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IONIC CURRENTS IN MOLLUSCAN SOMA

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INTRODUCTION

The present understanding of nerve excitability is founded on voltage clamp studies of axonal membranes. It has become clear, however, that the excitability of neuronal somata (cell bodies), dendrites, and terminal regions involves processes substantially different from those evident in axons. Somata, for instance, can exhibit a variety of slow oscillatory firing patterns and long lasting aftereffects of activity that are not observed in axonal preparations. In addition, voltage dependent calcium currents may play a major role in nerve excitability everywhere except the axon, perhaps in connection with the widespread intracellular messenger role of this ion (e.g. excitation–secretion coupling). The giant somata of the gastropod molluscs have so far yielded the most fundamental insights into the mechanisms of the distinctive electrical activity of nonaxonal membrane. It is our purpose here to review these insights.

The giant neurons in ganglia of the gastropods have approximately spherical somata, which range to over 500 μm in diameter, and most have

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only a single axo-dendritic process. The somata are arrayed accessibly in a cortical layer on the ganglion. A richly varied range of excitability phenomena can be observed in these cells under conditions ranging from largely intact, behaving animals (Willows, Dorsett & Hoyle 1973, Kandel 1976) to internally dialysed, isolated cells (Kostyuk, Krishtal & Pidoplichko 1975b). These phenomena include action potential firing, spike frequency encoding of constant current stimuli, frequency adaptation, spontaneous pacemaking, burst firing, and a variety of spike or tetanic afterpotentials. Oscillations and afterpotentials with characteristic time courses ranging from milliseconds to minutes are readily observable.

The suitability of gastropod somata to voltage clamp was first demonstrated by Hagiwara & Saito (1959). Studies of the ionic basis of giant soma excitation under voltage clamp account for the major advances in our understanding of this system. The recent developments of intracellular ion indicators and internal dialysis techniques are opening a new range of questions concerning molecular mechanisms of ion transport and the relationship between membrane state and intracellular milieu.

This review is concerned with the properties of ionic conductance systems activated directly or indirectly by changes in membrane potential. Ionic conductances sensitive to a variety of neurotransmitters and other chemical agonists are also observed in gastropod somata. Although some of these phenomena may ultimately prove to reflect different aspects or modulations of the same mechanisms discussed here, such agonistic effects are outside the scope of the present work. Agonist effects have been reviewed by Gerschenfeld (1973), Kupfermann (1979), and Barker & Smith (1979).

Soma Voltage Clamp Arrangements

Three general requirements for voltage camp analysis of ionic current are: (*a*) the ability to impose a specified potential uniformly over a membrane area, (*b*) the ability to measure current flow through that area, and (*c*) the ability to change the composition of solutions outside or inside the cell. All voltage clamping arrangements have limitations in each of these regards and the extent to which these requirements are satisfied must be considered carefully in interpreting any voltage clamp observations.

POTENTIAL CONTROL Control of soma membrane potential is usually accomplished by means of a feedback amplifier and pairs of intracellular and extracellular electrodes: one pair for measuring potential, another for passing current (Cole 1968, Katz & Schwartz 1974). Sometimes a single extracellular electrode is used both for current return and potential reference, but the method is prone to errors due to electrode resistance and

polarization. Intracellular electrode contact is made either through micropipettes (Hagiwara & Saito 1959), or through a disrupted membrane area sealed beneath a suction orifice (Kostyuk et al 1975b, Lee, Akaike & Brown 1977). The latter method allows a low resistance contact for both intracellular electrodes but increases the effective series resistance. Newly developed twin orifice and orifice-micropipette methods circumvent this difficulty. These arrangements appear to hold the potential uniform within a few millivolts over the entire soma membrane in most cells (Hagiwara & Saito 1959, Connor & Stevens 1971a; but see Kado 1973). This maintenance of isopotentiality, even during the flow of large ionic currents, must reflect the low internal resistances characteristic of sphere-like soma geometry. The rate at which membrane potential will follow the command signal is limited by preparation series resistance (Hodgkin, Huxley & Katz 1952, Katz & Schwartz 1974, Adams & Gage 1979a). Typical cellular series resistance and membrane capacitance values limit potential settling to exponential time constants on the order of 20 μsec.

