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

I. MODULATION OF THE PYLORIC MOTOR OUTPUT

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SUMMARY

In the lobsters *Jasus lalandii* and *Palinurus vulgaris*, the rhythmical activity of the pyloric pattern generator of the stomatogastric nervous system is strongly modified by the firing of a single identified interneurone, whose activity we have recorded from the cell body, *in vitro*.

The cell body of this interneurone, the anterior pyloric modulator (APM), is located in the oesophageal ganglion and sends two axons to the stomatogastric ganglion *via* the inferior oesophageal nerves, the commissural ganglia, the superior oesophageal nerves and the stomatogastric nerve.

Firing of neurone APM modifies the activity of all the neurones of the pyloric network, including pacemaker and follower neurones. Its effects are both quantitative (increase in the frequency of the rhythm and in the frequency of spikes within cell bursts) and qualitative (modifications in relative efficacies of the synaptic relationships within the pyloric network, which in turn lead to changes in the phase relationships between the discharges of the neurones).

The effects on pyloric activity induced by firing of neurone APM are established slowly (one or two seconds) and are of long duration (ten times the duration of APM's discharge). These modifications most probably involve muscarinic cholinergic receptors.

APM's influences on the activity of pyloric neurones appear to be characteristic of a neuromodulatory process and are such that they may be of behavioural significance in the intact animal.

INTRODUCTION

A number of rhythmic sequences of behaviour are programmed by central neuronal networks, the endogenous activities of which are determined by the synaptic interactions between neurones in the network and/or by the cellular properties of neurones in the network (see reviews by Fentress, 1976; Grillner, 1977; Delcomyn, 1980). The

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identification of the neurones in such a network and the determination of their synaptic relationships is, however, insufficient to explain either the flexibility of the behavioural sequences controlled by such networks or the manner in which such sequences are initiated and terminated. In fact, it appears that even those properties determining the 'endogenous' activity of pattern generators can be modulated. For example, the bursting activity of oscillatory neurones can be induced, amplified or eliminated (Parnas, Armstrong & Strumwasser, 1974; Barker, Ifshin & Gainer, 1975), and the efficacy of synaptic relationships between neurones can be modified by certain slow synaptic potentials (see review by Hartzell, 1981). Thus, it is of interest to study the mechanisms controlling central pattern generators.

Aspects of the control of pattern generators are examined in the present study, in which we show that the activity of a single identified interneurone can profoundly modify the output of the pyloric network, which is a motor pattern generator in the crustacean stomatogastric nervous system. This neuronal network, which controls the rhythmic movements of the pyloric stomach, is composed of 14 identified neurones, whose synaptic connections are known (Maynard, 1972; Maynard & Selverston, 1975; Selverston, Russell, Miller & King, 1976; Selverston & Miller, 1980). The rhythmic activity of this motor pattern generator is largely due to the fact that some of the neurones are endogenous oscillators and act as pacemakers. In addition, reciprocal inhibitory synapses between the various neurones of the network contribute to the generation of the pyloric rhythm. However, the rhythm and the intensity of the pyloric pattern are also strongly dependent upon input from higher nervous centres (Russell, 1979). This dependence is illustrated by the observations that the oscillatory activity of the pacemaker neurones can be induced or amplified by such inputs (Russell & Hartline, 1981; Moulins & Cournil, 1982) and that such inputs may also control regenerative membrane properties of the non-oscillatory neurones of the network that contribute to the generation of the pyloric pattern (Russell & Hartline, 1978). The source of these inputs has, however, been unknown.

The present study shows that an effective 'higher centre' input is a single interneurone which is located in an anterior ganglion and projects directly to the stomatogastric ganglion. We here report that discharge of this neurone profoundly modifies the activity of all the neurones of the pyloric network. The activity of this neurone both increases the frequency of the rhythm and the frequency of spikes within bursts of the neurones (quantitative modifications) and modifies phase relationships between the discharges of the neurones (qualitative modifications). These modifications are established slowly and are of long duration. Thus it appears that a single interneurone can alter a rhythmic motor output. In certain cases, these modifications of the pyloric pattern resemble those which occur when feeding starts in the intact animal. In the accompanying paper (Dickinson & Nagy, 1983) we shall show that these modifications of the pyloric rhythm by a modulatory interneurone are based primarily on the induction and amplification of the regenerative membrane properties which are responsible for the 'burstiness' of all the neurones of the pyloric pattern generator.

A preliminary report of this work has been published elsewhere (Nagy, Dickinson & Moulins, 1981).

MATERIALS AND METHODS

Both male and female Cape lobsters, *Jasus lalandii*, (41 animals) and red lobsters, *Palinurus vulgaris*, (6 animals) were used. Similar results were obtained from the two species; the data illustrated are from *Jasus* except where otherwise indicated. Animals were maintained in aerated, flowing sea water before use, at a temperature of 16–20 °C.

Experiments were conducted on isolated preparations of the stomatogastric nervous system, pinned on Sylgard-covered Petri dishes and constantly superfused with oxygenated saline (temperature around 20 °C). The isolated preparation used here corresponds to the 'combined preparation' used by Selverston *et al.* (1976; Fig. 1A) and consists of the stomatogastric ganglion (STG), the oesophageal ganglion (OG), the two commissural ganglia (CG) and the connecting nerves. The four ganglia were desheathed to allow access to the neuronal cell bodies. Activity of neurones in the stomatogastric ganglion was recorded extracellularly on three pairs of motor nerves: the dorsal and ventral lateral ventricular nerves (*dlvn*, *vlvn*) and the medial ventricular nerve (*mvn*). Intracellular recordings were made from cell bodies of the neurones, and their identities were established by the presence of action potentials in various motor nerves of the stomatogastric ganglion, the time of discharge in the pyloric sequence and synaptic relationships with other neurones of the network (Maynard, 1972; Maynard & Dando, 1974; Maynard & Selverston, 1975).

Details of the dissection, the experimental arrangements and recording techniques have all been previously described (Moulins & Nagy, 1981). Intracellular recordings were made using glass microelectrodes filled with 3M-KCl (resistances of 20–30 MΩ). The same electrodes were used for current injection *via* a bridge circuit. Platinum wire electrodes were used for extracellular recordings and stimulations of the various nerves. In some experiments, the ganglia were surrounded by petroleum jelly walls so that each ganglion could be bathed in a separate solution. The normal saline used was artificial sea water. To block synaptic activity within a ganglion, the Ca^{2+} ions in the saline were replaced with Mg^{2+}, and 12 mM-Co^{2+} was added; this solution is called $0Ca^{2+}+Co^{2+}$ saline throughout the paper. For pharmacological experiments, the drugs to be tested were dissolved in saline (pH 7·45) just before use. These solutions were continuously perfused through the Vaseline chamber (volume approximately 1 ml) and the effects were recorded after 20–30 volume changes (about 30 min). The following drugs were used: atropine sulphate (Sigma), eserine (Sigma), hexamethonium bromide (Sigma), picrotoxin (gift from Fluka A.G.), tetrodotoxin (Sigma) and *d*-tubocurarine chloride (Sigma).

RESULTS

The basic organization of the pyloric network in *Panulirus* was first described by Maynard (1972) and Maynard & Selverston (1975). In *Jasus*, this organization is quite similar, except that the pyloric rhythm has a mean frequency of about 1 Hz, instead of about 2 Hz as in *Panulirus* (see Selverston *et al.* 1976). The pyloric network consists of 14 neurones (Fig. 1B) whose cell bodies are located in the stomatogastric ganglion (Fig. 1A, STG). The rhythmic output of the network (i.e., the pyloric rhythm, Fig. 1C) is

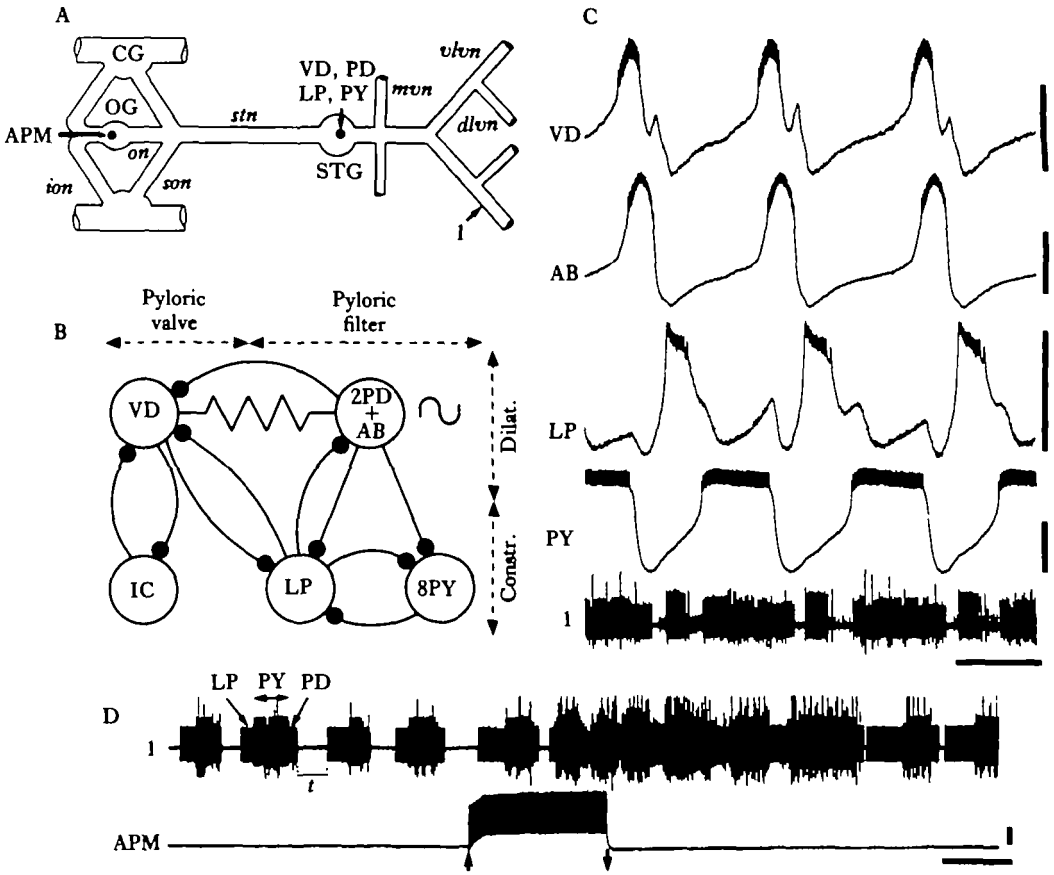

Fig. 1. The pyloric network and the APM neurone. (A) Diagram of the isolated stomatogastric nervous system preparation; arrows indicate intracellular recording electrodes. (B) Synaptic interactions within the pyloric network. Filled circles represent inhibitory connections; the resistance symbol (⁓) indicates an electrotonic synapse. The dilator neurones PD and AB are endogenous bursters (∿) and are the pacemaker neurones of the system. (C) The pyloric rhythm was recorded intracellularly in four pyloric neurones (VD, AB, LP, PY) and extracellularly on a motor nerve (*vlvn*, 1). Three successive pyloric cycles are shown. The phase relationships between the firing of the pyloric neurones is determined by the synaptic connections shown in (B). (D) Pyloric activity, recorded extracellularly on the *vlvn* (1), is modified by a 4 s discharge of APM (induced by intracellular current injection; arrows). *t*, silent period between the bursts in PD and LP. Calibrations: Horizontal bars, (C) 1 s; (D), 2 s: vertical bars, 20 mV. AB, anterior burster neurone; APM, anterior pyloric modulator neurone; CG, commissural ganglion; Constr., constrictor neurones; Dilat., dilator neurones; *dlvn*, dorsal lateral ventricular nerve; IC, inferior cardiac neurone; *ion*, inferior oesophageal nerve; LP, lateral pyloric neurone; *mvn*, medial ventricular nerve; OG, oesophageal ganglion; *on*, oesophageal nerve; PD, pyloric dilator neurone; PY, pyloric neurone; *son*, superior oesophageal nerve; STG, stomatogastric ganglion; *stn*, stomatogastric nerve; VD, ventral dilator neurone; *vlvn*, ventral lateral ventricular nerve; 1, recording electrode on the *vlvn*.

