in the presence of glucose, the duration of the plateaus being a function of the extracellular glucose concentration (Meissner, 1976).

A second aspect of APM's actions is to amplify these properties when they are already present and active. This is reflected in the longer plateaus and the higher spike frequency within plateaus recorded after an APM discharge. The prolongation of the plateaus is largely due to the fact that APM slows down their repolarization phase. Although we tested the ability to produce plateau potentials under the influence of activity in APM only for the constrictor (non-pacemaker) neurones, indirect observations suggest that the interneurone APM is also able to augment the pacemaker neurones' capacities for rhythmic activity. Thus an APM discharge provokes an increase both in amplitude of oscillation and in spike frequency within bursts in these neurones when the pyloric rhythm is normally active, (Nagy & Dickinson, 1983) and it provokes a ten-fold increase in plateau duration when the pacemaker neurones are isolated from other synaptic influences (present paper). It has recently been shown that an amplification of plateau properties specifically in the pacemaker neurones can also be induced by the electrical stimulation of a pair of nerve fibres afferent to the stomatogastric ganglion (Russell & Hartline, 1981). The amplification, under the influence of APM, of the rhythmic firing capabilities which are spontaneously manifested by all the pyloric neurones bears similarities to the control of endogenous rhythmic activity which has been examined in several preparations, including the cardiac ganglion of crustaceans (Watanabe, Obara & Akiyama, 1969; Cooke & Hartline, 1975; Lemos & Berlind, 1981) and neurone R15 of the parieto-visceral ganglion of *Aplysia* (Parnas, Armstrong & Strumwasser, 1974; Barker *et al.* 1975; Mayeri, Brownell, Branton & Simon, 1979). The control of the amplitude and duration of the vertebrate cardiac action potential (and particularly of its plateau phase) by various substances implicated in the nervous control of cardiac activity may also be comparable (Vassort *et al.* 1969; Reuter, 1974; Giles & Nobel, 1976; Ten Eick, Nawrath, McDonald & Trautwein, 1976).

Consequently, it appears that the types of effect induced by the interneurone APM are not restricted to the stomatogastric nervous system, but are instead fundamental to the control of several excitable systems. However, the system described here has a number of advantages as a model system for the study of this sort of control. Firstly, the modifications observed are due to the activity of a single neurone which can be found and identified from animal to animal. Secondly, it is possible to attribute a functional significance to the effects of APM: these effects allow the neurone to modulate both quantitatively and qualitatively the expression of a motor pattern generator (Nagy & Dickinson, 1983). In fact, these various modifications of the

pyloric pattern can all be explained by the modifications of the plateau properties of the neurones of the pyloric network.

*Modifications of the plateau properties of the pyloric neurones underly modulation of the pyloric output by the interneurone APM*

The modulation of the pyloric activity is seen in three principal effects: (1) an activation of previously silent neurones and an increase in the firing of active neurones; (2) an increase in the frequency of the pyloric rhythm to a maximum of around 1 Hz; (3) a modification of the efficacy of synaptic relationships within the network (Nagy & Dickinson, 1983).

The first effect is easily explained by the fact that the plateau properties form the basis for the bursting activity of the pyloric neurones. Thus the induction or amplification of these properties by activity in APM can either permit or augment the bursting activity of these neurones.

The second effect of an APM discharge is more complex. The pyloric network receives rhythmical excitation from a pair of command oscillators (the commissural pyloric oscillators, CPO) which have a rapid (approximately 1 Hz) and constant rhythm in *in vitro* preparations (Robertson & Moulins, 1981). These oscillators are able to entrain the rhythm of the pyloric pacemaker neurones (Nagy, 1981), although the coupling of the CPO to the pyloric oscillators is labile (Robertson & Moulins, 1981). It is this lability that is responsible for the existence of two distinct states of the pyloric rhythm, both in intact animals (Rezer & Moulins, 1980) and in isolated preparations (Nagy, 1981). A rapid and regular rhythm in which all the pyloric neurones participate corresponds to a tight coupling of the pyloric neurones and the CPO, whereas a slow and irregular rhythm appears to result from looser coupling. As was shown in the preceding paper (Nagy & Dickinson, 1983), an APM discharge can provoke an acceleration of the pyloric rhythm, the extent of which is dependent on the rhythm. In all cases, the maximum frequency attained is about 1 Hz (the frequency of the CPO); if the pyloric rhythm already has this frequency, activity in APM does not cause it to accelerate. In addition, an acceleration of the pyloric rhythm is always accompanied by an activation of all the formerly quiet neurones of the network. A reasonable explanation for these observations is that the modification of membrane properties by an APM discharge renders all of the pyloric neurones more sensitive to the rhythmic inputs from the CPO. In other words, the modulatory interneurone APM favours the coupling of the pyloric neurones to the command oscillators. This explanation is corroborated by the following observation: when synaptic activity in the commissural ganglia is experimentally blocked, the pyloric rhythm slows considerably or stops altogether (present paper), probably as a result of the suppression of the rhythmic inputs from the CPO, as well as other inputs. Under these conditions, an APM discharge still profoundly modifies the activity of pyloric neurones, but it never leads to the initiation or acceleration of a rhythmic activity. In the intact animal, the transition from a slow and irregular to a fast and regular pyloric rhythm occurs abruptly when the animal eats, and the rapid rhythm continues for up to several hours after the meal (Rezer & Moulins, 1980). These authors attribute this transition to an increased coupling of the CPO and the pyloric rhythm upon feeding. The characteristics of the action of the interneurone APM

suggest that it could play a role in such a transition. The experimental model that we have presented thus suggests that the central nervous system can initiate a behavioural sequence simply by modifying certain membrane properties of the motoneurones in such a way that the motoneurones become more sensitive to inputs from a command oscillator.

The third aspect of the modulation of pyloric activity by the activity of the interneurone APM is the modification of the relative efficacies of synapses within the pyloric network. These modifications qualitatively change the pyloric rhythm by altering the phase relationships between the discharges of the different neurones. These modifications are due both to changes in the intensities of the inhibitions received by the neurones and to changes in the sensitivity of the postsynaptic neurones to the inhibitions resulting from the activity of APM. These effects can be explained by the fact that an APM discharge modifies the temporal characteristics of the repolarization phase of the plateau potential, which is normally triggered by inhibitory synaptic inputs in these neurones. The repolarization is retarded and this retardation is voltage dependent. Thus, as the membrane potential is taken passively by the input to more negative levels (corresponding to stronger inhibitory inputs), the retardation is less pronounced. Consequently, strong inputs will repolarize the membrane rapidly whereas weak inputs will do so only slowly. Thus, even a brief stimulus of sufficient intensity will repolarize the membrane. However, for a less intense input to repolarize the membrane, it must be of longer duration. One effect of the active repolarization is to amplify the inhibitory inputs a neurone receives; thus the effects of the largest inhibition (in either intensity or duration) received by a neurone will be those that are amplified when APM fires. Consequently, an APM discharge leads to an increase in the contrast of synaptic effects by augmenting the response to strong inputs and decreasing the response to weak inputs. It has previously been suggested that plateau properties can act as the basis for a synaptic amplifying mechanism (Russell & Hartline, 1978). The interneurone APM, by modifying these properties, provides the pyloric neurones with a means of filtering these synaptic inputs by specifically amplifying only certain of the inputs they receive.

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