CURRENT MEASUREMENT The presence of an unclamped axo-dendritic process complicates the measurement of soma membrane current. With intact cells, total clamping current consists of significant contributions from both soma and axon. The total current, therefore, has limited usefulness as an indicator of current flow in the well-clamped soma region. Measurement of any rapid somatic conductance change is impossible due to the prolonged (on the order of 10 msec) and nonlinear step charging response of the unclamped axon. Axonal action potentials and other artifacts of poor spatial control complicate interpretation of all clamping current measurements. These problems have been dealt with in two different ways: (*a*) by patch or focal current measurement at the soma membrane (Frank & Tauc 1964, Neher & Lux 1969, Kado 1973), and (*b*) by total clamp current measurement after ligation or removal of the axonal process (Connor & Stevens 1971a, Kostyuk, Krishtal & Doroshenko 1974, Connor 1977). With very careful application of either method, capacity transient settling to 0.1% of maximum can be achieved within less than 500 μsec of step onset. At least at low temperatures, this allows resolution of sodium conductance, the fastest known gating process in these neurons (e.g. Adams & Gage 1979a).

SOLUTION CHANGES Most voltage clamp analysis involves either adding a pharmacological agent or changing the ionic composition at the external surface of the cell. This is often performed by exchanging the external perfusate, since this allows quantitative specification of dosage or concentration, at least in principle. Molluscan somata lend themselves well

to this operation, being on the outside of their ganglia, but certain limitations must be recognized. There may be prolonged diffusion delays, and for some substances such as ions, accumulation or depletion effects may displace concentrations near the cell away from bulk perfusate values. Such effects are normally minimized by removing the sheath of connective tissue overlying the ganglion, and can be further reduced by isolated cell techniques. Even in desheathed preparations, however, there is evidence for restricted diffusion access (Eaton 1972, Neher & Lux 1973, Ahmed & Connor 1979). Such restriction probably reflects the highly infolded geometry of the surface membrane and the presence of satellite cells closely associated with the soma (Coggeshall 1967, Graubard 1975). Recently developed techniques for internal dialysis (Kostyuk et al 1975b, Lee et al 1977) and internal ion replacement (Russell, Eaton & Brodwick 1977, Tillotson & Horn 1978) allow alteration and control of the intracellular ionic environment.

Composition of the Total Ionic Current

Corresponding to the diversity of electrical behavior observed in unclamped gastropod somata, the ionic current under voltage clamp is exceedingly complex. After step changes in membrane potential, ionic current relaxations occur on time scales ranging from milliseconds to minutes, and these relaxations may take widely different forms depending on the specific voltage trajectory imposed. The abstraction of order from this diverse phenomenology has proceeded by identification of distinct, relatively simple components of ionic current. The original precedent for this approach is Hodgkin & Huxley's (1952) analysis of axon membrane ionic current.

As is the case for the axon, the most striking features of the voltage clamp current in the soma appear to be due to voltage dependent membrane permeabilities to specific ions. Several major extensions to the axon framework have been necessary, however, to encompass observations of soma currents. First, calcium ions carry a significant fraction of the ionic current in giant somata, while calcium current in squid axons appears to make only a negligible contribution to the total current. Second, the activation kinetics and pharmacology of the soma membrane potassium permeability are far more complex than those observed for the axon potassium permeability. Third, the time dependence of permeability change in voltage clamped somata extends to a range orders of magnitude slower than that encompassed by the squid axon analysis. Finally, there is good evidence that part of the voltage dependence of potassium permeability is actually mediated by intracellular calcium accumulation, while axonal permeability changes appear to be due directly to an action of the electrical field on membrane macromolecules.

The major features of the ionic current in molluscan somata can be summarized by a scheme representing six distinct components as indicated in Table 1. This scheme is a conjunction of components identified by numerous workers over the years upon which we have imposed our own terminology in the hopes of a more coherent presentation. There is no claim that it represents a consensus of opinions even among those workers whose discoveries it purports to encompass. It is adopted mainly as a vehicle for collection and discussion of findings obtained in diverse experimental contexts. The scheme is not exhaustive, since there is no consideration of metabolically driven ionic currents or of currents carried by anions. Neither does it encompass the currents observed with large hyperpolarizations from the resting potential.

Each current component is characterized as an ionic conductance with fixed ion specificity but variable magnitude. The individual components have been distinguished experimentally on the basis of differences in ionic and pharmacological sensitivities with the intent of establishing the simplest possible description of the overall dependence of ionic conductance on voltage and time. It is tempting to suppose that the six distinct current components may correspond to six populations of ionic channel macromolecules, but no such conclusion can be rigorously supported at this time.

