4 Cellular and Synaptic Properties

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Little had been published about the cellular properties of STG neurons at the time of a previous review (Silverston et al. 1976). Since then we have learned much about rhythm activation, plateau potentials, synaptic modulation, nonspiking transmission, and the geometrical properties of the neurons, which are reviewed in this chapter and the appendix. New data on voltage clamp and photometric analyses are also summarized.

4.1 Passive Electrotonic Properties and Neuronal Geometry

Edwards and Mullooney (1984) have analyzed the geometry of the MG (GP) neuron of the gastric system, using intracellular dye injection and compartmental analysis (Jack et al. 1975). Hyperpolarizing step currents injected into the soma gave membrane potential trajectories that behaved as the sum of three exponentials with time constants of 35–53, 10–17, and 0.7–0.9 ms, suggesting a 3-compartment model. Given that the input and output synapses are distributed at numerous sites on the distal segments of the dendrites (King 1976b), calculations were made of the attenuation of synaptic and action potentials between the soma, axon, and dendrites using a multicompartment model. The IPSP from Int 1 (CI) was modeled as 8 ms, 5 nS conductances at each of 28 terminal dendrite compartments. The amplitude of the IPSP was calculated to be 30% larger in the terminal dendrites than in the soma. Action potentials initiated in the axon were calculated to have amplitudes of ca. 70 mV in the terminal dendrites, and 60 mV in the soma. The reported structure of the MG (GP) neuron is unusual in that the secondary processes arise from one point along the primary neurite; other STG cells may have a more distributed arrangement of secondary processes, which could affect their cable properties.

4.2 Repetitive Firing and Rebound

When a pyloric cell is depolarized with step currents, the firing rate adapts in a manner that can be described as a two-component exponential (Fig. 4.1; Hartline and Gasse 1979). Time constants for the rapid and slow adaptation have values of 0.2–0.4 s and 3–4 s respectively. Adaptation in gastric cells has not been studied
systematically. Such data pertain to the transformation of motoneuronal oscillations into the control of muscles.

The rebound that follows a hyperpolarization, due to either synaptic inhibition or injected current, is a prominent characteristic of many STG neurons both pyriform and gastric (Selverston et al. 1976, Perkel and Mulloney 1974). In pyriform neurons, Hartline and Gassie (1979) reported that rebound firing often has a delayed onset, increases in frequency over the initial few spikes, and then declines linearly in frequency over a few hundred ms, followed by a slower decay. Conditioning hyperpolarizations need to continue about 5 s for maximal rebound to develop. Pyriform neurons typically show "paradoxical excitation" after being phasically inhibited by the PD cells, probably due to rebound. For example, the average firing rate of the LP cell (in the isolated STG) is actually higher when it receives phasic PD inhibition, followed by rebound, compared to its steady firing rate in the absence of inhibition.

4.3 Graded Synaptic Transmission

Many STG cells release synaptic transmitters below the membrane potential threshold for spikes. During the slow oscillations that underlie the stomatogastric motor patterns, these neurons release transmitter tonically, as a smoothly graded function of presynaptic voltage over much of the oscillation range. When spikes occur, the additional stimulus to transmitter release causes FSPs to be superimposed on this underlying smooth synaptic potential. Graded (or chemotonic, nonspiking, or non-impulse-mediated) synaptic transmission is the common mode of synaptic function in all nonspiking neurons (reviewed in Roberts and Bush 1981, Siegel 1984, Pearson and Fourtner 1975) but has been only rarely described for spiking cells during normal function (e.g., Nicholls and Wallace 1978). However, even chemical synaptic pairs that normally generate obligatory spike-for-spike transmission, can be made to behave in a graded fashion by blocking spikes with tetrodotoxin (TTX) and manipulating the presynaptic membrane potential (Katz and Miledi 1967, Llinás et al. 1981, Martin and Ringham 1975, Wojtowicz and Atwood 1984).

Graded synaptic transmission (GST) is ordinary chemical synaptic transmission. A variety of tests can be used to distinguish graded chemical from graded electrical synaptic transmission (D.M. Maynard and Walton 1975, Graubard et al. 1983); in the GST, the sign inversion of inhibition simplifies the distinction. Other conventional chemical properties shared by FSPs and GST are synaptic delay, reversal potential, conductance increase (Fig. 4.2F), block in low calcium – high magnesium solutions, and the picrotoxin block of several synapses including EX1-to-GM, and the LP-to-PD graded connections. Where tested, GST and FSPs have similar reversal potentials and the same sensitivity to blockers (D.M. Maynard and Walton 1975, Bidaut 1980, Graubard et al. 1983).

4.3.1 Input-Output Properties of Graded Transmission

4.3.1.1 Cells Studied

GST properties have been examined in detail for the cell pairs EX1-to-GM, LP-to-PD, PL-to-LP, and PD-to-LP, -PE, and -PL. All studies used TTX to block spikes and to eliminate membrane potential oscillations (this is not necessary for the EX1-to-GM connection, since EX1 cells apparently do not spike). These studies used presynaptic somatic depolarizations and examined the inhibitory postsynaptic responses as recorded from the soma. In the gastric mill system, substantial graded inhibition of the LPG (LG) neurons is evoked by subthreshold depolarization of the LG (LC) and MG (GP) neurons; the discrete IPSPs evoked by stronger stimulus sum with the graded inhibition (Mulloney and Selverston 1974a, Russell 1985b).

4.3.1.2 Waveform

When a presynaptic neuron is depolarized with a large constant current pulse, the postsynaptic neuron responds with a hyperpolarizing shift to a peak value which then decays to a maintained plateau (Fig. 4.2A, B; D.M. Maynard and Walton 1975, Graubard 1978, Graubard et al. 1980, 1983). There is a synaptic delay (Fig. 4.2C) which always exceeds the delay for a spike-evoked PSP between the same cell pair.

4.3.1.3 Release Threshold

There is an apparent threshold membrane potential for GST (Fig. 4.2D, H; Graubard 1978, Graubard et al. 1980, 1983). Below the threshold, depolarizations do not evoke transmitter release; above the threshold, both the peak and the maintained plateau augment with increasing presynaptic depolarization (Fig. 4.2D, H). The GST threshold has been measured for EX1-to-GM as ~47 mV, a value which is outside the normal range of EX1 somatic voltages in the isolated STG (Graubard 1978).
reduce transmitter release and so depolarize postsynaptic cells by disinhibition (Fig. 4.2E, H). Thus the "resting potential" of cells can be biased by tonic inhibition even in TTX preparations.

