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INTRINSIC ELECTROPHYSIOLOGICAL REGULATION OF FIRING PATTERNS OF BURSTING NEURONS IN APLYSIA

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INTRODUCTION

Endogenous generation of action potential bursts occurs commonly among peptidergic neurosecretory cells (Barker and Gainer, 1975; Gähwiler and Dreifuss, 1977; Cooke, 1982; Andrew and Dudek, 1984). Burst generation has also been observed in more typical central neurons (Miller and Selverston, 1982; Grace and Bunney, 1984; Legendre et al., 1985), indicating that it is a property of many kinds of neurons. It is easily imagined that burst generation functions to enhance transmitter release, which by synaptic facilitation may be particularly sensitive to spike frequency, but it has been difficult to prove this point (Dutton and Dyball, 1979). Bursting neurons have also been implicated in the organization of rhythmic motor patterns (Miller and Selverston, 1982).

MEMBRANE CURRENTS REGULATING BURSTING

The origin of repeated spike bursts in a neuron isolated from all synaptic input has intrigued neurobiologists for a long time. The most extensive studies of this process have been on molluscan neurons, particularly cells R15 and L2-L6 in the abdominal ganglion of Aplysia californica. Many voltage-clamp studies (reviewed in Adams and Benson, 1985) have revealed a complex array of membrane currents which are involved in generating spike bursts. These currents flow through different channels from the usual voltage-dependent sodium and potassium channels involved in shaping each action potential. I shall summarize the most important of these currents involved in burst generation in Aplysia, and the evidence for our interpretation of the responsible ionic mechanisms.

The Pacemaker Current

Bursting neurons are endowed with an inward current that is partly activated at rest (i.e., at potentials prevalent in the interval between bursts). Although not a strong current, it is nevertheless the dominant current at potentials near -40 mV, leading to a negative slope in the steady-state current-voltage relation of bursting neurons. It is often called a "negative slope resistance current." As the dominant current leading to a weak net inward current, it slowly depolarizes cells until they reach spike threshold and begin to fire a burst. It is often referred to as the "slow inward current," not because its kinetics are slow, but because its effect on charging the membrane capacitance and depolarizing the neuron accumulates slowly.

The most comprehensive and coherent study of this current's role in burst generation is that of Gorman et al., (1982). We have repeated and expanded many of their observations (Kramer and Zucker, 1985b). The pacemaker current activates rapidly with depolarization, and deactivates rapidly with hyperpolarization. It is partially activated (and therefore incompletely inactivated) at "resting potentials." In addition to rapidly activating components (Chad et al., 1984), components with slower kinetics have been observed (Eckert and Lux, 1976; Kramer and Zucker, 1985b), suggesting the involvement of more than one type of channel (Chesnoy-Marchais, 1985)

The current through these channels is blocked by calcium antagonists (divalent ions such as Co^{2+} and Mn^{2+}), but not by sodium blockers such as tetrodotoxin. The current is little affected by short exposures to low sodium medium, but disappears when external calcium is chelated. It persists at potentials above the sodium equilibrium potential, and has an extrapolated reversal potential not far from that of calcium. It is apparently predominantly (and perhaps exclusively) a calcium current.

The pacemaker current is blocked by intracellular calcium injection and enhanced by EGTA injection. It is therefore not activated by internal calcium. Since reducing internal calcium should not directly affect the driving force or the net influx of current (from the Goldman single-ion equation), these effects of internal calcium and EGTA probably reflect modulation by calcium-dependent inactivation (Eckert and Chad, 1984).

Prolonged treatment with low sodium reduces the pacemaker current (reviewed in Adams and Benson, 1985), but this effect is prevented when EGTA is injected (Gorman et al., 1982). Sodium removal prevents Na/Ca exchange, which probably leads to elevated intracellular calcium and partial inactivation of the calcium pacemaker current. Low sodium also blocks a second depolarizing current (see below), which in early studies was not distinguished from the pacemaker current.