driven by endogenous oscillations of the membrane potentials of three electrically coupled pacemaker neurones. One of these, the anterior burster (AB) is an interneurone whose axon enters the commissural ganglia (Fig. 1A, CG); the other two are motoneurones which drive the dilator musculature of the pyloric filter (pyloric dilator neurones, PD). Because they are electrically coupled, these three neurones have very similar and synchronous activity and can be considered as a functional unit. Th

onstrictor muscles of the pyloric filter are controlled by the lateral pyloric neurone (LP) and eight pyloric neurones (PY); the latter are electrically coupled and hence will be considered as a single functional element. The dilator muscles of the cardiopyloric valve, which controls the entrance to the pyloric filter, are under the control of the ventral dilator motor neurone (VD), whilst the constrictor muscles of this valve are driven by the inferior cardiac motoneurone (IC). The pacemaker neurones rhythmically inhibit the activity of the constrictor motoneurones, resulting in a regular alternation of dilator and constrictor discharges (Fig. 1C). These inhibitory synapses are generally considered to be functionally the most important synapses in determining the expression of the pyloric sequence (Selverston *et al.* 1976). The other synaptic relationships within the network are shown in Fig. 1B.

We have identified an interneurone, the anterior pyloric modulator (APM) (Nagy *et al.* 1981), whose cell body is located in the oesophageal ganglion (Fig. 1A, OG) and whose discharge activates the pyloric network and leads to major modifications of the pyloric output. Fig. 1D shows extracellularly recorded activity of a pyloric motor nerve (the *vlvn*), illustrating the modifications resulting from a 4 s discharge of APM. The frequency of bursts increases, as do the number and frequency of spikes recorded in each burst.

The anterior pyloric modulator neurone (APM)

Activation of APM modifies the activity of the pyloric neurones, which are located in the stomatogastric ganglion (Fig. 2A, STG). Although only one afferent nerve (the stomatogastric, Fig. 2A, *stn*) enters the stomatogastric ganglion in the isolated preparation, two morphological pathways connect this nerve to the oesophageal ganglion and thence to the cell body of APM: a short pathway, *via* the oesophageal nerve (*on*), and a longer pathway, *via* the inferior oesophageal nerve (*ion*), the commissural ganglion (CG), and the superior oesophageal nerve (*son*). Cutting the *on* does not alter APM's effects on the pyloric neurones, suggesting that APM exerts its effects *via* the longer pathway. Furthermore, cutting a single *ion* or *son* diminishes, but does not eliminate, the effects of APM on the pyloric neurones, whereas cutting both *ion*s or both *son*s does eliminate APM's effects. Thus, it appears that APM influences the pyloric neurones *via* a bilateral pathway, which passes through both *ion*s and both *son*s to the *stn*, and thence to the stomatogastric ganglion.

Three lines of evidence, shown in Fig. 2, indicate that two bilaterally symmetrical axons of APM, both entering the stomatogastric ganglion, are responsible for the effects of APM on the pyloric neurones. There is a one-to-one correspondance of action potentials recorded in the soma of APM and those recorded in the *ion* and then in the *stn* (Fig. 2B). In addition, antidromic spikes are recorded in the soma of APM (Fig. 2C) in response to stimulation of the *ion* (S_1), the ipsilateral *son* (S_2) and the *stn* (S_3). The time required for these antidromic spikes to reach the soma increases as the stimulus is moved from *ion* to *son* to *stn*. Lastly, one can generate spike collisions between antidromic spikes provoked by stimulating one *ion* (Fig. 2D, S_1) and the ipsilateral *son* (S_2). The recordings shown here are from a single side, but either side gives identical results, confirming the bilateral symmetry of APM's axons. To eliminate the possibility that the axons of APM do not actually enter the stomatogastric ganglion, but instead synapse on and activate interneurones in the

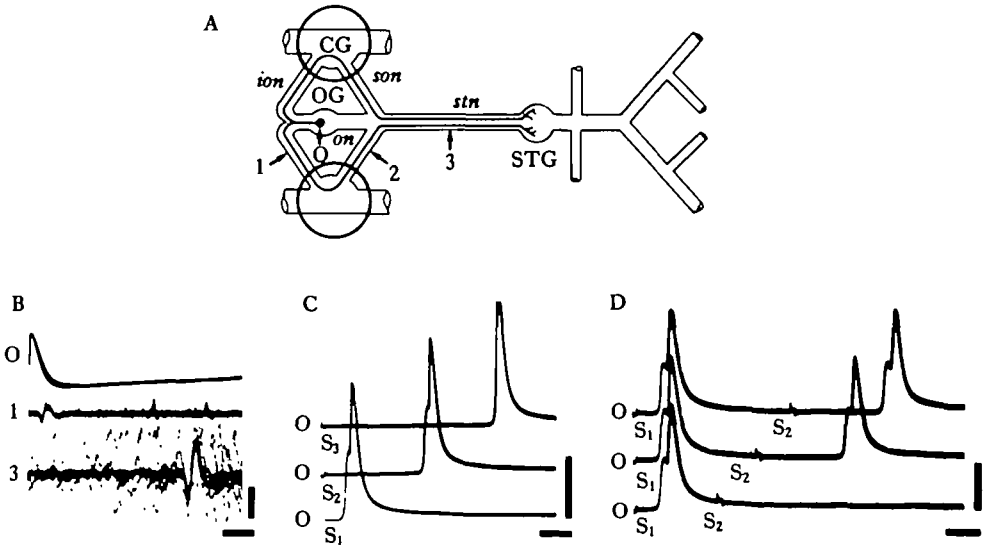

Fig. 2. The branching pattern of APM axons entering the stomatogastric ganglion. (A) Diagram of the preparation; the circles represent areas bathed in $0Ca^{2+}+Co^{2+}$ saline to block synaptic activity. O, intracellular electrode in the soma of APM; 1,2,3, extracellular electrodes used for recording (as in B) or stimulation (as in C, D). (B) Each action potential recorded in the soma of APM (O) is correlated with an extracellularly recorded spike on the ion (1) and then on the *stn* (3). Superimposed oscilloscope sweeps triggered by the intracellularly recorded spike (the three traces were recorded simultaneously). (C) Electrical stimulation of the *ion* (S_1), the *son* (S_2) and the *stn* (S_3), respectively, provokes antidromic spikes, after increasing delays, in the soma of APM. Superimposed oscilloscope sweeps triggered by the electrical stimulation. (D) Collisions can occur between antidromic spikes provoked by stimulation of the *ion* (S_1) and the *son* (S_2). For each trace, stimulation S_1 (the *ion*) triggered the superimposed sweeps and provoked an antidromic action potential which is recorded in the soma of APM (first potential). The antidromic spike provoked by subsequent stimulation S_2 (the *son*) is recorded in the soma of APM (second potential) only if the delay between S_1 and S_2 is sufficiently long (upper and middle traces). When this delay is decreased (lower trace), the second potential does not appear in the soma; it has been blocked by collision with the first antidromic potential somewhere between the two electrodes (1 and 2; see diagram in A). In (C) and (D) the three traces were recorded successively. Note the shoulder on the rising phase of the intracellularly recorded action potential (see also Fig. 3). For abbreviations, see legend to Fig. 1. Calibrations: horizontal bars, 10 ms; vertical bars, 40 mV.

commissural ganglia (see Fig. 2A), synaptic activity in the commissural ganglia was blocked with a $0Ca^{2+}+Co^{2+}$ (12 mM) saline during the experiments described above.

We can therefore conclude that APM is a 'T'-shaped neurone, sending one axon down each *ion*; these axons traverse the commissural ganglia to enter the *son*s, and then the single *stn*. The two axons, which are about 4 cm in length, thus enter the stomatogastric ganglion, where they exert their effects. APM is the only one of the 12 neurones in the oesophageal ganglion which has this morphology and which can modify the pyloric activity. Consequently this neurone is relatively easy to identify using electrophysiological tests.

The shape of the APM action potentials in Fig. 2C and D shows a certain complexity: the spikes are overshooting (reaching +15 to +20 mV) and have a shoulder on the rising phase. If the axon of APM is stimulated repetitively, the shoulder becomes increasingly pronounced (Fig. 3B, C, D). After a number of stimuli, the overshooting spike is replaced by a much smaller spike (about 40 mV), the amplitude of which corresponds to that of the shoulder on the larger action potential (Fig. 3D, E). B[illegible]

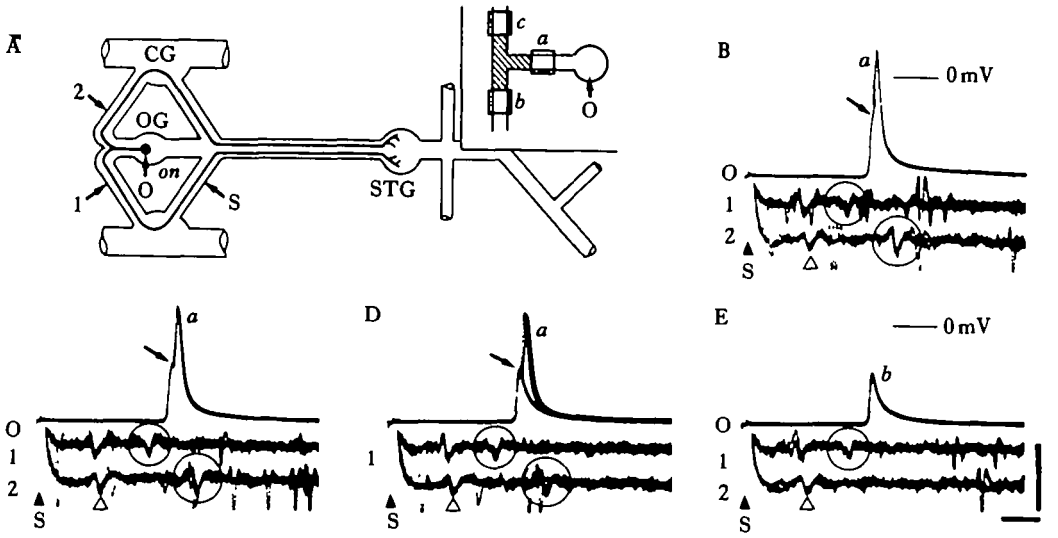

Fig. 3. Each somatic action potential in APM is correlated with an action potential in each *ion*. (A) Diagram of the preparation. O, intracellular electrode in the soma of APM; 1,2,S, extracellular electrodes. Inset: theoretical representation of APM; the cross-hatched area indicates an inexcitable zone, which is surrounded by three spike initiating zones (squares *a*,*b*,*c*). (B) Stimulation of a *son* (S) provokes a 90 mV (overshooting) antidromic spike (*a*) in the soma of APM. Note the shoulder on the rising phase (arrow). (C) After several *son* (S) stimulations, the shoulder on the intracellular action potential is more pronounced. (D) and (E) After further stimulations of the *son* (S), the excitability of the APM soma decreases and only a small (40 mV) antidromic action potential (*b*) is recorded (axon spike). In all cases, stimulation of the left *son* (S) provokes an action potential in the left *ion* (B, C, D, E; circle in trace 1). This potential is recorded in the right *ion* (circle in trace 2) only when the soma excitability is sufficient to allow an overshooting action potential to invade the soma; in (E) it is no longer present. In (B), (C), (D) and (E) the superimposed oscilloscope sweeps were triggered by the *son* stimulation. Action potentials marked with an open triangle are from the axon of an unidentified neurone stimulated in the *son* which enters the two *ion*s via the *on* and the oesophageal ganglion (OG). Calibrations: horizontal bar, 10 ms; vertical bar, 50 mV. For abbreviations see Fig. 1.