The detailed properties of each component listed in the table and the rationale for its separation from total ionic current are discussed in sections on individual components below.

INWARD IONIC CURRENTS

In some molluscan neurons influxes of both Na^+ and Ca^{2+} contribute to the rising phase of the action potential. The soma membrane continues to produce action potentials in either sodium-free or calcium-free solutions but becomes inexcitable if both sodium and calcium ions are removed from the external solution (*Aplysia:* Junge 1967, Geduldig & Junge 1968, Carpenter & Gunn 1970, Wald 1972, *Helix:* Gerasimov et al 1965, Meves 1968).

Table 1 Summary of ionic currents

Symbol	Name	Selectivity
I_{Na}	Sodium current	Na^+
I_{Ca}	Calcium current	Ca^{++}
I_B	Slow inward current	Na^+, Ca^{++}
I_A	Transient potassium current	K^+
I_K	Voltage-activated late current	K^+
I_C	Calcium-activated late current	K^+

Evidence for the independence of sodium and calcium currents is obtained from kinetic, electrochemical, and pharmacological arguments. Differences in the time course and voltage dependence of inward sodium and calcium currents were reported for the *Aplysia* neuron, R_2 (Geduldig & Gruener 1970). Although studies of membrane currents in other cells suggested that the kinetics of Na^+ and Ca^{2+} ion fluxes might be more similar. Newer analyses with better suppression of potassium current have shown that the Na^+ and Ca^{2+} currents differ in gating kinetics and voltage dependence of activation and inactivation. The substitution of impermeant organic cations such as Tris (tris-hydroxy-methylamino methane), choline, or tetramethylammonium, and the effects of varying the external sodium concentration indicate that the earlier, most rapidly rising, inward current recorded in molluscan neurons is carried by sodium ions (Geduldig & Gruener 1970, Kostyuk & Krishtal 1977a, Lee et al 1977, Adams & Gage 1979a). A slower and more sustained inward current is carried by Ca^{2+} ions. It can be blocked selectively by external application of the inorganic cations Ni^{2+}, La^{3+}, Cd^{2+}, Mn^{2+}, Co^{2+}, Mg^{2+} (Geduldig & Gruener 1970, Kostyuk & Krishtal 1977b, Akaike et al 1978b, Adams & Gage 1979a).

Independence of Na and Ca channels is also suggested from the study of gating currents. When all the ionic currents are blocked, subtraction of symmetrical capacitive and leakage currents reveal nonlinear displacement currents. Such displacement currents are believed to be due to intramembranous charge movements involved in opening and closing ion channels in response to changes in the electric field and are called gating currents (see review by Almers 1978). Detection of gating currents associated with activation of Na and Ca channels has been reported in *Aplysia* and *Helix* neurons (Adam & Gage 1976, 1979c, Kostyuk, Krishtal & Pidoplichko 1977). A rapid component is thought to represent Na gating current while a slower component is thought to represent Ca gating current. The amplitudes of gating currents vary with clamp potential in a way that corresponds to the voltage dependence of Na^+ and Ca^{2+} ionic currents.

The Sodium Current, I_{Na}

The activation of sodium current by voltage has been described in *Aplysia* (Adams & Gage 1979b) and in *Helix* neurons (Kostyuk & Krishtal, 1977a). Sodium current in the *Aplysia* neuron, R_{15}, is activated at potentials more positive than −25 mV and is steeply voltage dependent with half maximal conductance occurring at a potential of −8 mV. During maintained depolarization, sodium current activates and then inactivates as in axons. The peak current occurs within a few milliseconds while the time constant of inactivation is 10–20 msec (Adams & Gage 1979a, b, Kostyuk & Krishtal 1977a).

Rates of activation and inactivation are voltage dependent, increasing with depolarization. The time course of Na current in both *Aplysia* and *Helix* can be adequately described by m^3h kinetics where m and h are the Hodgkin and Huxley parameters for activation and inactivation (Adams & Gage 1979b, Kostyuk & Krishtal 1977a). The amplitude and duration of conditioning voltage pulses strongly effect the amplitude of sodium current during a test pulse by affecting the level of inactivation. Unlike sodium current in the squid axon, little if any additional inactivation is removed with conditioning potentials more negative than the resting potential. The voltage dependence of sodium inactivation is similar in *Aplysia* and *Helix* neurons with half-inactivation occurring at about −30 mV (Geduldig & Gruener 1970, Adams & Gage 1979b, Standen 1975, Kostyuk & Krishtal 1977a). Recovery from inactivation is exponential with a time constant of about 30 msec at the resting potential (*Aplysia:* Geduldig & Gruener 1970, Bergmann, Klee & Faber 1974, Adams & Gage 1979b; *Helix:* Kostyuk & Krishtal 1977a).