Calcium-Activated Potassium Current ($I_{K(Ca)}$)

Each spike in a burst is followed by a hyperpolarizing afterpotential lasting roughly 50-100 ms. This is slow compared to spike duration, but faster than subsequent afterpotentials discussed below. It participates in spike termination. This afterpotential is generated by an outward current of similar duration following a depolarization, which we have called the phase I tail current (Kramer and Zucker, 1985a,b). This current reverses at the potassium equilibrium potential, even when the latter is moved by altering potassium

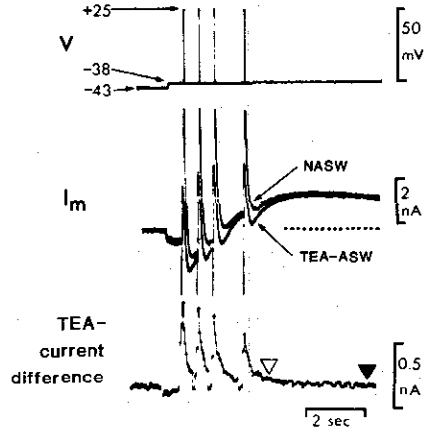

Fig. 1. Time course of $I_{K(Ca)}$, determined as the difference (bottom trace) between currents in the presence and absence of 50 mM TEA (middle traces), in response to a simulated spike burst (top trace) under voltage clamp.

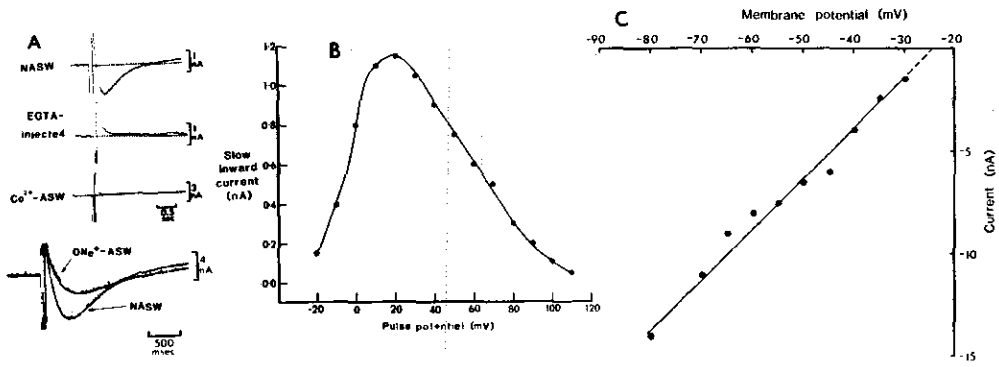

Fig. 2. Properties of $I_{NS(Ca)}$. A) The slow inward current is blocked by EGTA injection and calcium antagonists, and reduced in sodium-free sea water. B) The inward tail current magnitude has a bell-shaped dependence on the potential of the pulse used to evoke it. C) The current depends linearly upon membrane potential, and reverses at about -25 mV.

concentration. It is blocked by millimolar concentrations of external tetraethylammonium (TEA), and is clearly a potassium current (Fig. 1). Its magnitude shows a bell-shaped dependence on the potential during the pulse used to evoke it, with a peak at the same potential at which calcium influx is maximum during the pulse. Larger pulses, further from the potassium equilibrium potential, elicit smaller phase I tail currents. Its magnitude also depends strongly and nonlinearly on the potential at which it is measured after the pulse, being large at depolarized potentials and very small at potentials below the potassium equilibrium potential, so the underlying conductance is voltage-dependent. It is blocked by EGTA injection and calcium antagonists, so it is a calcium-activated potassium current.

Calcium-Activated Nonspecific Cation Current ($I_{NS(Ca)}$)