analogy to certain neurones in locusts (Heitler & Goodman, 1978; Hoyle & Dagan, 1978), we suspect that the larger spikes (Fig. 3B) are action potentials that fully invade the soma, whilst the smaller spikes are axonal spikes, which are revealed as the excitability of the soma progressively decreases during repetitive stimulation. This type of spiking pattern suggests the presence of an inexcitable zone at the junction of the two APM axons (Fig. 3A, inset; see also Heitler & Goodman, 1978). When antidromic stimulation produces an action potential which does not invade the soma, that action potential likewise fails to invade the contralateral *ion* (Fig. 3E); however, action potentials which do invade the soma also invade the contralateral *ion* (Fig. 3B, C; circle, bottom trace). Thus if the soma of APM is sufficiently excitable and a somatic spike is produced (spontaneously or with current injection), an action potential in each APM axon is propagated towards the stomatogastric ganglion. In other words, each action potential recorded in the soma of APM is accompanied by two action potentials which reach the stomatogastric ganglion *via* the *ion*s, *son*s and *stn*.

Modulation of the pyloric pattern depends on preceding pyloric activity

Activity in APM causes activation of all the neurones of the pyloric network (Fig. 4). This activation is accompanied by major modifications of the pyloric pattern

although the extent of these modifications is dependent in part upon the previous level of activity in the pyloric network.

It has been shown in both intact lobsters (Rezer & Moulins, 1980) and in isolated nervous systems (Nagy, 1981) that the pyloric rhythm can take one of two general forms: a slow, irregular rhythm with a cycle period greater than 2 s and in which some

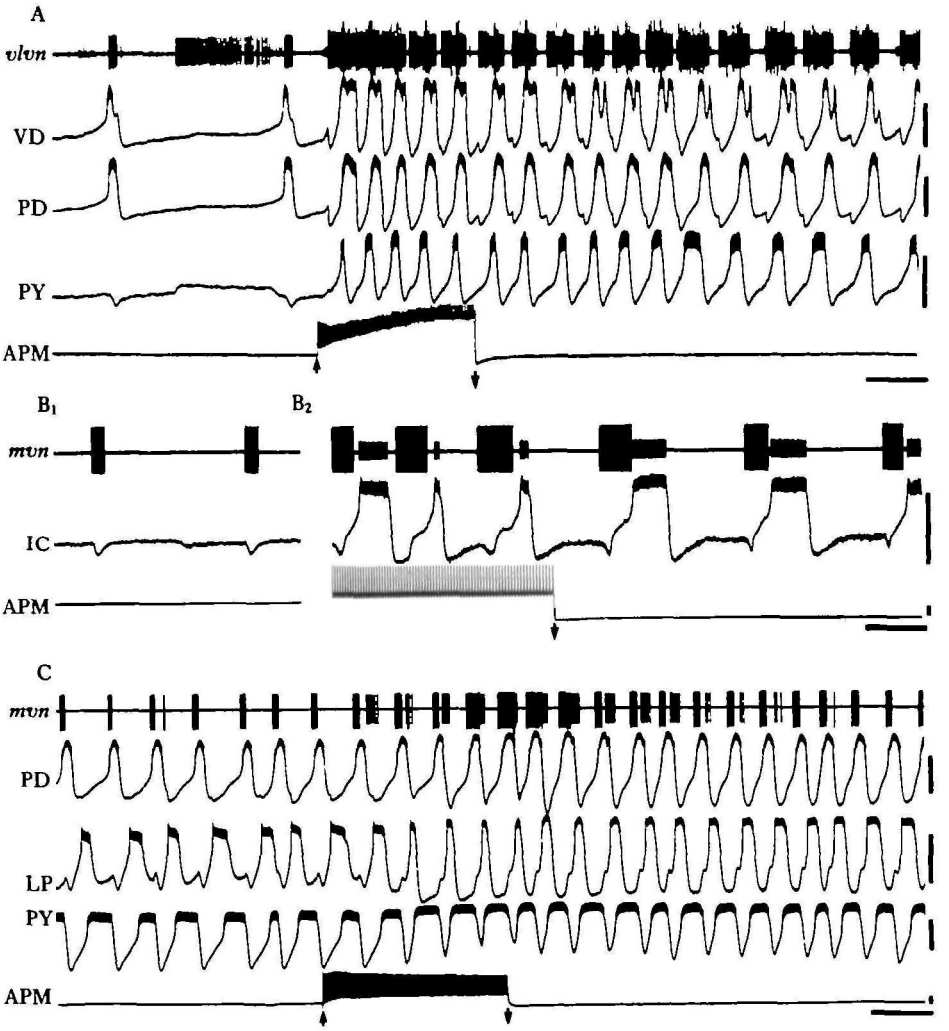

Fig. 4. The APM neurone activates all the pyloric neurones [VD, PD, PY in (A); IC in (B); PD, LP, PY in (C)]; its effects are dependent upon the preceding pyloric activity. (A) The pyloric rhythm is spontaneously slow; a 5 s discharge of APM accelerates the rhythm and activates silent neurones (PY). (B) The spontaneously slow pyloric rhythm (B_1) is accelerated only temporarily (B_2) by the discharge of APM (12 s); the neurones tend to fire in plateaus (IC, intracellular trace and small potential in *mvn*; VD, large potential in *mvn*). (C) The spontaneously active pyloric rhythm is changed only slightly by the discharge of APM (6 s). APM nonetheless modifies the amplitude of oscillation of the pyloric neurones, the form and intensity of bursts in the neurones, and the effects of various synaptic inhibitions between the different neurones. VD and IC are recorded in the *mvn* (large and small potentials, respectively). In all cases, firing in APM was elicited by intracellular current injection (arrows). See legend of Fig. 1 for abbreviations. Calibrations: horizontal bars, 2 s; vertical bars, 20 mV.

of the pyloric neurones may not be active (Fig. 4A, before APM firing; Fig. $4B_1$) and a fast, regular rhythm with a period of 1–2 s, in which all the pyloric neurones are active (Fig. 4C, before APM firing).

If APM is induced to fire when the pyloric rhythm is slow and irregular, its overall effect takes one of two forms, both of which are accompanied by extensive increases in spike frequency of all the pyloric neurones. Either the overall frequency of pyloric bursting is markedly increased (acceleration of the pyloric rhythm, Fig. 4A) or it is accelerated only slightly and temporarily (Fig. 4B). In the latter case, the pyloric neurones, especially the constrictor neurones (LP and PY), tend to fire in plateaus that are considerably longer than normal (see Fig. 10D, PY). If, on the other hand, the pyloric rhythm is already rapid and regular, the major effects of an APM discharge are increased amplitude of oscillation and increased spike frequency in all pyloric neurones, but little or no increase in pyloric cycle frequency (Fig. 4C).

Quantitative aspects of the modulation

As seen in Fig. 4A, activity in APM can provoke a strong acceleration of a spontaneously slow pyloric rhythm. This effect is also shown in Fig. 5B, in which the pyloric frequency is represented by the frequency of membrane potential oscillations in the pacemaker neurone PD. The increase in its oscillation frequency after a 6 s APM discharge is illustrated by plotting oscillation frequency as a function of time (Fig. 5D; open circles). Maximum frequency is reached 4–5 s after the start of APM spiking; the frequency of the pyloric rhythm then slowly decreases and is still greater than the control 45 s after the end of the APM discharge. It can thus be seen that the duration of this effect of APM is eight- to ten-fold longer than the duration of the discharge that provoked it. Fig. 5C shows that, in contrast to the strong acceleration seen in Fig. 5B, when the pyloric rhythm is rapid (Frequency 1–1·2 Hz), APM firing does not affect the pyloric frequency. This insensitivity of the frequency of the rapid pyloric rhythm is depicted graphically in Fig. 5D (filled squares). APM still affects the pyloric neurones, however, as seen in the other modifications it provokes (e.g. changes in amplitude of oscillations and in the efficacy of synaptic inputs, which will be discussed shortly; Fig. 5C, LP). Note that the maximum frequency reached by the slow rhythm (Fig. 5D, open circles) under the influence of APM is very close to that of the rapid rhythm (Fig. 5D, filled squares; approximately 1 Hz). Although the two cases represented in Fig. 5 are near the extremes of spontaneous pyloric frequency, the maximum frequency reached is around 1 Hz in all cases in which APM causes an acceleration of the pyloric rhythm.

A second effect of APM activity is an increase in the spike frequency within the bursts of all of the pyloric neurones. This is shown graphically in Fig. 6, which corresponds to the recording of Fig. 4C, in which the pyloric frequency was relatively high before APM was induced to fire. Two major differences between the effects of APM on the constrictor motoneurones (LP, PY) and on the pacemaker neurones (PD) can be seen. Firstly, the extent of the increase in spike frequency is considerably greater for the constrictors (80–90 % increase) than for the pacemakers (15–17 % increase). Secondly, the duration of the increase is greater for the constrictor than for the pacemaker neurones. Spike frequency of the constrictors is still greater than control 45 s after the APM discharge (it returns to pre-stimulation level in about