The measured reversal potential for sodium current is about +50 mV in solutions containing the normal external Na^+ concentration (*Aplysia:* Geduldig & Gruener, 1970; Adams & Gage, 1979a; *Helix:* Kostyuk et al 1975b, Lee et al 1977). Although no comprehensive study of the ion selectivity of the sodium channel has been undertaken, comparison of the relative permeabilities of monovalent cations in R_{15} of *Aplysia* indicates that the sodium channel is permeable to Li^+ ions but relatively impermeable to Cs^+ and K^+ ions as found in squid and frog axonal membranes (Adams & Gage 1979a; see review by Hille 1975). Tetrodotoxin (TTX) selectively blocks ion movements through the channel independent of ionic species (Geduldig & Gruener 1970, Kado 1973, Adams & Gage 1979a). In most gastropod neurons, the sodium current is less sensitive to TTX than in squid axon, and TTX-insensitive channels have been described in *Helix* (Kostyuk et al 1974, Kostyuk & Krishtal 1977a). In some cases, relative insensitivity may be related to the prior use of trypsin to dissociate cells, since this treatment has been shown to inhibit the action of TTX in *Helix* (Lee et al 1977, but see Connor 1977).

The Calcium Current, I_{Ca}

Compelling evidence for a calcium current in gastropod somata comes from the ionic and pharmacological sensitivities of voltage clamp current, and from chemical indicators of the intracellular free calcium level. The gating characteristics of the calcium inward current are quite different from those of the sodium current, beyond the fact that both are activated by membrane depolarization.

MEASUREMENT OF CALCIUM CURRENT Though the presence of a calcium conductance system is demonstrated readily, separation of the current for quantitative study has proven difficult. Separation by time dependence or voltage dependence is possible only for a limited range of activation conditions, and the diverse effects of calcium outside of its role as charge carrier complicate the interpretation of ion substitution experiments (see section on calcium-activated potassium current below; see also Frankenhaeuser & Hodgkin 1957, Hille, 1968). Direct electrical observation of calcium current at positive potentials is normally thwarted by the activation of much larger currents carried by sodium and potassium ions. The sodium current can be suppressed in several different ways but potassium current poses a less tractable problem, particularly because of evidence that part of the potassium current depends on intracellular calcium. This dependence rules out the procedure of subtracting current after substitution for Ca^{2+} as an assay of calcium current.

This section discusses characteristics of the calcium current when studied after separation by one of several alternative methods:

1. Examination of early inward current in sodium-free medium or in the presence of TTX (Geduldig & Gruener 1970). Calcium current activation occurs somewhat more rapidly than potassium activation, so that early measurements should reflect calcium current characteristics. Separation by this method is only partial and does not allow observation of the true time course of calcium current during maintained depolarizations.

2. Examination of calcium tail currents in axotomized neurons upon repolarization to the potassium equilibrium potential (Connor 1977, Adams & Gage 1979b). This method requires speed and good spatial uniformity of the voltage clamp arrangement, because the tail current is very rapid and any poorly clamped membrane area could result in confusion of calcium and potassium currents.

3. Pharmacological suppression of outward potassium current with high concentrations of tetraethylammonium ions, TEA (Adams & Gage 1979a, Connor 1979). The applicability of this method is limited, however, since TEA does not totally suppress potassium current (see Pharmacology of Potassium Currents).

4. Replacement of intracellular potassium by a relatively impermeant cation, thus eliminating or reducing current through potassium channels (see section on I_K). Two methods have been employed: internal dialysis (Kostyuk et al 1975b, Lee et al 1977) and nystatin-mediated monovalent cation exchange (Tillotson & Horn 1978). A disadvantage of either method is uncertainty about possible effects of the ion replacement procedures on the calcium current itself.