Each spike in a burst is followed by a one-second-long depolarizing afterpotential which triggers the next spike in the burst. This afterpotential is mirrored in a second tail current which we have called phase II, a slow inward current which follows each spike and accumulates in a burst (Lewis, 1984; Adams, 1985). This leads to acceleration of spike frequency until a slower outward current begins to predominate (see below). The afterpotential and underlying current are sensitive to both external sodium and calcium concentrations (Thompson and Smith, 1976; Kramer and Zucker, 1985a; Smith and Thompson, 1987), and in R15, the current is partially triggered by the depolarization from axon spikes (Adams and Levitan, 1985). When axon spikes are prevented, this current has a bell-shaped dependence upon the voltage of the pulse used to evoke it; it is mimiced by calcium injection when $I_{K(Ca)}$ is blocked with TEA; and the current is blocked by EGTA injection; so it is a calcium-activated current (Fig. 2). When isolated from $I_{K(Ca)}$, it has a reversal potential at about -25 mV in Aplysia, and is normally carried by sodium, calcium and potassium ions. Changes in concentrations of these ions shift the reversal potential and affect the conductance magnitude. Large cations like TEA, tris, choline, glucosamine, and tetramethylammonium can substitute, at least partially, for sodium (Swandulla and Lux, 1985). Barium and lithium also penetrate the responsible channel, although cobalt, nickel, manganese, hydrogen and chloride do not. Preventing potassium accumulation by reducing potassium current does not affect either depolarizing afterpotential or slow inward current, nor do treatments such as lithium ringer or ouabain which block Na/Ca and Na/K exchange. Unlike $I_{K(Ca)}$, this current is linearly dependent upon the membrane potential during its measurement, indicating a conductance independent of voltage. We therefore have a relatively nonspecific cation current activated by intracellular calcium helping to boost the burst.

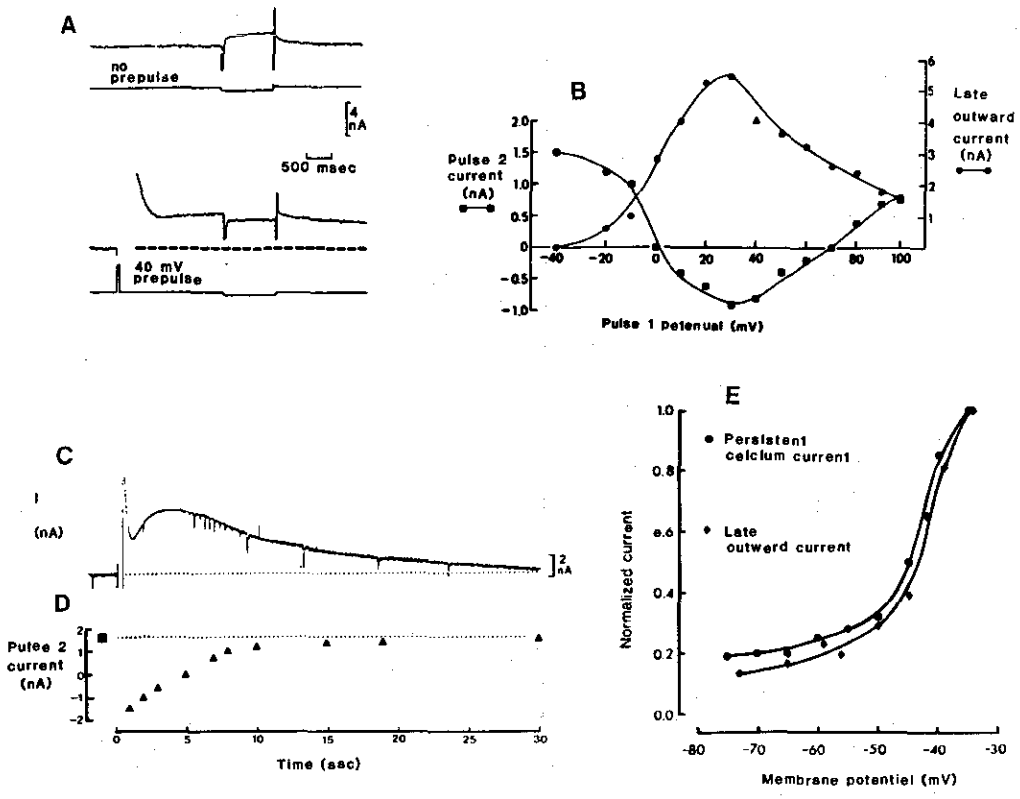

Fig. 3. Properties of the late outward current. A) A small hyperpolarization normally elicits an outward current at -40 mV, due to the negative resistance of the membrane at this potential. A larger prepulse which elicits the late outward current blocks the negative resistance. B) The negative resistance (pulse 2 outward current) is blocked with the same bell-shaped dependence on pulse amplitude as the late outward current. C) Decay of late outward current after a pulse. D) Decay of negative resistance after an identical pulse. E) Voltage dependence of persistent calcium pacemaker current and late outward current.