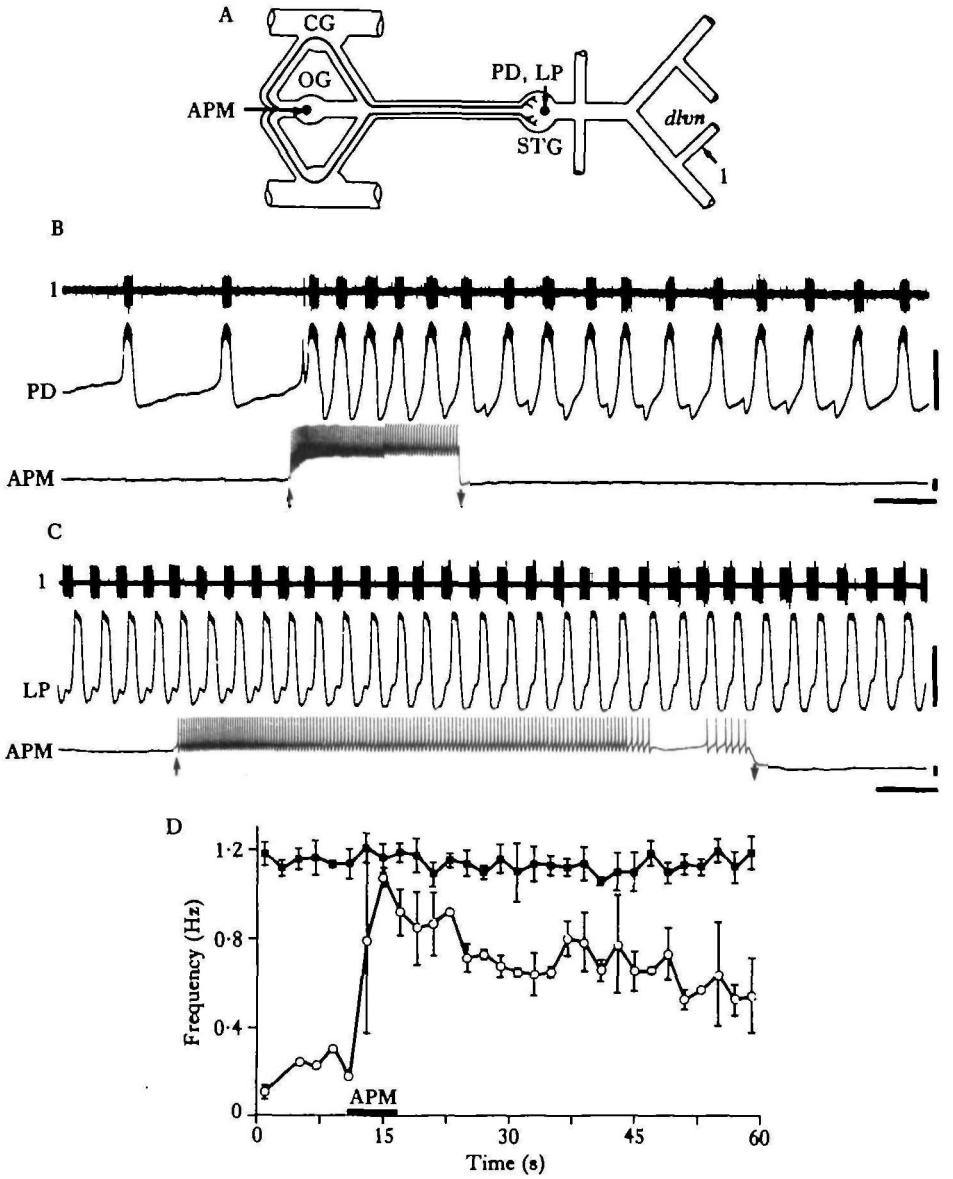

Fig. 5. Activity in APM increases the frequency of a slow pyloric rhythm to that of a spontaneously rapid pyloric rhythm. (A) Diagram of the preparation (see Fig. 1 for abbreviations). (B) A slow pyloric rhythm, represented by the activity of the pacemaker PD [intracellular, PD and extracellular (1) recordings] is strongly and lastingly accelerated by a discharge of APM (5·5 s). (C) A rapid pyloric rhythm, represented by the extracellular activity of PD (1) is not accelerated by an APM discharge (15 s). That APM still effectively modifies the pyloric network is shown by the changes in oscillation amplitude, in form and intensity of bursts of the constrictor neurone LP and in the inhibition LP receives from the pacemakers PD (see also Fig. 8). In (B) and (C), the APM discharge is elicited by intracellular current injection (arrows). (D) Graphic representation of the effect of an APM discharge (6 s, marked by solid line) on the frequency of the pyloric rhythm as a function of time. Open circles represent a spontaneously slow pyloric rhythm [analogous to (B)]; filled squares represent a rapid rhythm [analogous to (C)]. The ordinate is the instantaneous frequency of bursts in PD (inverse of the interburst interval). Each point is the frequency calculated from an interval of 2 s; the values are means from two APM discharges; vertical bars are standard deviations. Note that the frequency of the rapid rhythm is unchanged by APM, whilst that of the slow rhythm is increased to a maximum which is similar to the rapid frequency. The frequency of the slow rhythm decreases gradually after the APM discharge. Calibrations: horizontal bars, 2 s; vertical bars, 20 mV.

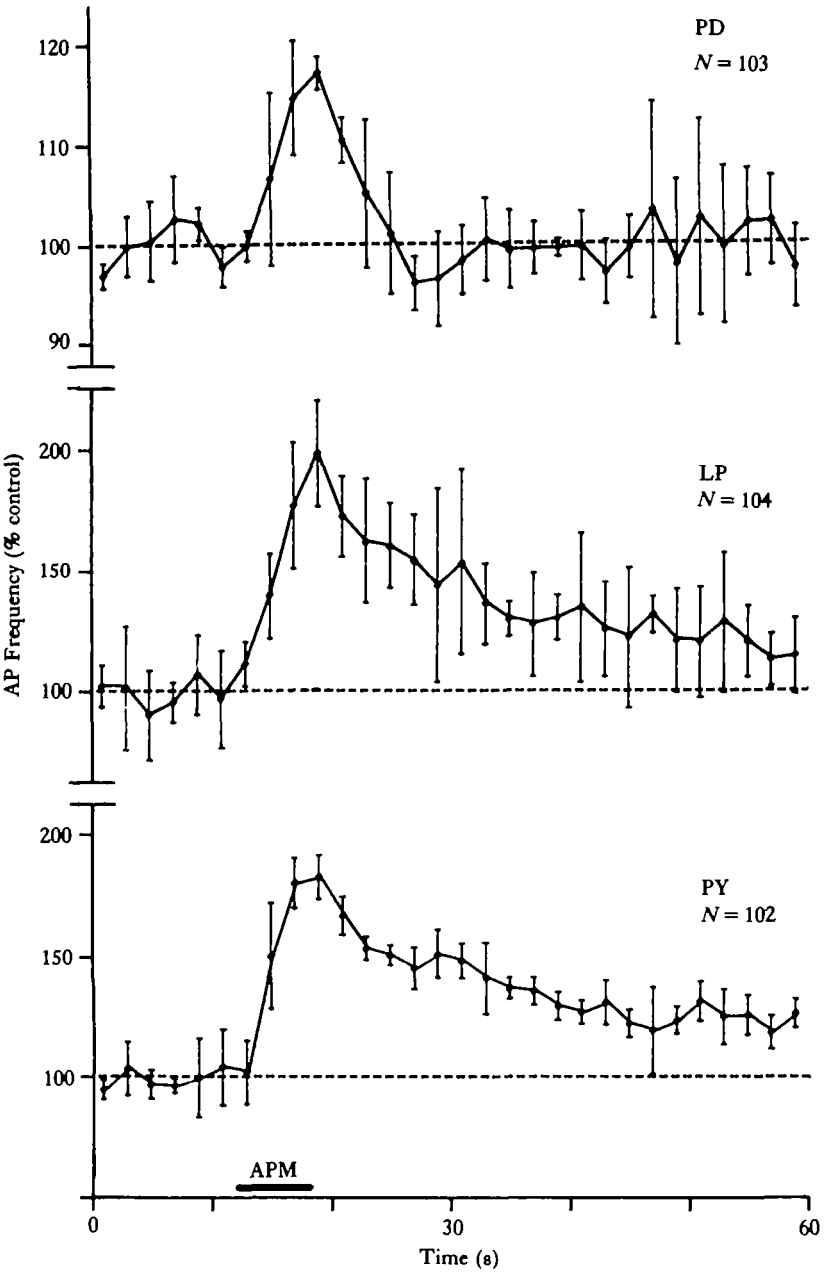

Fig. 6. An APM discharge lastingly increases the frequency of spikes within bursts of the pyloric neurones. Spike frequency is expressed as percentage of the control frequency (calculated over the 10 bursts before the APM discharge) for the PD, LP and PY neurones, respectively. Each point represents the mean calculated for bursts contained within a 2 s interval; each curve was calculated from two discharges of APM; (sequences analysed are those shown in Fig. 4C) vertical bars are standard deviations; the APM discharge (6 s) is marked by a black line. Note that the increased spike frequency after the APM discharge is both more extensive and longer lasting for the constrictors LP and PY than for the pacemaker PD (note the difference in scale).

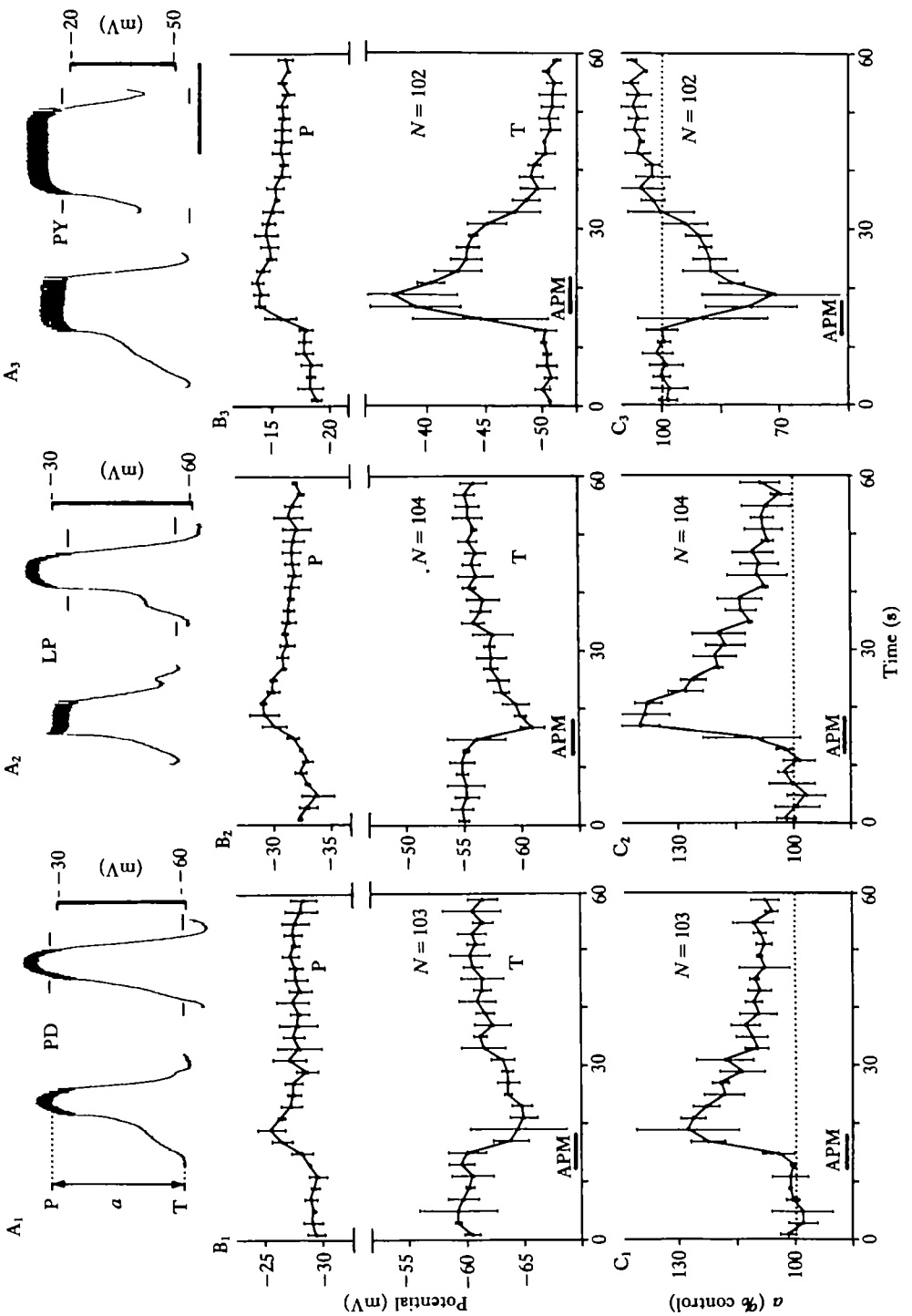
A1
PD
(mV)
-30
-60
P
a
T
A2
LP
(mV)
-30
-60
A3
PY
(mV)
-20
-50
B1
B2
B3
C1
C2
C3
P
T
N = 103
N = 104
N = 102
APM
Potential (mV)
a (% control)
Time (s)
0
30
60
100
130
70

Fig. 7.

min); here again the effects last eight to ten times the duration of the APM firing. In the pacemakers, on the other hand, the increase in spike frequency lasts only 4–5 s after the APM discharge. This stronger activation of the constrictors is also seen when the pyloric rhythm is spontaneously slow (Fig. 10D, see also Dickinson & Nagy, 1983).