4.3.1.4 Rebound

Following the removal of hyperpolarization, many STG neurons rebound transiently to a more depolarized level than their antecedent resting potential (Fig. 4.2E, for a PD cell under TTX). Rebound in a presynaptic neuron can cause a wave of inhibition postsynaptically by evoking GST. The amplitude of the GST can be augmented by increasing the presynaptic overaholt (Graubard 1978).

Fig. 4.2 A–H. Graded transmission at pyloric synapses under TTX. A Current and voltage electrodes were placed in a presynaptic neuron while recording from a postsynaptic neuron; B Inhibition of a PF cell from depolarization of a PD cell; C initial peak was followed by a maintained plateau; C Faster sweep showing the delayed onset of inhibition; same calibrations as B; D The amplitude of both the peak and plateau inhibition were graded with the amplitude of presynaptic depolarization; E Hyperpolarization of the presynaptic (PF) cell caused a depolarization of the PD, indicating tonic release of transmitter at the resting potential under TTX; postsynaptic (LP) cell; constant-current pulses to the presynaptic (LP) cell; sweep duration was 4 s; G Somasial stimulation of the PD cell evoked an inverted, double-rectified response in the presynaptic PF cell; H Input-output curve; postsynaptic depolarization in a PD cell was plotted against peak postsynaptic hyoperpolarization in a LP cell (Modified from Graubard et al. 1980, 1983)

The threshold for GST is about 460mV for presynaptic PD and LP neurons (Table 4.1; Graubard et al. 1980, 1983). Under TTX, LP neurons have a more negative resting potential than 460 mV and so must be depolarized to produce GST. However, PD neurons often have a TTX resting potential above the threshold for GST and are capable of maintaining continuous inhibition of postsynaptic cells over the several-hours duration of a TTX-GST experiment. For these PD neurons, hyperpolarizations

Fig. 4.3 A–C. Use dependence of the peak, but not the plateau, components of graded synaptic transmission from EX1 to GM cells. A A short conditioning EX stimulus was followed at various intervals by a longer EX stimulus, recording inhibition of a GM cell, such a short conditioning pulse had little effect; B Similar data using a longer (200 ms) EX conditioning stimulus. The initial peak of the GM inhibition (open squares) was depressed at short C-T interval, whereas there was little effect on the amplitude of the maintained inhibition; C Effect of duration of the conditioning stimulus, for a constant C-T interval of 290 ms. Larger and longer conditioning stimuli caused a reduction in the peak amplitude of the test response. (From Graubard 1978)
4.3.1.5 Conditioning

Peak GST is use-dependent. When test presynaptic depolarizations are preceded by conditioning depolarizations, the peak (but not the maintained) GST is reduced as a function of the amplitude and duration of the conditioning pulse (Fig. 4.3; Graubard 1978). The peak response is readily reduced to the level of the maintained plateau unless the stimulus interval is many seconds.

4.3.1.6 Inferences from Input-Output Properties

A comparison of the membrane potential values during pyloric cycling, to the GST thresholds obtained in TTX (Table 4.1), indicates that PD neurons should release transmitter during virtually all of the pyloric cycle while LP neurons should release transmitter over the more depolarized half of their cycle. Thus a deeper oscillation trough could reduce or halt PD transmitter release during the trough, whereas the release from LP neurons would be less affected. However, the rebound data indicate that a deeper trough could lead to a rebound increase in GST during the next cycle depolarization.

The GST input-output curves suggest that a larger presynaptic depolarization should increase the maximal GST. However, the conditioning data indicate that sustained presynaptic depolarization (e.g., oscillation without much trough), or high frequency oscillations where the cycle peaks occur close together, could result in a reduction in maximal GST, from peak to plateau levels. During high frequency cycling, a very negative trough might help to maintain maximal GST (during cycle depolarization) by reducing any low-level conditioning effect during the trough and by activating rebound GST when the trough ends.

Table 4.1. Threshold for graded release compared to the membrane potential range of the oscillations in pyloric system neurons.

<table>
<thead>
<tr>
<th>Presynaptic neuron</th>
<th>Oscillation trough</th>
<th>Oscillation peak</th>
<th>Spike threshold</th>
<th>Resting potential threshold</th>
<th>Graded transmission threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD</td>
<td>58.6±2.2</td>
<td>47.0±2.9</td>
<td>49.6±2.3</td>
<td>55.9±2.4</td>
<td>58.7±4.4</td>
</tr>
<tr>
<td>LP</td>
<td>68.3±4.9</td>
<td>52.6±2.9</td>
<td>53.4±2.8</td>
<td>65.4±4.3</td>
<td>60.1±3.2</td>
</tr>
</tbody>
</table>

Values give the absolute membrane potential, in millivolts negative, as the mean ± S.D.; sample sizes were 5–6. Electrodes were placed in somata. Spike threshold was measured as V_m of the first spike during spontaneous bursts.

Excludes the inhibition from LP in measuring the PD oscillation amplitude. From Graubard et al. 1983)

4.3.2 GST and the Oscillation Cycle

4.3.2.1 Current-induced Cycling Under TTX

Injections of sinusoidal waveforms of current into a presynaptic neuron, in an otherwise quiescent preparation, can mimic the pyloric rhythm. The postsynaptic response is then almost entirely due to GST and, in most cell pairs, yields a waveform resembling a half-wave rectified, inverted version of the presynaptic stimulating waveform (Fig. 4.2G; Graubard et al. 1980).

4.3.2.2 Drug-induced Cycling Under TTX

Nonspiking oscillations in pyloric neurons have been achieved by bath application of dopamine (or other transmitters or precursors) + TTX to the isolated STG (Anderson 1980, 1986a, b, Anderson and Barker 1981, Raper 1979a and b). Bath application of TTX alone abolishes oscillations, but they resume upon addition of modulators. All the major cell types undergo slow depolarizations in the phase sequence of PD-LP-PL, resembling the typical in vitro pattern (Fig. 4.4A). Under these conditions, it can be difficult to estimate the relative contributions of GST, electrical coupling, and active membrane properties to the complex waveforms. For example, the oscillations in the AB pacemaker neuron are voltage-dependent: by taking advantage of the reduction of active membrane conductances at hyperpolarized voltages, Anderson (Fig. 4.4A) and Raper (1976b) have demonstrated that GST is responsible for almost all of the voltage oscillation in LP neurons under dopamine + TTX, since a mirror image oscillation is obtained when LP is hyperpolarized (Fig. 4.4B, B2). The nonpolarized waveform is flatter, probably due to shunting by K^+ conductances during the depolarized phase (Fig. 4.4B2). The waveform in PL neurons can also be inverted.