The Late Outward Current

Bursts are terminated by a deep hyperpolarization, due to a slow outward current that accumulates gradually during each burst, and only comes to match the other slow currents late in the burst (Lewis, 1984; Adams, 1985). This current correlates with the intracellular accumulation of calcium measured spectrophotometrically with the dye arsenazo III (Gorman and Thomas, 1978). It is blocked by calcium antagonists and EGTA injection, and shows a bell-shaped dependence on pulse potential (Gorman et al., 1982; Kramer and Zucker, 1985b), so it is evidently another calcium-dependent current. Originally thought to be a calcium-activated potassium current, it is distinguished from $I_{K(Ca)}$ at room temperature by its insensitivity to TEA and charybdotoxin and changes in potassium concentration and its failure to reverse at the potassium equilibrium potential (Adams and Levitan, 1985; Kramer and Zucker, 1985; A. Hermann, personal communication). Only at low temperatures and in some dorid bursting neurons does a slow component of $I_{K(Ca)}$, insensitive to TEA, appear to contribute significantly to the late outward current (Deitmer and Eckert, 1985; Thompson et al., 1986). A TEA-insensitive component of outward current in response to calcium injection can also be detected. This current is unaffected by blocking Na/Ca and Na/K transport and replacing chloride in the ringer.

If not by affecting anion flow, electrogenic transport, or potassium conductance, how can calcium cause an increased outward current? A reduced inward current would resemble an increased outward current. Both would appear as an increase in steady-state conductance to voltage or current pulses (Junge and Stephens, 1973; Barker and Gainer, 1975). The persistent inward current most eligible to be reduced by calcium is the calcium pacemaker current, since it is subject to calcium-dependent inactivation (Eckert and Chad, 1984). If the late outward current is a reduction in pacemaker current, they should show the same sensitivity to voltage during the current. They do (Kramer and Zucker, 1985b). The negative resistance characteristic that serves to mark the presence of pacemaker current is reduced during the late outward current. As the latter decays, the former recovers. Both effects show the same bell-shaped dependence upon activating pulse potential preceding the current (Fig. 3). Apparently, bursts are terminated as calcium accumulating during a burst shuts off the pacemaker current.

KINETICS OF CALCIUM-DEPENDENT CURRENTS

At this point, we see that bursting is initiated by a calcium current activated at potentials subthreshold for action potentials, and boosted by a calcium-activated nonspecific cation current triggered by spikes in the burst. A more rapidly decaying calcium-activated potassium current acts mainly to assist spike termination, while a slower late outward current reflecting calcium-dependent inactivation of the pacemaker current terminates bursts. The slow decay of this block of pacemaker current sets the interburst interval, until the pacemaker current recovers and initiates a new burst (Fig.4)

Calcium entering the neuron during a burst has three effects: activation of $I_{K(Ca)}$, activation of $I_{NS(Ca)}$, and block of the pacemaker current. If all three effects are due to the activity of submembrane intracellular calcium, why do they decay at different rates? I initially thought $I_{K(Ca)}$ decayed faster than $I_{NS(Ca)}$ because the former was activated cooperatively by calcium, and followed some power greater than one of calcium concentration. However, in recent experiments (Landò and Zucker, in preparation), we have studied the calcium dependence of $I_{K(Ca)}$ and $I_{NS(Ca)}$ in bursting neurons by activating these conductances directly by intracellular calcium release from the photodynamic chelator nitr-5 (Adams et. al., 1986; Tsien and Zucker, 1986). We found both currents to be activated linearly and without saturation by cytoplasmic calcium increments similar to those occurring during bursting (Fig. 5). One calcium ion appears to activate each current at all voltages tested.