The third major effect of an APM discharge is a modification of the amplitudes of oscillations of the pyloric neurones (Fig. 7). An APM discharge (6 s, about 30 Hz) causes an increase in overall oscillation amplitude in both the pacemaker PD and the constrictor LP (25 % and 35 % increases, respectively) (Fig. $7C_{1,2}$). In the constrictor PY, however, amplitude first decreases (to a maximum of 30 %), then increases (8 %) (Fig. $7C_3$). These effects are more easily understood if one examines separately the temporal evolution of the peaks and troughs of the oscillations (Fig. 7B). For all three neuronal types, the peak reaches a potential that is 3–5 mV less negative (more depolarized) than normal (Fig. 7B, P). The increase in spike frequency in all three neurones corresponds to this change in the peaks of oscillation. In contrast, the troughs of the oscillations reach a potential that is 5–6 mV more negative (more hyperpolarized) in the pacemaker PD and the constrictor LP, but is 12 mV less negative (more depolarized) in the constrictor PY (Fig. 7B, T). In PY the peaks remain depolarized after the troughs have returned to the control level, thus explaining the double effect on the overall amplitude. It is noteworthy that the temporal evolution of the amplitude effects of APM's discharge is analogous to that of both spike frequency and the frequency of the pyloric rhythm (long latency, long duration). Because the bursting activity of the pyloric neurones is due both to synaptic connectivity within the network and to endogenous properties of the neurones (see Selverston *et al.* 1976; Russell & Hartline, 1978), it may be asked which of these two factors is modified by APM's discharge and which results in the observed changes in the amplitude of oscillation of the pyloric neurones. The parallel depolarizations of the peaks of oscillation in the three neuronal types may result from modifications APM provokes in the properties of all three neurones (see Dickinson & Nagy, 1983). In contrast, as will be shown below, the evolution of the troughs of the oscillations is at least partly due to changes in the efficacy of synapses within the pyloric network after an APM discharge.

APM modifies the efficacy of synapses within the pyloric network

Inhibitory chemical synapses

In addition to the quantitative modifications of the pyloric rhythm discussed above, APM provokes qualitative modifications that involve changes in the phase relationships

Fig. 7. An APM discharge lastingly modifies the amplitude of oscillation in the pyloric neurones. (A) Recording of control bursts (first burst in A_1, A_2, A_3) and bursts at the peak of APM's effects (second burst in A_1, A_2, A_3) for the PD (1), LP (2) and PY (3) neurones. Amplitude of oscillation (*a*) is measured between the most negative point in the oscillation (trough of the oscillation, T) and the base of the action potentials (peak of the oscillation, P). The horizontal traces on the second bursts mark the trough and peak levels of the corresponding controls (first bursts). (B) Membrane potential (in mV) of the peaks (P) and troughs (T) of the oscillations of the PD (1), LP (2) and PY (3) neurones as a function of time after a 6 s APM discharge (bar). (C) Total amplitude of oscillation of neurones PD (1), LP (2) and PY (3) as a function of time after a 6 s discharge of APM (marked by black line). Amplitude is expressed as percentage of the control amplitude (mean of 10 bursts before the APM discharge). In (B) and (C), each point represents the mean of values calculated for bursts contained within a 2 s interval; each curve corresponds to two APM discharges; vertical bars are standard deviations. Sequences analysed are those shown in Fig. 4C. For conclusions, see text. Calibration: horizontal bar, 1 s.

amongst the various neurones. These changes are caused by modifications in the efficacy of synapses within the network. Amongst those affected are the inhibitory synapses between the pacemaker neurones (PD) and the constrictor neurones (LP and PY), synapses which are thought to be functionally the most important in determining the phase relationships between the firing of the neurones in the pyloric network (Selverston *et al.* 1976; Selverston & Miller, 1980).

We first considered the effects of APM on the inhibition of LP by PD (Fig. 8A). The apparent efficacy of this synapse is reflected in the absolute value to which the membrane potential of LP falls due to the inhibition by PD (Fig. 8B, filled triangles). This value is much less negative after an APM discharge than is the control (compare B_2 with B_1) indicating that the efficacy of the synapse has decreased. The development of this potential before, during and after APM firing is shown in Fig. 8C (LP). The decreased efficacy of the inhibition of LP by PD is established slowly (maximum decrease 4–6 s after the start of APM firing) and is of long duration (lasting for at least 30 s after a 6 s APM discharge). Fig. 8C also shows simultaneous changes in action potential frequency in PD (curve marked PD, same curve as Fig. 6A). The observed decrease in synaptic efficacy is unexpected for two reasons: firstly, it occurs when the spike frequency in the presynaptic neurone PD increases; secondly, it occurs when the membrane potential of the postsynaptic neurone LP is less negative (and hence further from the reversal potential for the *ipsp*s), which should increase the apparent hyperpolarization of LP.

The most negative values of LP's membrane potential (troughs of LP oscillations) are normally determined by the inhibition LP receives from PD (Fig. $8B_1$, filled triangles). We have seen that the LP potential resulting from the inhibition by PD is less negative after an APM discharge; however, after such a discharge, the absolute value of the troughs of LP's oscillations is more negative (Fig. $7B_2$, T). This apparent

Fig. 8. An APM discharge causes long term modification of the efficacy of inhibitory chemical synapses of neurones PD and PY on the constrictor neurone LP. (A) Schematic diagram of the inhibitory synapses (filled circles) whose efficacy is modified by APM. (B) Intracellular recordings of PD, LP and PY before (B_1), during (B_2) and 33 s after (B_3) a discharge of APM (6 s, 30 Hz). The modification of the LP potential caused by the inhibition from PD is marked with a filled triangle; that due to the inhibition from PY is marked with an open triangle. (C) The curve LP is the absolute value (in mV) of LP's membrane potential during inhibition by PD [filled triangles in (B)] after a 6 s APM discharge (black line). The curve PD shows spike frequency in PD during the same time period (curve as in Fig. 6A). In spite of the increased spike frequency in PD (after APM discharge) the potential reached by LP as a result of inhibition by PD is less negative; hence this inhibition is functionally less efficacious after an APM discharge. (D) The curve LP represents the absolute value (in mV) of LP's membrane potential during inhibition by PY [open triangles in (B)] after an APM discharge (6 s; black line). The curve PY shows spike frequency in PY during the same time period (curve as in Fig. 6C). After the APM discharge, the potential reached by LP during inhibition by PY is more negative; hence this inhibition is functionally more important after an APM discharge. In (C) and (D) each point is the mean of values calculated for the bursts occurring within a 2 s interval; each curve represents two APM discharges, vertical bars are standard deviations. (E), (F) APM firing modifies the phase relationships between the discharges of the pacemaker neurone PD and the constrictor neurones LP and PY. (E) In a control recorded 10 s before an APM discharge, the phase relationships of LP and PY discharges in the pacemaker cycle are respectively $L_1/P \approx 0{\cdot}46$ and $L_2/P = 0{\cdot}69$. Note the silent period (t) between PD and subsequent LP bursts, which is a characteristic of the pyloric pattern. (F) 4 s after a 6 s imposed discharge of APM, the phase relationships of LP and PY discharges in the pacemaker cycle are, respectively, $L_1/P = 0{\cdot}27$ and $L_2/P = 0{\cdot}42$. The silent period t has now disappeared (arrow). L_1, latency of LP burst in PD oscillatory period; L_2, latency of PY burst in PD oscillatory period; P, period of PD oscillation. Calibration: horizontal bars, (B) 1 s, (E), (F) 0·5 s; vertical bars, 20 mV.

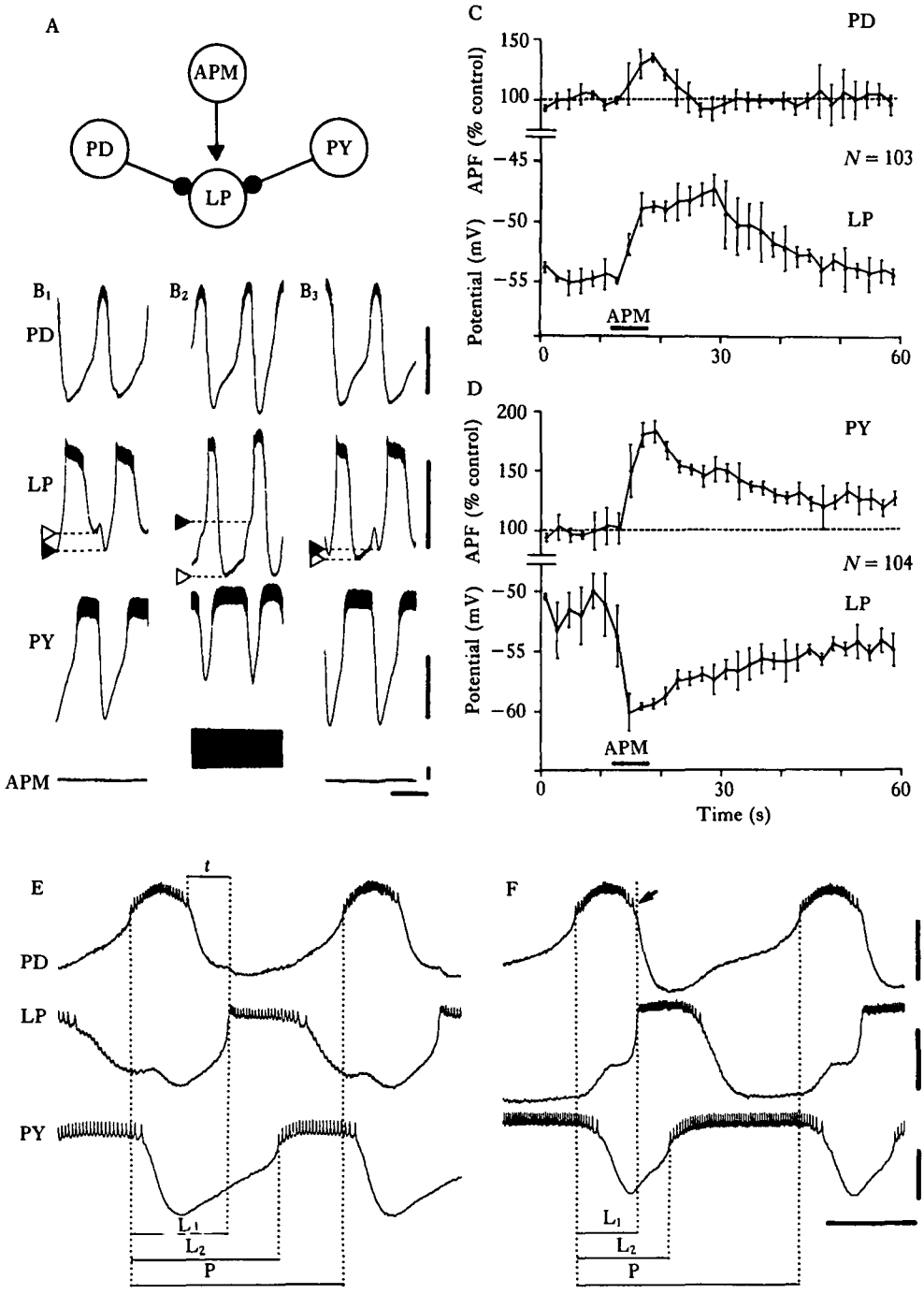
A
APM
PD
PY
LP
B1
B2
B3
PD
LP
PY
APM
C
PD
150
100
APF (% control)
N = 103
−45
−50
−55
Potential (mV)
LP
APM
0
30
60
D
200
150
100
PY
N = 104
−50
−55
−60
LP
APM
Time (s)
E
t
PD
LP
PY
L1
L2
P
F
L1
L2
P