![Fig 4.4A Nonspiking pyloric oscillations under 50 μM dopamine and 0.2 μM TTX; intracellular records in an isolated STG; note that AB oscillations are later than those of the PD cell; B1 LP oscillations were inverted when the cell was hyperpolarized, and showed a reversal potential; see text; B2 Superposition of the nonpolarized LP waveform (solid line) and the waveform under hyperpolarizing current (dotted line) after inversion and normalization of amplitude; note the flattening of the nonpolarized waveform. Calibrations = A 20 mV, 1 s; B1 20 mV, 20 nA, 1 s; B2 10 mV, 0.5 s. (Modified from Anderson 1986a, b)
Reducing the graded inhibitory transmission from the LP cell, by hyperpolarizing it, results in a shallower trough in the PD oscillation (Fig. 4.4B1, dashed line), and also causes the positive-going wave in PL neurons to become steeper (dashed lines, PL trace). Reciprocally, the LP cell is known to be inhibited by PL as well as PD-AB cells, and indeed shows two phases of inhibition during nonspiking oscillations (vertical lines "1", Fig. 4.4B1) corresponding to the peaks of the PL and PD-AB waves respectively. Both inhibitory components can be inverted ("2"), and show an equilibrium potential ("3" and "4"). Anderson (1986b) has suggested that the total amount of inhibition between pyrlic cells remains about the same after blockade of discrete IPSPs by TTX, apparently due to the IPSPs being relatively small compared to the graded inhibition, along with the abolition of a depolarizing component of what appear to be biphasic IPSPs at certain synapses.

4.3.2.3 Focal TTX Block

Raper (1979a), by applying TTX locally to the major output nerve leaving the ganglion, was able to block most of the spike activity in the ganglion without blocking plateau potentials or cycling (Fig. 4.5). Elimination of most of the spikes had little effect on the overall oscillation pattern, suggesting that GST was adequate to maintain cycle phases in the absence of spike-augmented synaptic transmission.

Fig. 4.5.A: Nonspiking pyrlic rhythm from local perfusion of TTX over the motor nerve axons at the caudal end of the STG; B1 Typical rhythm following activation by stimulation of the stomatogastric nerve; intracellular records in an isolated STG; B2 Same after TTX perfusion to abolish motor spikes; C: Impulse generation failed during the course of a plateau potential in the LP cell, allowing comparison of a spike-mediated IPSP and the plateau-evoked graded inhibition of the PD cell. Calibrations = B 10 mV, 0.5 s; C 10 mV, 1 s. (Modified from Raper 1979a)

4.3.2.4 Intact Spiking Ganglia

It is possible to demonstrate GST effects without using TTX, although the relative importance of GST and PSPs cannot be readily assessed. Graded depolarizations of EXI cells cause GST that can reduce or eliminate GM spike activity in preparations with active gastric cycling (Graubard 1978). However, the role of GST in the pyrlic system during weak-to-moderate cycling has been examined for the LP and PD neurons (Graubard et al. 1980, 1983). When the LP cell is not firing, depolarizing stimuli that straddle its spike threshold can be used to demonstrate significant GST responses on other cells. PD neurons can be demonstrated to release transmitter during the trough of their cycle by giving a hyperpolarizing stimulus and thus disinhibiting postsynaptic neurons (Fig. 4.6).

4.3.3 Conclusion

During cycling, the effectiveness of GST will be influenced by the voltage levels of the oscillations, as well as their frequency. All of these are known to be affected by neuromodulators. Thus it is likely that synaptic strengths for GST will not remain fixed during different motor patterns. Finally, the strengths of GST and of spike-evoked PSPs need not change in parallel.

4.4 Plateau Potentials

Current-injection tests have demonstrated that many STG cells undergo slow regenerative depolarizations whose expression is synaptically unmasked by inputs from the CNS (Russell and Hartline 1977, 1978, 1981, 1982, 1984, Hartline and Russell 1978, 1984, Hartline 1979, Anderson and Barker 1981, Moulais and Council 1982, Wiens 1982, Dickinson and Nagy 1983, Moulais and Nagy 1983, Anderson 1986a). The term "plateau potential" is per se simply descriptive, although it is often used to denote the prolonged depolarized phase of other slow action potentials, e.g., in heart muscle. Plateaus appear to derive from a persistent negative resistance characteristic (Noble 1966). Similar potentials have been observed in neurons of the crustacean cardiac ganglion (Tazaki and Cooke 1979a) and in several types of mammalian CNS neurons (Llinda and Sugimori 1980, Schwindt and Crill 1980, D"schenes et al. 1984).
4.4.1 Criteria for Regenerative Plateaus

Current-injection tests for plateaus derive from early studies on axons and vertebrate cardiac muscle (Weidmann 1951). Plateaus can be suppressed by steady hyperpolarization, triggered by a brief depolarization, are stimulus-independent once started, display a threshold and metastability near threshold, give rise to a discontinuous \( I-V \) curve, and can be terminated in a threshold-dependent manner by brief hyperpolarizing stimuli; there is also a decreased input resistance during a plateau (Fig. 4.7; Russell and Hartline 1978, 1982, 1984, Gola and Selverston 1981, Hartline and Russell 1984, Dickinson and Nagy 1983, Anderson 1986a). The combination of a hyperpolarizing offset to suppress bursting and a depolarizing stimulus to trigger a response is effective for demonstrating regenerative plateaus during ongoing rhythms. The ability to trigger or terminate plateau responses at different phases of the rhythm cycle, and their continuation when presynaptic cells are silenced by hyperpolarization, or when inhibitory synapses are blocked with picrotoxin, is evidence that plateau behavior can be independent of synaptic network interactions.