5. Measurement of intracellular free calcium concentrations by a chemical indicator. This provides an alternative to electrical techniques for detection of calcium entry. Both the photoprotein aequorin (Stinnakre & Tauc 1973, Eckert, Tillotson & Ridgway 1977, Lux & Heyer 1977) and the metallochromic dye arsenazo III (Gorman & Thomas 1978, Ahmed & Connor 1979) have been used as indicators of intracellular calcium, but the latter has now been shown to provide both better calcium detection sensitivity and more readily quantifiable results (see Ahmed & Connor 1979). Indicator measurements have the advantage of providing excellent specificity for calcium movement without the use of blocking agents or ionic substitutions. On the other hand, quantitative inferences about the calcium current are restricted by imperfect knowledge of the cellular calcium clearance mechanisms that affect the accumulation monitored by an indicator response.

ION PERMEATION The reversal (zero-current) potential obtained for the calcium current in neurons exposed to high external TEA concentrations or loaded with Cs^+ ions is between +60 and +70 mV (Connor 1979, Tillotson & Horn 1978, Adams & Gage 1979a). A major discrepancy exists between this and the value estimated from voltage dependent changes in the intracellular calcium concentration measured with aequorin and arsenazo III, which is about +130 mV (Eckert et al 1977, Ahmed & Connor 1979). A similar high value for the reversal potential was obtained after removal of internal K^+ by the dialysis technique (Kostyuk & Krishtal 1977a, Akaike et al 1978b). The indicator and dialyzed cell values are in good agreement with that predicted for a calcium-selective channel. Factors that may contribute to these differences in reversal potential measurements are: (*a*) the block of potassium current by TEA becomes ineffective at large positive potentials, and (*b*) the calcium channel is permeable to some extent to the monovalent cations Na^+ and K^+, which move out of the cell at positive voltages. Evidence suggesting that monovalent cations permeate the Ca channel is provided by single channel conductance measurements in the presence of sodium ions.

Although no comprehensive study of the ion selectivity of the calcium channel has been completed, there is information concerning permeability of some divalent cations. Sr^{2+} and Ba^{2+} have been found to be permeable while no measurable current is obtained on the replacement of Ca^{2+} by Mg^{2+} (Gola et al 1977, Magura 1977, Connor 1977, 1979, Akaike et al 1978b, Adams & Gage 1979b). The selectivity sequence obtained from reversal potential measurements in dialyzed *Helix* neurons is $Ba^{2+} \cong Sr^{2+} > Ca^{2+}$ (Akaike et al 1978).

Calcium currents are blocked by some other external cations in a concentration dependent and reversible manner (Geduldig & Gruener 1970, Kostyuk & Krishtal 1977a, Akaike et al 1978a, Adams & Gage 1979a). The sequence of effectiveness from dose-response curves in *Helix* neurons is $Ni^{2+} > La^{3+} > Cd^{2+} > Co^{2+} > Mg^{2+}$ (Akaike et al 1978b). Organic calcium antagonists such as verapamil and D-600 are effective in blocking calcium current though nonspecifically (Kostyuk & Krishtal 1977a, Akaike et al 1978b, Adams & Gage 1979a). This pharmacology corresponds closely to that of calcium currents in other excitable membranes (see reviews by Baker & Glitsch 1975, Hagiwara 1975). Internal dialysis of *Helix* neurons has shown that calcium current is blocked irreversibly by internal perfusion with fluoride ions and is blocked by a high intracellular free-calcium ion concentration (Kostyuk et al 1975b, Kostyuk & Krishtal 1977b, Akaike et al 1978a).

The relationship between peak calcium current amplitude and external calcium concentration exhibits saturation with calcium concentrations above normal (Standen 1975, Akaike et al 1978b, Adams & Gage 1979a). Furthermore, Akaike et al (1978b) provide evidence for voltage dependent binding of calcium to membrane sites in the channel such that the affinity of the site for calcium is decreased with depolarization. Competitive block of calcium channels by various metal cations and saturation at modest elevations of external calcium ion concentration are consistent with the external site binding model proposed for the Ca channel in barnacle muscle fibers by Hagiwara (1975).

Noise measurements presumed to represent calcium channel gating fluctuations have been obtained by measuring current fluctuations before and after blocking calcium channels. Measurements obtained by this method in internally dialyzed *Helix* neurons provide a single channel conductance for the Ca channel of 0.5 pS or less when Ca^{2+} is the charge carrier and a value of 1.1 pS for Ba^{++} transfer through the channel (Akaike et al 1978a). A theory relating the power spectrum of current fluctuations to the macroscopic gating behavior of the calcium conductance has been proposed by Akaike et al (1978b).