Fig. 8

contradiction can be explained by the observation that the hyperpolarization of LP results from two separate inhibitions (Fig. 8A): that from PD (Fig. $8B_1$, filled triangle) and that from the PY neurones (Fig. $8B_1$, open triangle). After an APM discharge, the efficacy of the inhibitory synapse of PD onto LP decreases, but that of the inhibitory synapse of PY onto LP increases, and the value of the LP membrane potential determined by the inhibition from PY becomes more negative (Fig. $8B_2$, open triangle). Fig. 8D (LP) shows the evolution of this potential as a function of time after an APM discharge, as well as the parallel evolution of spike frequency in PY (Fig. 8D, PY; same curve as Fig. 6C). Consequently, when APM fires, it is the inhibition from PY rather than that from PD that determines the extreme values of the LP troughs. Thus, a discharge of APM causes a reversal of the relative importances of the inhibitions produced in LP by the pacemaker neurones PD and by the constrictor neurones PY. Functionally, this allows LP to maintain or increase its oscillatory activity, yet to modify substantially the phase relationships between its discharges and the discharges of the pacemaker neurones (see below).

Another synaptic relationship which is functionally important in determining the pyloric rhythm is the chemically mediated synaptic inhibition exerted by the pacemaker neurones (PD) upon the constrictor neurones PY. Again the apparent efficacy of the synapse can be characterized by the membrane potential of PY at the maximum of the inhibition. This corresponds to the troughs of the PY oscillations, which were shown in Fig. $7B_3$ (T) to become less negative after an APM discharge. At the same time, the spike frequency of the presynaptic neurone (PD) increases (see Fig. 8C, PD). Thus, the efficacy of the inhibition of PY by PD decreases in a manner analogous to that of the inhibition of LP by PD (compare Fig. $7B_3$, T and 8C, LP).

Overall, an APM discharge is accompanied by a marked decrease in the apparent efficacy of those synapses which are functionally of greatest importance in the pyloric network, the inhibitory synapses of the pacemakers on the constrictor motoneurones. This results in changes in phase relationships between the discharges of the various neurones (Fig. 8E, F). The phases of discharge of the constrictor neurones (LP and PY) in the pacemaker cycle are significantly advanced when APM fires (see legend of Fig. 8E, F for explanation of phase relationships). This modification of the phase relationships induced by APM discharge can lead to the suppression (Fig. 8F, arrow) of the characteristic silent period which usually occurs between the firing of the antagonistic neurones PD and LP (Fig. 8E, *t*; see also extracellular record in Fig. 1D, before APM firing).

In addition, the APM discharge is accompanied by an increase in the efficacy of another inhibitory synapse, that of one constrictor (PY) on another (LP). This modification can be explained by the strong activation (and resultant increased spike frequency) of the presynaptic element (PY). In contrast, the decreased synaptic efficacies appear paradoxical because they occur when spike frequency (and amplitude of oscillation) in the presynaptic neurone (PD) increases. We will show in the following paper (Dickinson & Nagy, 1983) that this can be explained by modifications of the membrane properties of the postsynaptic neurones.

Double synaptic relationships

Activity in APM inverts the relative efficacy of two synapses of the same type

inhibitory chemical) that a single neurone (LP) receives from two others (PD and PY). Firing in APM can likewise invert the relative importance of two types of synapse (electrical and inhibitory chemical) which exist simultaneously between two neurones.

Within the set of dilator neurones such a double synaptic relation exists between the pacemaker neurone (PD) and the ventral dilator neurone (VD) (Maynard, 1972; Maynard & Selverston, 1975; Fig. 9A). They are linked by an electrical synapse, and the pacemaker neurones inhibit neurone VD by a chemical synapse. The electrical synapse between PD and VD tends to synchronize the cyclically recurring depolarizations of the two neurones (Fig. 9C). However, at the peak of firing, chemical inhibition from PD causes VD to stop firing, thus maintaining a difference in phase

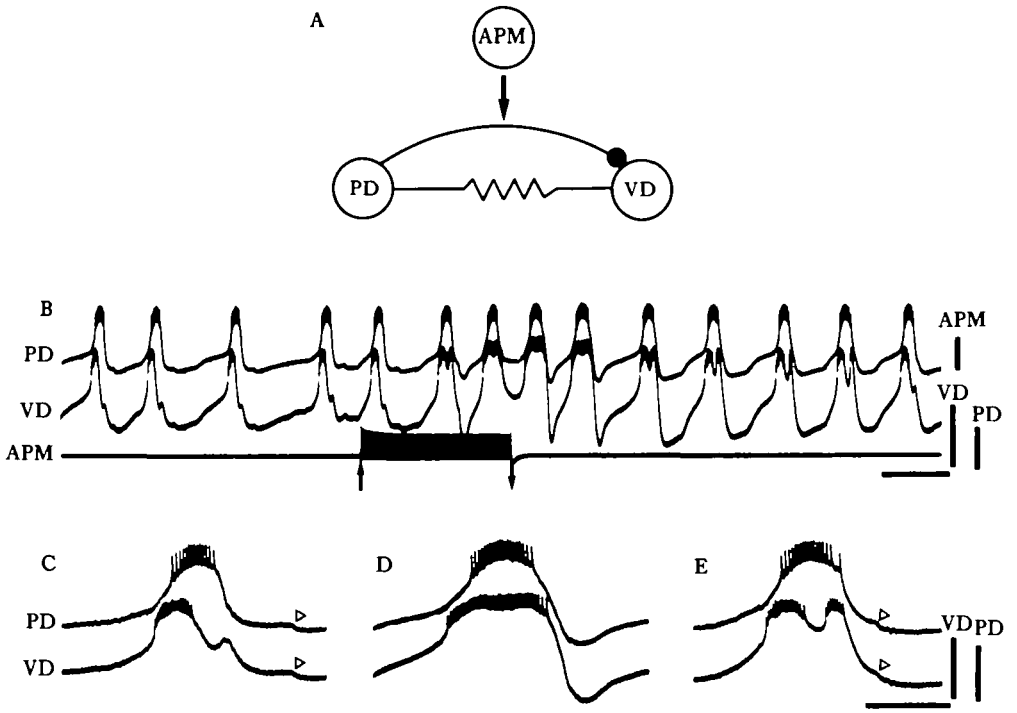

Fig. 9. An APM discharge modifies the phase relationship between the discharges of PD and VD by shifting the balance between the effects of the inhibitory chemical synapse and those of the electrotonic synapse between these two neurones. (A) Diagram of the synaptic relationships between PD and VD; the filled circle represents an inhibitory chemical synapse, the resistance (∿) an electrical synapse. (B) At the beginning of the recording, PD and VD show their usual phase relationship [shown at higher speed in (C)]. The electrical synapse synchronizes the depolarizations of the two neurones; VD is then inhibited by the chemical synapse from PD, causing a phase difference between their discharges. A 4·5 s discharge of APM (by intracellular current injection, arrows) activates both neurones, but decreases the efficacy of the chemical synapse. The bursts in PD and VD thus become synchronous. Several seconds after the APM discharge, the inhibition of VD by PD gradually recovers its efficacy, and the bursts in PD separate the VD bursts into two parts. (C) Bursts in PD and VD, showing their phase relationship, before an APM discharge. (D) Bursts in PD and VD, showing their phase relationship, just after an APM discharge. (E) Bursts in PD and VD, showing their phase relationship, 10 s after an APM discharge. Open triangles, inhibition from LP neurone simultaneously received by PD and VD. Calibrations: horizontal bars: (B) 2 s; (C), (D), (E), 0·5 s; vertical bars: APM, 60 mV; PD, VD, 20 mV.

between these two neurones. The exact phase relationship of PD and VD discharge at any time is determined by the dynamic balance between the effects of the electrical synapse and those of the inhibitory chemical synapse. When APM fires, the efficacy of the inhibitory synapse from PD onto VD decreases to almost zero and the bursts in PD and VD become synchronous (Fig. 9D). Thus, activity in APM results in a shift of the balance between the two synapse types in favour of the electrical synapse. This effect continues for some time after the end of the APM discharge (Fig. 9B), and then progressively recovers. During the recovery, intermediate cases in which VD fires double bursts can be seen (Fig. 9E). In these cases, at the peak of its discharge PD can briefly inhibit VD (*via* the chemical synapse), but after that the electrical synapse (which depolarizes VD) predominates again, and the burst of VD resumes. The decreased efficacy of the chemical synapse cannot be accounted for by changes in firing of the presynaptic element, for both spike frequency and amplitude of oscillation of PD increase rather than decrease during and after an APM discharge.

In summary, it appears that APM can modify the phase relationships between the discharges of the pacemaker neurones and VD by shifting the dynamic balance which exists between the effects of an electrical and an inhibitory chemical synapse.

The intensity of APM activity controls the expression of the pyloric pattern

Firing of the APM neurone engenders major and lasting modifications of the activity of the pyloric neurones. The expression of these modifications is in part dependent upon the previous activity level of the pyloric network, as discussed earlier, but is also dependent upon the intensity of the activity in APM (Fig. 10). Increasing spike frequency in APM does not result in a simple increase in activity of the pyloric neurones, but rather modifies, to differing extents, the various characteristics of the discharges of the pyloric neurones (amplitude of oscillation, spike frequency, frequency of the rhythm, etc.). This more or less alters the pyloric rhythm. As shown in Fig. 10B, firing of APM at 2·5 Hz causes increased spike frequency and amplitude of oscillation in the PY neurone (compare to Fig. 10A), but has little effect on the overall frequency of the pyloric rhythm (see the extracellular recording of pacemaker neurone PD in the *dlvn*, Fig. 10B). When APM fires at 6 Hz, however, the rhythm is substantially accelerated (Fig. 10C). Finally, after a 20 Hz discharge of APM (Fig. 10D), the pyloric rhythm first accelerates, then stops and the PY neurone fires in long plateaus. Thus, in the example shown, activity in APM results in a slow but intense rhythm, a rapid and intense rhythm and an intense non-rhythmic activity of the pyloric neurones, respectively, as firing frequency in APM is increased. These effects have been observed repeatably throughout the same experiment, and from one preparation to another.

In this example, APM spike frequencies as low as 2·5 Hz produce distinct modifications of the pyloric pattern (compare Fig. 10B and A). It is interesting to note that APM will, at least in isolated preparations, fire spontaneously at frequencies equal to or greater than 2·5 Hz. One such example is shown in Fig. 10E, in which APM fires in irregular bursts. We have frequently recorded such bursting patterns of activity in both *Jasus* and *Palinurus*. However this rhythm is variable from one animal to another and thus far we have not been able to correlate it with any other rhythm or to identify any sources of afferent input which might trigger this activity in APM.