4.4.2 Cell Types Exhibiting Plateaus

All the cell types of the pyloric system produce regenerative plateaus by the above criteria (Russell and Hartline 1982). Among the gastric-system neurons, plateaus have been demonstrated during ongoing rhythms in the AM, DG (CP), and MG (GP) neurons (Figs. 4.7F, 11, 14), in the LG (LC) and Int 1 (CI) neurons when strongly activated, but not in the GM neurons (Russell and Hartline 1984). Thus the neurons of the gastric system appear more heterogeneous than those of the pyloric system, although the negative results on the GM cells may be methodological in origin since they produce plateaus under Ba\(^{2+}\) (Russell and Hartline 1984; see Fig. 4.8).

4.4.3 Functional Roles of Regenerative Plateaus

The plateau mechanism drives repetitive spiking during bursts. It is also likely to amplify synaptic input, evoke graded release of transmitter, and sharpen transitions as the plateau mechanism turns on or off regeneratively (Russell and Hartline 1978, 1982, 1984, Hartline 1979, Dickinson and Nagy 1983). A spectrum of interactions between plateaus and synaptic potentials are seen in different cells. For example,
plateaus in the VD pyloric cell can be triggered much earlier in the cycle than they normally commence (Fig. 4.9), indicating that the burst timing of VD is set by a rather delicate interaction of synaptic and intrinsic mechanisms. Plateaus in the DG (CP) gastric cell appear to amplify the periodic synaptic depolarizations that are revealed when the cell is hyperpolarized (Fig. 4.7F). The AB-to-LP and AB-to-PL inhibition seen in Fig. 4.4A during nonspiking oscillations (Anderson 1986b), and the graded LP-to-PD inhibition in Fig. 4.5C (Raper 1979a), represent GST evoked by plateau depolarizations in the presynaptic cells. The 10–40 mV regenerative plateau no doubt drive GST during normal cycling, as well-matched cellular and synaptic mechanisms.

4.5 Synaptic Modulation of Neuronal Properties

It has been known for some time that stimulation of the stomatogastric (input) nerve produces a long-lived activation of the pyloric rhythm (Hartline and D.M. Maynard 1975). Both the pyloric and gastric rhythms are active if the STG is left attached to the CNS commissural ganglia, but removal of the latter usually causes the gastric rhythm to stop and the pyloric rhythm to decay (Selverston et al. 1976, Russell 1975a, 1976a, 1977, 1979, Anderson 1980, 1986a, Anderson and Barker 1981, Moulins and Coulrin 1982, Moulins and Nagy 1983, Hartline and Russell 1984, Russell and Hartline 1984, Russell 1985a). We now know that synaptic and possibly hormonal modulation of cellular properties is an important class of mechanisms contributing to the extrinsic control of the pattern generators. Analogies can be drawn to the action of norepinephrine on vertebrate cardiac muscle (Reuter and Scholz 1977), modulation of smooth muscle properties (Bolton 1979), transmitter control of action potential currents (Dunlap and Fischbach 1977, Hoer and McAfee 1980), and the modulation of other bursting neural rhythms (Watanabe et al. 1969, Sullivan and M.W. Miller 1984, W.B. Adams and Benson 1985). Attempts to study the cellular mechanisms of modulation are reviewed here; other investigators have used transmitter application and photoactivation at a different level of analysis (Chap. 9).

4.5.1 Synaptic Induction of Regenerative Plateaus

This is one, but probably not the only, mechanism contributing to activation of the rhythm. Supporting evidence includes the correlation between bursting and the ability to evoke plateau responses in experiments of stimulating or blocking the input nerves (Russell and Hartline 1977, 1978, 1981, 1984, Hartline 1979, Hartline and Russell 1978, 1984, Moulins and Coulrin 1982, Dickinson and Nagy 1983, Moulins and Nagy 1983, Anderson 1986a). The correlation is rather good for neurons in the pyloric system since, e.g., cutting the input nerve abolishes in parallel the bursting and plateau responses of most neurons (Fig. 4.10), although the PD-AB pacemaker neurons may continue bursting. The correlation is less complete for the gastric system. Gross stimulation of the stomatogastric nerve usually does not drive gastric rhythms (Hartline and D.M. Maynard 1975), yet can induce plateaus in
4.5.2 Plateau Induction by Identified Inputs

A few identified inputs have been found which, when selectively stimulated, synaptically induce the plateau property of specific STG cells.

4.5.2.1 Dopaminergic Inputs

Fluorescence histochemistry reveals two axons (or tracts) entering the STG from the CNS (Kushner and E.A. Maynard 1977). Their cells of origin appear to be in the commissural ganglia (Kushner 1979a, b). Dopamine is synthesized and released in the STG (D.L. Barker et al. 1979, Kushner and Barker 1983). Dopamine precursors exert physiological actions similar to those of bath-applied dopamine, namely activation of bursting in the AB, LP, and PL neurons of the pyloric system (Fig. 4.4), and the appearance of voltage-dependent potentials in the AB neuron (Anderson 1980 1986a, Anderson and Barker 1981). An interesting finding was that the PD ("pacemaker") neurons are much less activated than AB (Fig. 4.4A). Similarly, dopamine has little effect on the pyloric VS, PE, or IC neurons or the gastric neurons.

4.5.2.2 APM

Intracellular stimulation of a neuron in the oesophageal ganglion termed APM (anterior pyloric modulator) drives bursting in the pyloric system and induces the plateau property of all its neurons (Nagy and Dickinson 1983, Dickinson and Nagy 1983, see Chap. 8). The most extreme effects are seen when the pyloric rhythm has decayed and then APM is fired (Fig. 4.12). APM does not normally fire in combined preparations, and hence the spontaneous pyloric activation and plateau induction are due to other inputs. Similar effects are obtained by bath application of muscarinic agonists (Dickinson and Nagy 1983, Anderson 1980, 1986a).

4.5.2.3 Multiaxial Synapses from inn TF

These two neurons have somata in the "brain" (Claiborne and Seiverston 1984a), have axons in the inferior ventricular nerve which continue to the STG (Dando and Seiverston 1972), use histamine as a transmitter, evoke C1 dependent inhibition in several STG cells (Claiborne and Seiverston 1984b), and exert additional synaptic effects (Sigvardt and Muloney 1982b). Selective stimulation of the inn TF axons evokes...
EPSFs and increases the amplitude and frequency of bursting in the PD and AB pacemaker neurons, correlated with an augmentation of their plateau responses (Fig. 4.13). The EPSF can be blocked with curare yet the plateau induction remains (Russell and Hartline 1981). This, and its other synaptic effects (Sigvardt and Muloney 1982a), indicate that the iv TF make multiaction synapses onto the PD-cells, resembling in principle the multiaction synapses in other species (Kohoe 1972). The plateau induction appears to be specific for the PD-AB cells since it was not demonstrable in other pyloric cells such as VD. How widespread are multiaction synapses in the STG system is not known.