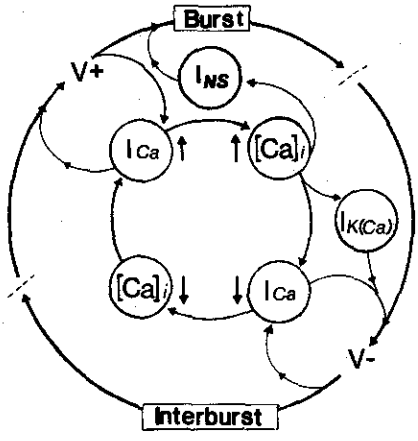

Fig. 4. Schematic model of the bursting mechanism. Cycles of membrane potential are on the outer circle, and cycles of calcium current and internal calcium levels are on the inner circle. Calcium-activated currents are also shown. The drop in I_{Ca} corresponds to the late outward current.

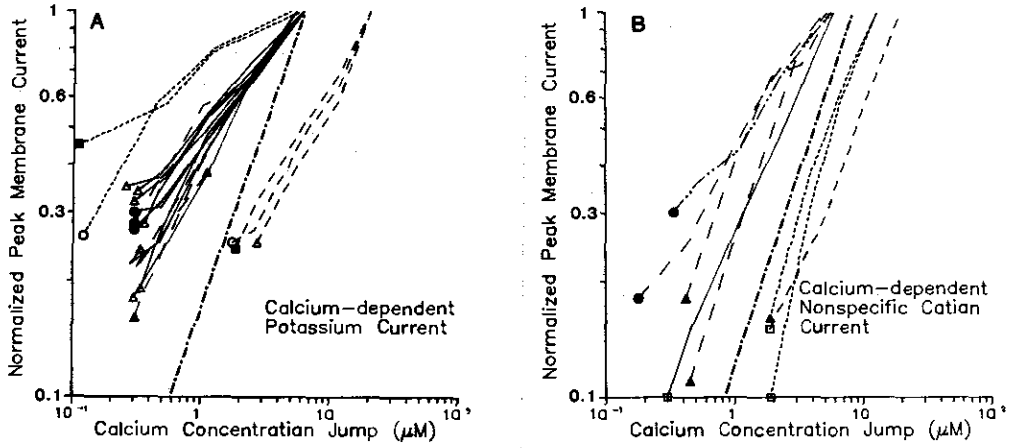

Fig. 5. Dependence of $I_{K(Ca)}$ (in A) and of $I_{NS(Ca)}$ (in B) upon increments in intracellular calcium concentration caused by flash photolysis of nitr-5 or nitr-7. Each line is from a different experiment. The dot-dash line has unity slope.

Membrane currents activated by flash photolysis of nitr-5 decay after the flash, mostly due to diffusional equilibration of calcium (released mainly at the surface facing the light) and the buffer constituents. These currents, measured at a constant voltage, decay at the same rate in any single cell, again indicating an equal degree of calcium cooperativity in activating both currents. However, as indicated above, $I_{NS(Ca)}$ channels are voltage-insensitive while $I_{K(Ca)}$ are strongly affected by voltage. After a depolarization, $I_{K(Ca)}$ will show a voltage-dependent relaxation, while $I_{NS(Ca)}$ will only decay as submembrane calcium drops. We have measured these voltage-dependent relaxations of $I_{K(Ca)}$, and found that they, combined with the expected fall in submembrane calcium following a burst, are sufficient to explain the more rapid decay rate of $I_{K(Ca)}$ compared to that of $I_{NS(Ca)}$.

The very slow time course of pacemaker current inactivation and its recovery are still a matter of speculation. A very high calcium sensitivity might explain its long persistence, but not its slow build-up during a burst. However, in preliminary experiments we have been unable to block calcium currents during depolarizing pulses, or the negative resistance characteristic in the subthreshold range, by releasing calcium photolytically from nitr-5 injected intracellularly into bursting pacemaker neurons. This suggests that calcium-inactivation of the pacemaker current is the calcium-dependent process least sensitive to calcium accumulation. A similar conclusion was reached by considering the high local activity of calcium in the small "domains" surrounding calcium channel mouths, where calcium presumably acts to shut down the channels (Chad and Eckert, 1984). Perhaps the slow decay of late outward current reflects the time course of protein phosphorylation thought to be involved in recovery from calcium-dependent inactivation of calcium current (Eckert and Chad, 1984).

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