The modulation of the pyloric network occurs via *muscarinic cholinergic receptors*

We have shown that APM modifies the expression of the motor pattern produced by the whole pyloric network. These effects are established slowly (seconds) and persist longer (five to ten times) than the duration of the APM discharge provoking them. In addition, as is shown in the following paper (Dickinson & Nagy, 1983), APM modifies voltage-dependent properties of its postsynaptic neurones. This suggests that APM does not exert classical synaptic effects, having short actions (milliseconds), but is instead modulatory in nature (see Discussion). It was therefore of particular interest to determine the kind of receptors (and the neural transmitter) involved. The pharmacological tests presented below suggest that APM acts *via* muscarinic cholinergic receptors.

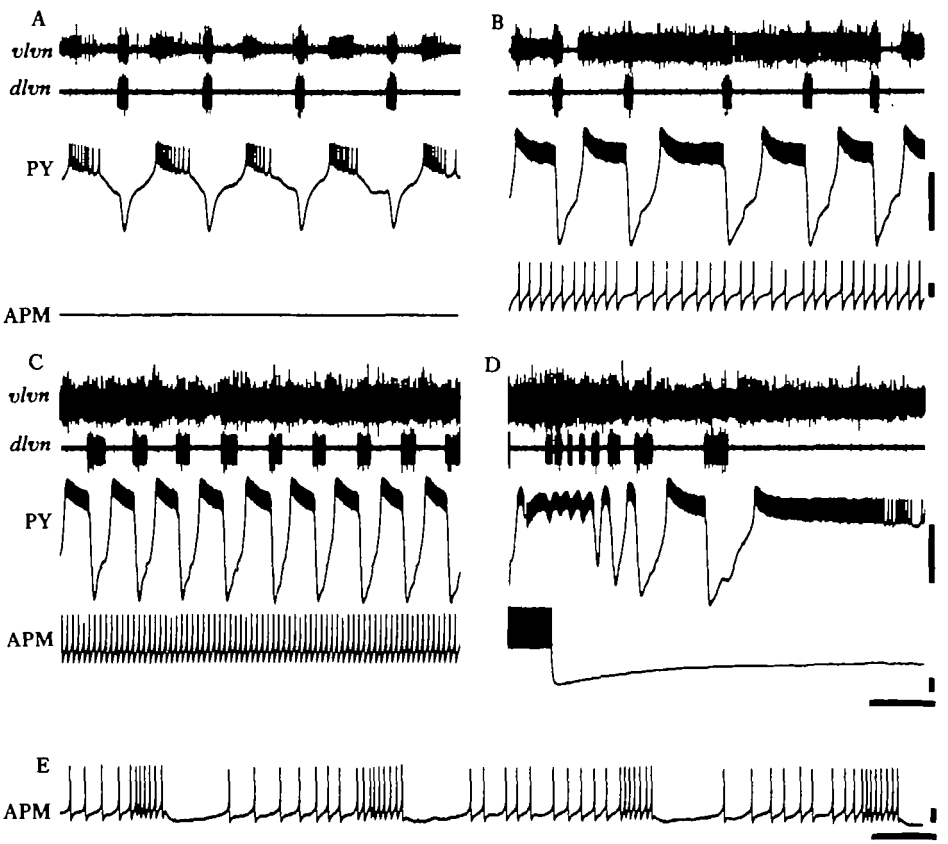

Fig. 10. Modifications of the pyloric pattern depend on the frequency of spiking in APM (*Palinurus vulgaris*). Pyloric activity is represented by the extracellular activity of several neurones recorded in the *vlvn*, by the extracellular activity of PD recorded in the *dlvn* and by the intracellular activity of PY. (A) Spontaneous pyloric activity before the APM discharge. (B) Low frequency (2·5 Hz) firing of APM activates the constrictors without noticeably modifying the pyloric rhythm. (C) A 6 Hz discharge of APM activates all the pyloric neurones and strongly accelerates the rhythm. (D) After a 4 s, 20 Hz discharge of APM, the temporarily increased pyloric rhythm (*dlvn*) stops and the strongly activated constrictors fire in long plateaus. Recordings (A), (B), (C) and (D) were taken from the same preparation in a single experiment. APM was induced to fire by intracellular current injection. (E) Spontaneous bursting activity in APM. Calibrations: horizontal bars, 2 s; vertical bars, 20 mV.

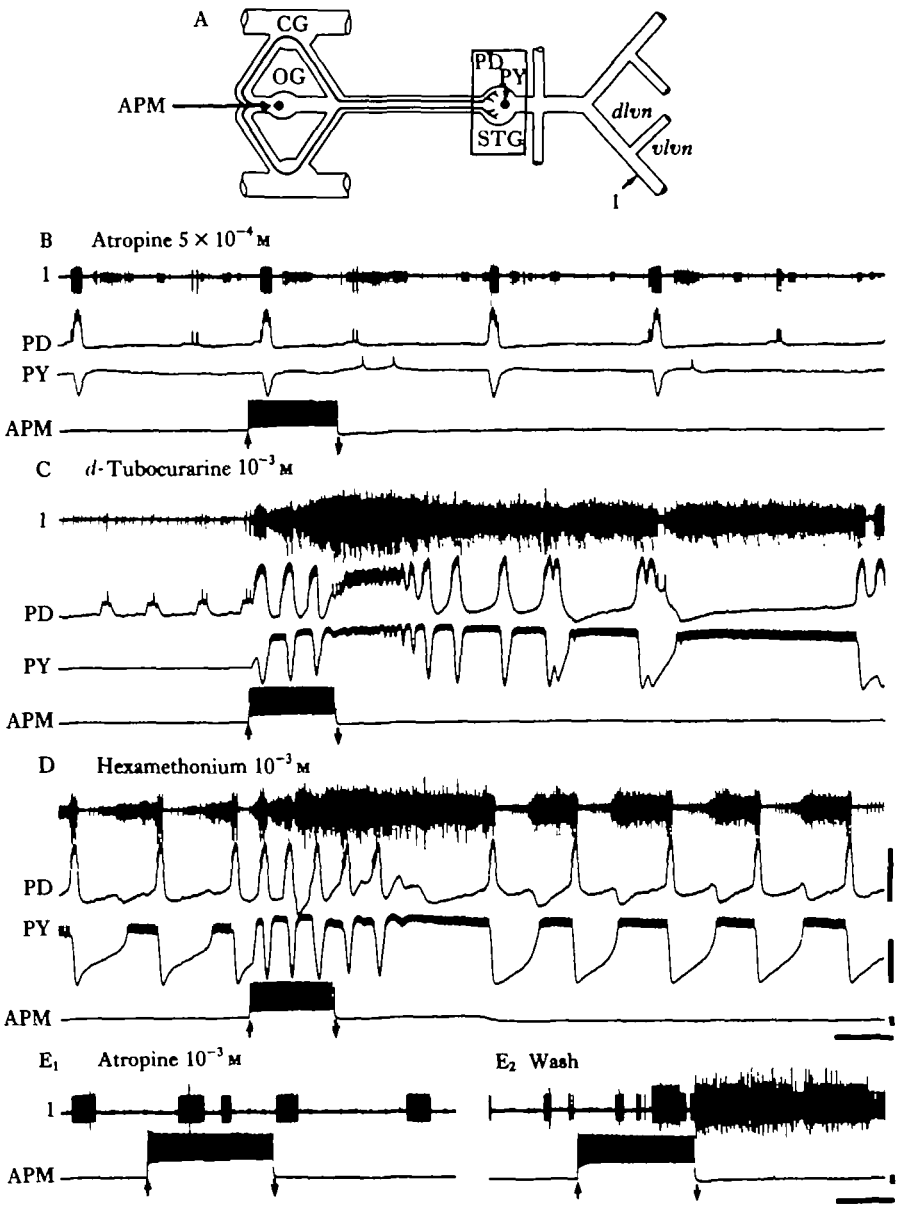

Fig. 11. The effects of APM on the pyloric rhythm are blocked by atropine and not by *d*-tubocurarine or hexamethonium. (A) Diagram of the preparation; the rectangle represents the area bathed in the various drug solutions. (B) When the STG is bathed in 5×10^{-4} M-atropine, firing in APM no longer affects the pyloric activity (extracellular activity on the *vlvn*, intracellular activity in PD and PY). (C) APM continues to affect the pyloric activity when the STG is bathed in 10^{-3} M-*d*-tubocurarine. This recording is from the same preparation as (B) taken after 30 min washing with saline. (D) APM continues to affect the pyloric rhythm when the STG is bathed in 10^{-3} M-hexamethonium. This recording is from the same preparation as (B) and (C) (*Palinurus vulgaris*). (E) The blocking of APM's effects with atropine is reversible. (E_1) When the STG is bathed in 10^{-3} M-atropine, APM has no effect. After a 1 h wash with saline (E_2), the effects of APM reappear (*Jasus lalandii*). In all cases, APM was driven by intracellular current injection (arrows). Calibrations: horizontal bars, 2 s; vertical bars, 20 mV.

The effects of APM on the pyloric neurones are blocked by atropine, a cholinergic antagonist which is known to block muscarinic receptors in vertebrates. When the stomatogastric ganglion is bathed in 5×10^{-4} M-atropine (in saline), the effects of APM are reversibly blocked (Fig. 11B) (in $2{\cdot}5 \times 10^{-4}$ M-atropine, the effects of APM are greatly decreased). In contrast, solutions of *d*-tubocurarine and hexamethonium (cholinergic antagonists which block nicotinic receptors) do not block the effects of APM, even at concentrations as high as 10^{-3} M (Fig. 11C, D). Note that atropine, although it completely blocks APM's effects, does not modify synaptic activity within the pyloric network, as shown by the fact that PD still inhibits the constrictor neurones (Fig. 11B, PY). The effects of atropine are completely reversible, as seen in Fig. 11E in which the effects of APM, blocked by 10^{-3} M-atropine (Fig. $11E_1$), returned after 1 h of perfusion with normal saline (Fig. E_2).

That the effects of APM on pyloric neurones involve cholinergic muscarinic receptors could also be shown using muscarinic agonists. Perfusion of the deafferented stomatogastric ganglion with muscarinic agonists (pilocarpine, oxotremorine) has been shown to induce rhythmic activity in previously silent pyloric pacemaker neurones (Marder & Paupardin-Tritsch, 1978; Anderson, 1980). This activation resembles to some extent the activation induced by APM. However, other afferent fibres to the stomatogastric ganglion, known to activate the pacemaker neurones, are also thought to be cholinergic (Russell & Hartline, 1981; Sigvardt & Mulloney, 1982) thus rendering the actions of muscarinic agonists difficult to interpret.

DISCUSSION

The activity of a single identified interneurone, the anterior pyloric modulator (APM), in the oesophageal ganglion of the rock lobsters *Jasus lalandii* and *Palinurus vulgaris*, can modify the output of the pyloric pattern generator. Thus a single neurone can alter a rhythmic motor behaviour. These extensive effects appear to be characteristic of a neuromodulatory process.