4.6 Pacemaker Neurons

It used to be thought that among the STG neurons, only the PD and AB cells possessed pacemaker properties for endogenous repetitive bursting. This conclusion has been revised in several respects.

4.6.1 Conditional Bursters

It can be debated whether bursting is "endogenous" if it is conditioned by modulatory input from the CNS. After isolating the STG, the PD-AB group reportedly stops bursting in certain species (e.g., crayfish: Hermann 1981; Appendix B, Chap. 5; Homarus: Moulins and Cournil 1982), behaving as "conditional bursters". The continued bursting in other species, e.g. Penaeus interruptus and argus (D. M. Maynard and Selverston 1975, Hartline and D.M. Maynard 1975, Russell 1979), might still be conditioned by spontaneous (MEFP-like) release of modulatory transmitters from input fibers (Krashen and Barker 1983, Anderson 1986a; see Appendix B, Chap. 5, for further discussion). This latter point is potentially applicable to other systems, e.g., slice preparations, in which spontaneous release of transmitters might steadily modulate neuronal properties. Observed bursting may not be an adequate assay for deciding whether or not the underlying conductances decay to zero after spinal isolation since bursting derives from complex interactions of several mechanisms, incompletely understood.

4.6.2 AB Cell

This cell can burst without the PDS, but the PD cells apparently do not burst repetitively unless AB is active, as shown by selective photostimulation of neurons (J.P. Miller and Selverston 1982a), or by bath application of dopamine and TTX (Anderson and Barker 1981, Anderson 1986a).
4.6.3 LP Cell

This cell and perhaps other pyloric “follower” neurons can burst endogenously after the PD-AB “pacemaker” group is photoactivated (J.P. Miller and Selverston 1982a) or hyperpolarized (Russell and Hartline 1978). Such bursting disappears in the isolated STG and hence is conditioned by inputs.

4.6.4 DG (CP) Cell

The DG cell of the gastric mill system can burst endogenously and independently when modulatory inputs are stimulated, or when octopamine (10^{-8} M) is applied to the bath, provided that entraining synaptic barrage from other gastric cells are absent (Russell and Hartline 1978, Hartline and Russell 1984, Wadepuhl and Selverston 1984). This sometimes occurs spontaneously in the isolated STG (Fig. 4.14). Criteria for endogenous bursting included the reset behavior, slower bursting when DG (CP) was hyperpolarized, and the absence of synchronized bursting in presynaptic cells. However, the DG (CP) cell is strongly entrained and behaves much like a follower cell when the entire gastric network is bursting (Fig. 4.7F).

1. ENDOGENOUS BURSTING

2. TRIGGERED BURST

3. TERMINATED BURST

Fig. 4.14. Spontaneous endogenous bursting in the DG (CP) gastric cell after sucrose blockade of the input nerve; lower traces show that other gastric neurons fired tonically; a hyperpolarizing offset current was applied. Upper control. Middle, Lower brief depolarizing or hyperpolarizing stimulus could trigger or terminate bursts to reset the DG (CP) burst timing, as evidence that it was endogenously generated. (Modified from Hartline and Russell 1984)

4.7 Analysis of Membrane Currents

4.7.1 Pyloric Pacemaker Neurons

Gola and Selverston (1981) employed voltage clamp and ion substitution techniques to analyze the endogenous oscillations in the PD-AB group. An increase in input resistance was observed during the interburst interval (Fig. 4.15) as in molluscan pacemakers. A slow wave of inward current was observed when clamping a single PD soma (i.e., the pacemaker was not completely clamped), with fast inward spikes superimposed. They reported that V_m during the postburst hyperpolarization behaved as a K^+ electrode but was not reduced by tetraethylammonium. TTX blocked peripheral spikes at 1–5× 10^{-9} M, leaving slow waves without spikes; the slow waves stopped under 10^{-7} M TTX. The cells tended to depolarize and fire tonically in zero Ca^2+ saline, although bursting resumed if hyperpolarizing offsets were applied. Co^{2+} or Mn^{2+} had effects similar to zero Ca^2+ saline. Replacement
of Ca\(^{2+}\) with Ba\(^{2+}\) gave large TTX-insensitive slow waves; the PD cells appeared to become uncoupled and could burst independently. A model was proposed in which the burst depolarization would be due to entry of Na\(^{+}\) and Ca\(^{2+}\), a TEA-sensitive K\(^{+}\) conductance would build up during the burst, a Ca\(^{2+}\) activated K\(^{+}\) conductance would produce the postburst hyperpolarization, and its decay would produce the pacemaker potential during the interburst interval (Fig. 4.16). This model resembles certain models for molluscan endogenous bursters (reviewed in W.B. Adams and Benson 1985).

4.7.2 Inward Current

Regenerative plateaus may involve a voltage-dependent slow inward current; the alternative of an inward leak and a depolarization-reduced outward current seems less likely. Both Na\(^{+}\) and Ca\(^{2+}\) dependent plateau potentials have been found in other systems (Eckert and Lux 1976, Llinas and Sugimori 1980, Schwindt and Crill 1980, Stafstrom et al. 1982). A slow inward Ca\(^{2+}\) current has been demonstrated in neurons of the crustacean cardiac ganglion (Tazaki and Cooke 1977).

Grubard and Ross (1985) reported using intracellular injection of the calcium indicator dye arsenazo III and a photodiode array and compound microscope to monitor Ca\(^{2+}\) entry. Depolarization of the somata of gastric or pyloric neurons to fire spikes (by injected current) evokes Ca\(^{2+}\) entry apparently throughout the cell, including the soma and fine neurite processes but not the axon (Fig. 4.17).

During pyloric cycling, the burst depolarizations in the VD or LP neurons are associated with Ca\(^{2+}\) transients in the neuropil, but no Ca\(^{2+}\) entry is observed in the soma.

4.7.3 Outward Current

Recent voltage clamp studies by Grubard and Hartline (1984) and Hartline et al. (1985) have characterized at least three types of outward currents in intact or ligatured pyloric somata. There was little sign of inward current in isolated somata. The outward currents include a fast-inactivating K\(^{+}\)-dependent current resembling molluscan I_{A} (Connor and Stevens 1971a), a nonactivating current, I_{g}, and a calcium-dependent inactivating current, I_{H}. Hartline (1979) demonstrated a "delaying conductance" in certain pyloric neurons (Fig. 4.18) and suggested that it resembled the molluscan I_{A} and may contribute to the phasing of the pyloric burst pattern.

4.7.4 Modulation by Transmitters

Modulatory transmitters that appear to "induce" plateaus could act either by enhancing an inward current or reducing an outward current, the latter being the case in certain molluscan neurons (Klein and Randel 1980, Paupardin-Tritsch et al. 1985).
A parallel case has recently been reported for the activation of pyloric bursting by oxytocin, a muscarinic agonist (Dickinson and Nagy 1983). Nagy et al. (1985) report that muscarinic agonists act to reduce a "subthreshold" K⁺ current to permit the activation of voltage-dependent Ca²⁺ current, citing that oxytocin still evokes a depolarization under TTX plus Mn²⁺ or Cd²⁺, and that intracellular injection of TEA into PD-AB neurons mimics the action of oxytocin.

Dopamine is reported to activate voltage-dependent conductances in the AB pacemaker neuron (Anderson 1980; Anderson and Barker 1981, Anderson 1986a). The larger oscillations under 50-200 µM dopamine are abolished by hyperpolarizing offset currents, and are accompanied by a decrease in the input resistance as estimated by decreased voltage coupling to the PD or VD cells or by decreased amplitude of conventional PSPs.

Hermann (1981) reported that 10-30 mM caffeine can activate pyloric bursting transiently and increase the permeability of PD neurons to Na⁺, possibly due to a direct membrane effect.

4.7.5 Implications of Modulation for Studies on Ionic Mechanisms

Transmitter regulation of membrane currents raises certain problems for designing experiments to study their ionic basis. Substitution of an ion in the bath could alter a given membrane current by affecting either the state of modulation, or the ionic gradient, or both. For example, reduced Ca²⁺ in the bath might cause a decreased plateau current in a STG neuron by blocking the synaptic release of modulatory transmitters from input fibers. Both application of drugs may be more tractable to study, yet examples can be found of receptor binding studies in which the binding of a transmitter or peptide was found to be ion-dependent (Childers 1980, Hulme et al. 1980). The ability of Mg²⁺ to make the receptors to certain excitatory amino acids appear voltage-dependent (Nowak et al. 1984, Mayer and Westbrook 1985) may be a further complication for the analysis of ionic mechanisms.

4.8 Conclusions

Our understanding of the STG system has evolved considerably since the mid-1970's when the literature was previously reviewed. At that time, most studies had used isolated STG preparations, only the PD neurons were thought to possess special cellular properties related to bursting, and the operation of the gastric and pyloric oscillators tended to be thought of in terms of PSP-mediated synaptic interactions. The roles in pattern generation of graded synaptic transmission, qualitative and quantitative variation of cellular properties among the different identified cell types, and synaptic modulation of cellular properties, are more accepted at this date.

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Appendix: Ionic Basis of Pacemaker Activity in Stomachogastic Neurons

A. HERMANN and M. WADEPUHL

The study of mechanisms underlying the spontaneous activity of nerve cells and the modification of their activity by biogenic amines, peptides or other hormones is of particular interest to neurobiologists because these cells may constitute important elements in the nervous system. Two major types of rhythmically active "pacemaker neurons" are generally found: (1) bursting cells, where periods of discharge of action potentials interchange with silent periods and, (2) beating cells, which generate action potentials repetitively and continuously. The term pacemaker has been adopted as a functional term from cardiac tissue, where regular cycles of electrical activity in some cells command the "pace", i.e., the discharge frequency of other cells in the tissue. It is used now in a more general, mechanistic sense comprising all types of cells that produce spontaneous activity in the absence of external synaptic or humoral input (Carpenter 1982). Cells which exhibit rhythmic activity only after being appropriately activated have been termed "conditional pacemakers" (Selverston et al. 1983). Most bursting cells in the stomachogastic ganglion appear to be conditional pacemakers where the external stimulus exerts a de- or hyperpolarizing shift of the membrane potential to turn on cyclic conductance changes. Presently it is not known, however, which of the membrane properties compared to spontaneous pacemaker cells are altered.

In this section we compare available data on the ionic mechanism of pacemaker activity of stomachogastic neurons and of molluscan nerve cells. The comparison to molluscan cells appears well suited because of similarities in the wave-form of the discharge activity and because evidence suggests similar underlying ionic mechanisms.

Localization of the Pacemaker Site. From the about 15 neurons driving the pyloric filter apparatus in decapod crustaceans, three cells (2 pyloric dilators, PD; 1 anterior burster, AB) are the most conspicuous bursting pacemakers although most of the other pyloric cells can burst as well. Intracellular recordings from the somata shows bursting activity with typically nonoverlapping action potentials and underlying slow membrane potential fluctuations. Experiments to isolate the soma in order to demonstrate pacemaker activity as a property of a single cell (as previously shown for molluscan neurons; Alving 1968) are not feasible in stomachogastic neurons. The reason is that the site where the potential oscillations are generated is located at some distance from the soma within the neuropil. This also prevents the use of voltage clamp techniques to study the underlying currents because it is difficult to keep the entire cell under quiescent conditions. In other words, although the soma may be under proper voltage control, the site of pacemaker activity is not and therefore oscillating voltage fluctuations produced at this site will be superimposed on the voltage recorded from the soma.