Possible direct effect of APM on all the neurones of the pyloric network

It seems that APM directly activates each of the neurones of the pyloric network. Its two axons project directly into the stomatogastric ganglion. Although these axons pass through the two commissural ganglia and may make synaptic connections there, such connections are not necessary for the activation of the pyloric neurones. This is indicated by the fact that APM activates the pyloric neurones even when synaptic activity in the commissural ganglia is blocked. Moreover, all of the 30 neurones in the stomatogastric ganglion have been studied (Selverston *et al.* 1976) and it is known that there are no local interneurones presynaptic to the neurones of the pyloric network. Thus the effects of APM on pyloric activity cannot involve a local interneurone in the stomatogastric ganglion, and a direct action of APM on the pyloric neurones is a possibility that must be considered. This is also suggested by the observation that APM's effects involve cholinergic muscarinic receptors and that the existence of such receptors has been proposed at least for the pacemaker neurones PD (Marder & Paupardin-Tritsch, 1978). In addition, some recent experiments on *Palinurus vulgaris* (unpublished) suggest the presence of monosynaptic connections between APM

and the PY neurones. Excitatory postsynaptic potentials (*epsps*) were correlated one-to-one with spikes in APM. These *epsps*, in contrast to APM's modulatory effects, are sensitive to and can be reversibly blocked by *d*-tubocurarine (a nicotinic cholinergic blocker), indicating that APM is cholinergic. The simplest explanation for these results is that APM acts directly upon nicotinic receptors on the pyloric neurones to produce *epsps*, and that it simultaneously acts upon muscarinic receptors on the same neurones to produce the modulatory effects we have described in this paper.

Although we have not been able to exclude the possibility that APM acts presynaptically in the stomatogastric ganglion upon another fibre afferent to this ganglion (and to the pyloric network), the available evidence suggests a direct action of APM's axons on the pyloric neurones. We may now ask whether APM has synapses upon each of the pyloric neurones or whether it directly affects only certain of these neurones. Most of the synaptic connections within the pyloric network are blocked by either curare or picrotoxin (Marder & Paupardin-Tritsch, 1978; Bidaut, 1980). However, APM is able to influence all the pyloric neurones in the presence of either drug (see Dickinson & Nagy, 1983 for APM's effects in the presence of picrotoxin). Thus, APM must be directly and separately activating each of the pyloric neurones.

Functional consequences of activity in APM: possible modifications of the pyloric behavioural sequence

An APM discharge influences all the neurones of the pyloric pattern generator, thus modifying the output of the network and changing the entire pyloric motor pattern. Because the muscular activity controlled by the pyloric neurones is well known (Hartline & Maynard, 1975), it is possible to envisage a number of behavioural implications of this modulation by APM. The most common effect of an APM discharge is an increased activity in the pyloric neurones (seen as an increase in spike frequency within bursts), which would be likely to increase the strength of muscular contractions. This activation is particularly strong in the constrictor motoneurones, which control the muscles performing the power stroke of the pyloric movements.

More far-reaching functional consequences of an APM discharge could result from the qualitative alteration of the pyloric pattern that is produced by such a discharge. The motor pattern generated by the pyloric network is determined both by the oscillatory activity of the pacemaker neurones (Fig. 12A, PD-AB) and by the reciprocal inhibitory synapses within the network, the most important being those of the pacemakers onto the constrictors (Selverston *et al.* 1976). We have shown firstly that APM decreases the functional importance of these synapses relative to others in the network (Fig. 12C), and secondly that it can alter the oscillatory activity of the pacemaker neurones, either by transforming the pyloric pattern from a rhythmic to a non-rhythmic one, or by increasing the frequency of the pyloric rhythm. These effects of APM can influence three aspects of the pyloric behavioural sequence. The first of these is the phase relationship between the contractions of the different pyloric muscles. The decreased efficacy of the synaptic input from pacemaker to constrictor neurones after firing of APM leads to phase shifts of the discharges of pyloric neurones relative to each other. Therefore APM activity must lead to the same phase shifts between the contractions of the pyloric muscles, particularly the antagonistic dilator and constrictor muscles.

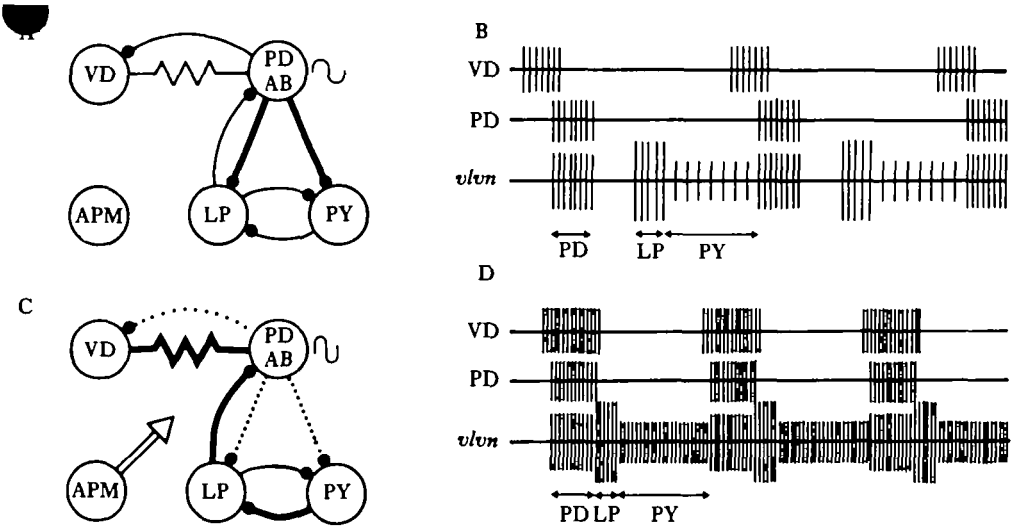

Fig. 12. The APM neurone modifies the relative efficacy of synapses in the pyloric network. This in turn modifies the expression of the pyloric pattern. (A) Diagram of synaptic relationships within the pyloric network (see also Fig. 1B). Heavy lines represent the synapses which are functionally the most important in producing the pyloric pattern. (B) Theoretical representation of spontaneous activity of the various pyloric neurones and their respective phase relationships. (C) Diagram indicating the relative functional importance of the synaptic relationships within the pyloric network after an APM discharge. Heavy lines indicate relationships which have become functionally the most important; dotted lines indicate synapses which have become functionally less efficacious. (D) Theoretical representation of activity of the various pyloric neurones and their respective phase relationships after an APM discharge. For abbreviations see Fig. 1.

The second modification which can be induced by APM is a temporary suppression of rhythmic behaviour and its replacement by strong firing in plateaus of the pyloric neurones, especially of the constrictor neurones (see Fig. 10D). This represents a profound alteration of the pyloric motor pattern. In the intact animal, rhythmic activity in the pyloric stomach would cease, and the strong and long-lasting contractions of the constrictor muscles could then push food out of the pyloric stomach.

The third type of modification which can be provoked by APM is an acceleration of a previously slow pyloric rhythm to a maximum frequency of about 1 Hz, an acceleration which is accompanied by recruitment of previously silent neurones. This corresponds to a shift from a slow, irregular rhythm to a fast, regular pyloric rhythm, which in the intact animal has been observed to occur abruptly when feeding starts, the fast rhythm continuing for several hours after the end of a meal (Rezer & Moulins, 1980). The long duration of the effects of APM suggests that it might be involved in this transition between the two kinds of pyloric rhythm during feeding.

APM's effects as a neuromodulatory process

APM's action on the pyloric activity is typical of a neuromodulatory action (see Kandel, 1976; Kandel, Krasne, Strumwasser & Truman, 1979; Dismukes, 1979; Kupfermann, 1979; Daly, Hoffer & Dismukes, 1980; review by Hartzell, 1981). The characteristics are (1) membrane properties of a relatively large number of neurones are modified; we have shown that APM influences all the neurones of the pyloric network; (2) effects are established slowly and have a long duration (tens of seconds

to hours); we have seen that the modifications provoked by APM are first visible about 1 s after the start of APM firing, that they peak 3–5 s later (although APM's spikes reach the stomatogastric ganglion in about 100 ms), and that they last eight to ten times longer than the discharge which provoked them; (3) effects are dependent on the voltage of the postsynaptic elements; all of the modifications of the pyloric pattern provoked by APM can be explained by voltage-dependent modifications of membrane properties of the pyloric neurones (Dickinson & Nagy, 1983); (4) the action modifies the effects of other synaptic inputs; we have seen that APM's discharge drastically alters the relative efficacy of all the synaptic relations within the pyloric network. Therefore, the influence exerted by APM on the pyloric motor pattern fulfills the main criteria for a neuromodulatory process.

We have seen that APM can profoundly change the expression of the pyloric activity in several ways, and that some of these changes resemble the modifications of the pyloric motor pattern which correspond in intact animals to the initiation of a behaviour adapted for feeding. Therefore, one might suggest that APM could contribute to the triggering of this pyloric pattern associated with feeding. APM might thus be an example of the theoretical concept of a modulatory element integrated into a command system (see Kupfermann & Weiss, 1978). A possible role for APM in a command system is suggested by the observation that it modifies the membrane properties of pyloric neurones and thus changes their sensitivity to synaptic inputs (Dickinson & Nagy, 1983). It is therefore possible that APM conditions the pyloric neurones to respond to input from other elements in a command system.

Implicated receptors

A number of studies have suggested that it is the receptors involved rather than the nature of the transmitter that determine a modulatory synaptic effect (see review by Dismukes, 1979). Thus, a transmitter used as a briefly acting neuromediator at a given synapse may also be involved in neuromodulation at another or even at the same synapse.

In the stomatogastric ganglion of lobsters, a number of substances may be involved in chemical synaptic transmission, including acetylcholine, GABA and glutamate, which are putative transmitters at synapses within the pyloric network (Marder & Paupardin-Tritsch, 1978; Bidaut, 1980). Others, such as dopamine (Kushner & Maynard, 1977; Raper, 1979; Anderson & Barker, 1981) and octopamine (Barker, Kushner & Hooper, 1979), are thought to mediate transmission from higher order nervous centres. Our experiments suggest that the modulation of pyloric activity provoked by APM is mediated by acetylcholine acting on muscarinic receptors. It is interesting to note that all of the known slow potentials mediated by acetylcholine utilize muscarinic receptors (e.g. parasympathetic ganglion cells in the mudpuppy, Hartzell, Kuffler, Stickgold & Yoshikami, 1977; sympathetic neurones in the toad, Schulman & Weight, 1976; Weight, Schulman, Smith & Busis, 1979 and in the rabbit, Dun, Kaibara & Karczmar, 1978).

In addition, studies of several non-neuronal cell types have shown that the activation of muscarinic receptors can cause modifications of regenerative membrane properties; thus, the plateau phase of cardiac action potentials in vertebrates is decreased (Giles & Noble, 1976; Ten Eick, Nawrath, McDonald & Trautwein, 1976

Purves, 1976), slow waves in intestinal muscles of mammals are increased (Bolton, 1971), and the plateau phase of activity in pancreatic endocrine cells is increased (Gagerman, Idahl, Meissner & Taljedal, 1978). In the pyloric network, activation of muscarinic receptors on the PD pacemaker neurones with muscarinic agonists (pilocarpine, oxotremorine) induces bursting activity in these neurones (Marder & Paupardin-Tritsch, 1978; Anderson, 1980). Moreover, electrical stimulation of the inferior ventricular nerve induces a long-term enhancement of bursting in the pacemaker neurones, and this effect might be mediated by muscarinic receptors (Russell & Hartline, 1981). But, before the present study, nothing was known about modifications of the activity of the non-pacemaker neurones. The long-term modulation by an APM discharge, which most probably involves muscarinic receptors, will be shown in the next paper (Dickinson & Nagy, 1983) to be due essentially to induction and amplification of membrane properties underlying the burstiness in all of the pyloric neurones.

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