Electrical Characteristics of Pacemaker Cells. Pyloric dilator and anterior burster cells generate regular bursts of action potentials with a frequency of about 1 Hz. The oscillating activity comprises a slow depolarization of the membrane potential, which eventually gives rise to a burst of action potentials which usually last 100-
200 ms. The action potentials are generally discharged in a parabolic manner with a frequency of 20–30 Hz and are followed by a postburst hyperpolarization of 10–20 mV. The slow oscillations of the membrane potential are accompanied by concurrent changes of the input resistance. Rectangular current pulses injected shortly after termination of the burst reveal low amplitude membrane voltage responses with a fast time constant of rise and fall. The amplitude of the membrane voltage responses increases during the interburst phase and their time constants are slowed. Furthermore, excitatory postsynaptic potentials elicited shortly after the burst are depressed in amplitude but increased during the interburst phase (Hermann 1979b; Fig. 4.19). Both of these findings have been interpreted previously as indicating a high conductance state of the membrane during the postburst hyperpolarization, followed by a progressive conductance decrease throughout the interburst period (Hermann 1979, Gola and Silverston 1981).

However, this is not the only interpretation of the data. It is possible that the postburst hyperpolarization is the result of a steady conductance that is turned off during the burst and the postburst hyperpolarization, but is slowly turned on during the interburst phase (W.B. Adams and Levine 1985, Kramer and Zucker 1985 a and b). This means that if the hyperpolarizing current pulse is applied in the presence of a voltage sensitive, persistent inward current, the pulse deactivates some current causing a regenerative membrane hyperpolarization. Membrane voltage responses caused by pulsed current injections therefore indicate a low conductance state during the postburst hyperpolarization, while they reflect a high conductance state during the interburst phase. This is exactly opposite to the above interpretation.

It implies that membrane conductance measurements by application of brief current pulses are no longer interpretable because some voltage-dependent membrane conductance may be turned on or off by the imposed current pulse. A noninvasive technique to measure membrane conductance is necessary to decide whether it increases or decreases during the various phases of the slow wave.

**Ionic Mechanism of Bursting Activity.** For reasons outlined earlier, the analysis of the ionic mechanism underlying the generation of burst activity in stomatogastric neurons has been limited to membrane potential measurements and experiments involving changes in ionic composition or application of pharmacological agents.

Reducing the Na⁺ current by the reduction of the external Na⁺ concentration abolished bursting (Fig. 4.20A); slow waves however, could be still initiated. Bursting was not prevented by application of low doses of tetrodotoxin (TTX; 1 nM). The hyperpolarization of the resting membrane potential induced by higher concentrations of TTX (10 µM) or by zero Na⁺-saline may not be specific but could be explained by an increase of the leakage current induced by TTX in high doses or by inhibition of the Na⁺/Ca²⁺ exchange (Dipolo and Bezou 1983). By this means the internal, free Ca²⁺ concentration could have been increased, activating a K⁺ conductance.

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**Fig. 4.19A, B.** Conductance changes indicated by constant current pulses (A) or by EPSP amplitudes elicited by stimulation of the stomatogastric nerve (B); 5-mm membrane resistance. A: Panulirus (modified after Gola and Silverston 1981); B: Cancer pagurus (Hermann 1979b).

**Fig. 4.20A, B.** Effects of ion substitution on burst activity. A: Reduction of Na⁺ ions (replaced by TRIS) does not completely abolish bursting, even in saline without Na⁺ ions a subthreshold slow wave can be initiated by current injection; B: Exposure to Ca²⁺-free saline (Ca²⁺ ions replaced by Mg²⁺ ions) transiently prolongs slow waves and causes repetitive discharge of action potentials. Bursting activity is reestablished after hyperpolarizing current injection. A and B from Panulirus (Gola and Silverston 1981).
which causes the hyperpolarization of the membrane potential. It is also possible that bursting was eliminated by blocking the synaptic input onto these cells (cf. Selverston et al. 1983). Recently, Nagy et al. (1985) found that pyridic activity in the cape lobster, <i>Junxus laevis</i>, is blocked by intermediate concentrations of TTX (0.1 μM). However, they were able to restore rhythmic activity by applying the muscarinic agonist oxotremorin. The oxotremorin effect was abolished in a solution containing the Ca²⁺ antagonist Mn²⁺. This supports the view that Na⁺ ions are not the main carrier of the inward current during the depolarizing phase of the slow wave under these conditions.

In normally Ca⁺⁺-free saline (Ca⁺⁺ ions replaced by Mn⁺⁺ ions) the cells depolarized 5 to 10 mV and bursting activity was changed to repetitive discharge activity (Fig. 4.20B). The injection of inward current in some cases restores bursting activity with an increased duration of the slow wave and a loss of the postburst hyperpolarization (Gola and Selverston 1981). If the inorganic Ca²⁺ antagonists, Co²⁺ or La³⁺ were added to a Ca⁺⁺-containing solution, the slow potential waves were increased in amplitude (up to 30–50 mV) and their duration was prolonged (Hermann 1979b, Gola and Selverston 1981). Eventually, the membrane potential remained depolarized after 15 to 30 min. However, if the Ca²⁺ antagonists were added to a Ca⁺⁺-free solution, bursting activity was quickly changed within 10 to 30s to a steady depolarization and the cells fired repetitively (Fig. 4.21). The data suggests that Ca⁺⁺ ions are a main source of charge carrier during the slow potential wave.

A change of the extracellular concentration of K⁺ ions causes an alteration of the amplitude of the postburst hyperpolarization as expected from the Nernst relation: the amplitude of the slow waves is increased by lowering the external K⁺ concentration and is decreased by increasing the external K⁺ concentration (Fig. 4.22A). A plot of the amplitude of the postburst hyperpolarization versus the external K⁺ concentration which provides an estimate of the Na⁺/K⁺ permeability indicates that during this phase the membrane becomes highly permeable to K⁺ ions (Gola and Selverston 1981).

External tetraethylammonium (TEA), which blocks K⁺ conductance in a variety of tissues (Stanfield 1983), increased the amplitude of the slow waves at a relatively low concentration of 1–2 mM but did not reduce the postburst hyperpolarization, even at higher TEA concentrations (Fig. 4.22A, B). From these data it has been concluded that (a) the K⁺ conductance involved in the postburst hyperpolarization in TEA insensitive (cf. Detmer and Eckert 1985) and (b) the increase in the amplitude of the slow wave is brought about by the blockade of a K⁺ conductance that is active during the depolarizing phase of the slow wave (Gola and Selverston 1981). As the increase of the amplitude of the slow wave appears rather sensitive to external TEA, it is possible that a Ca⁺⁺-activated K⁺ conductance (with a Kₒ of 0.4 mM in molluscan neurons; Hermann and Gorman 1981) contributes to the shaping of the slow wave.

These findings and their interpretations may also help to explain the effects of various divalent and trivalent cations on the generation of slow potential oscillations (see above). If the Ca⁺⁺ antagonists were added to a solution containing Ca⁺⁺ ions, the block of Ca⁺⁺ influx into the cells is not immediate and not complete. There are enough Ca⁺⁺ ions available as charge carriers to cause the membrane to depolarize but because of the greatly reduced influx of Ca⁺⁺ ions their rate of intracellular accumulation is reduced and accordingly less Ca⁺⁺-dependent K⁺ current is activated to repolarize the membrane potential. After prolonged depolarization and firing of action potentials, however, the internal, free Ca⁺⁺ concentration rises and eventually activates the K⁺ conductance to terminate the burst. If the Ca⁺⁺ antagonists were added to a Ca⁺⁺-free solution, a sustained depolarization develops more rapidly, since the Ca⁺⁺-activated K⁺ conductance is not activated (Hermann 1979b). Similar effects were obtained when methylated xanthines (i.e., caffeine) were added to Ca⁺⁺-containing or Ca⁺⁺-free solution (Hermann 1981).

The apparent TEA insensitivity of the postburst hyperpolarization may also be explained by a voltage-dependent block of K⁺ channels (cf. Hermann and Gorman 1981). The massive Ca⁺⁺ influx during the slow waves with their prolonged and positive state of membrane potential could reduce the effect of the blocking agent. The amount of Ca⁺⁺-activated K⁺ conductance necessary to cause the postburst hyper-
polarization may be small and, in addition, the effect on the membrane potential could be increased because of a higher input resistance of the cell in TEA solution.

The ionic mechanism for the generation of slow waves may be altered under various ionic conditions or after application of drugs or biogenic agents. A clue to this view is the conversion of bursting pacemaker activity to a type of plateauing discharge activity after TEA application (cf. Fig. 4.22C). In TEA solution the Na⁺ dependence of the slow wave is increased compared to that in normal saline (Fig. 4.23). This indicates that either the ionic mechanism for the generation of slow waves in TEA solution is altered or that the "slow wave" in TEA solution actually is a greatly prolonged action potential. It is possible that under these conditions the soma membrane becomes regenerative and the action potentials on top of the slow wave, being generated at a distant site, passively invade the soma. The hyperpolarizing phase of the slow wave in TEA solution hence may actually be the repolarizing phase of a prolonged action potential. This would also explain the modest sensitivity of the hyperpolarizing phase in TEA solution, since the voltage-dependent, delayed K⁺ conductance, responsible for the repolarization of action potentials is less TEA sensitive (with a Kd of 6.0 mM in molluscan neurons; Hermann and Gorman 1981) and processes like Na⁺ and Ca²⁺ inactivation may contribute to the repolarization. Hence the "phenomenon slow wave" may have a similar phenotype under various conditions but the underlying membrane conductances may change.

Models of Bursting Pacemaker Activity. On the basis of data obtained from molluscan neurons various models for the generation of bursting pacemaker activity have been developed. In the model of Gorman et al. (1981, 1982) the slow depolarization of the membrane potential in the pacemaker cycle is predominantly brought about by a slow inward Ca²⁺ current. This current shows voltage-dependent activation at relative negative membrane potentials (about −50 mV) and is slowly inactivating. The influx of Ca²⁺ ions during a burst increases the internal, free Ca²⁺ ions concentration, which initiates a Ca²⁺-dependent outward K⁺ current. The increase of the Ca²⁺-activated K⁺ current has been suggested to terminate the burst and to cause the postburst hyperpolarization. As the Ca²⁺-activated K⁺ conductance is voltage-dependent, which results in an increased activation of the current at depolarized potentials, this may further contribute to the features of burst termination (Hermann and Hartung 1982). Due to the negative potential during the postburst hyperpolarization, the voltage-dependent inward Ca²⁺ current is inactivated, which allows for the sequestration and extrusion of Ca²⁺ ions to reduce the internal, free Ca²⁺ ion concentration. By this means the Ca²⁺-activated K⁺ conductance is decreased, which causes the membrane potential to depolarize. Eventually, the voltage-dependent Ca²⁺ inward current is reactivated which will further depolarize the cell and initiate the following burst, and this way completes the cycle.

The model of bursting pacemaker activity as proposed by W.B. Adams and Levitan (1985), comprises a slow inward current component, carried by Ca²⁺ ions and in addition a nonspecific cation current, which initiates the depolarizing phase of the pacemaker cycle. The negative feedback to restore the membrane potential, i.e., the postburst hyperpolarization, is provided by a Ca²⁺-dependent inactivation of a persistent Ca²⁺ inward current. The Ca²⁺-activated K⁺ current plays no role in this model.

The model proposed by Kramer and Zucker (1985b) is basically similar to the model suggested by Adams and Levitan, but ascribes some role to the Ca²⁺-activated K⁺ conductance in causing the repolarization of action potentials. Recent data suggest that there may be some truth in all three of the hypothesis presented above. Experiments with charybotoxin (CTX), a scorpion venom, which specifically blocks the Ca²⁺-activated K⁺ conductance in molluscan neurons (Hermann 1985), have shown that the postburst hyperpolarization is somewhat diminished, but not blocked, the number of action potentials per burst is increased, and the last action potentials in the burst are prolonged in their duration. The effect of CTX on the duration of action potentials was more obvious if voltage-dependent K⁺ currents were blocked by 4-aminoypyridine (Hermann, unpublished). From these experiments it appears that the Ca²⁺-activated K⁺ conductance contributes to the repolarization of the action potential during a burst and to the postburst hyperpolarization, although it is not the sole factor in terminating the burst.

The data available for stomatogastric cells producing slow-bursting pacemaker potentials do not permit to decide which of these models applies to crustacean pacemaker cells. They suggest, however, that Ca²⁺ ions are a main carrier of inward current causing the depolarization of the slow wave. The K⁺ dependence of the postburst hyperpolarization supports the idea that the initiation of a Ca²⁺-activated K⁺ current causes the repolarizing phase. This view is contradicted by the apparent TEA insensitivity of the postburst hyperpolarization, although several arguments can be made to reconcile this fact with a K⁺ dependence of the burst termination. The use of newly developed drugs and toxins or voltage clamp of cultured cells may help to clarify some of the questions.

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