GATING CHARACTERISTICS A sufficient depolarizing step leads to activation of calcium current, which approaches a maximum with a time constant of 5 to 10 msec (*Helix:* Kostyuk & Krishtal 1977a, Akaike et al 1978b; *Aplysia:* Adams & Gage 1979b). If the depolarization is maintained, the calcium current declines along a relatively prolonged time course. Upon repolarization, calcium current deactivates exponentially with a time constant somewhat faster than the activation process. Some studies have suggested that calcium currents may facilitate or increase during trains of identical depolarizing pulses (Eckert et al 1977, Lux & Heyer 1977). It now appears that the facilitation observed in these studies probably reflects properties of the Ca^{2+} indicator (aequorin) employed rather than the calcium current itself (Ahmed & Connor 1979, Smith & Zucker 1979).

Activation of Ca conductance in *Aplysia* and nudibranch neurons occurs at potentials more positive than those required for activation of sodium conductance (Geduldig & Gruener 1970, Tillotson & Horn 1978, Connor 1977, 1979, Adams & Gage 1976, 1979a), although in *Helix* cells, Ca^{2+} and Na^{+} currents first begin to activate at about the same potential (Kostyuk et al 1974, Standen 1975, Kostyuk & Krishtal 1977a, Lee et al 1978). Conductance-voltage curves for marine and terrestrial snails increase sigmoidally with increasing depolarization and maximum calcium conductance is attained at potentials more positive than +30 mV.

The development of inactivation during maintained depolarization has been a point of uncertainty in the published data on Ca current. Many early microelectrode studies reported complete inactivation occurring within a fraction of a second after stepping to positive potentials. Slower or less complete inactivation has been reported where cells have been bathed in high concentrations of TEA (Connor 1979, Adams & Gage 1979b). Furthermore, in dialyzed cells after removal of intracellular potassium ions, a fraction of the calcium current failed to inactivate even at very positive potentials (Kostyuk & Krishtal 1977a, Lee et al 1978). The apparent dependence of calcium channel behavior on procedures designed to block or remove potassium current suggests two possibilities: (*a*) calcium channels become less prone to inactivation in cells treated with TEA and in internally dialyzed cells, or (*b*) suppression of potassium current in such cells reveals normal, incomplete inactivation of calcium channels. Arsenazo III studies suggest that the latter possibility is the case. Intracellular calcium is observed to accumulate progressively for several seconds during prolonged clamp steps, even in intact cells bathed in normal external media (marine: Gorman & Thomas 1978, Ahmed & Connor 1979; terrestrial: S. J. Smith, unpublished). Sustained accumulation strongly suggests that inward calcium current persists for the entire duration of the pulse. Ahmed & Connor (1979) have shown that external addition of TEA has negligible effect on the arsenazo response to depolarization, which suggests that the slow and incomplete inactivation observed in TEA reflects normal channel behavior. Considerable uncertainty remains concerning the presence of inactivation, its presumed kinetics and the amplitude of the effect. These are major questions, especially when the pervasive role of internal Ca^{2+} in nerve cells is considered. Most of the uncertainty stems from the extreme difficulty encountered in isolating Ca current and further complications arising from internal Ca^{2+} accumulation are anticipated. In the following paragraphs,

the data on inactivation are collected in the hope of stimulating further research into this issue.

Ca-current inactivation has been described in *Aplysia* (Geduldig & Gruener 1970, Adams & Gage 1979b) and *Helix* neurons (Standen 1975, Kostyuk & Krishtal 1977a, Akaike et al 1978b). Inactivation develops during depolarizing with an approximately exponential time course that depends on the conditioning prepulse potential. Inward calcium currents are reduced by prepulses or at holding potentials more positive than the resting potential with half-inactivation at about –20 mV. The Ca inactivation curve obtained in *Helix* neurons is less steeply voltage dependent than in *Aplysia*. The Ca^{2+} inactivation curve in the *Aplysia* giant neuron, R_2, exhibits an anomalous depression of the calcium current at hyperpolarized holding potentials (Geduldig & Gruener 1970). Studies in *Helix* cells suggested that the apparent depression of Ca current generated from hyperpolarized holding voltages is due to the simultaneous activation of a transient potassium current, *A*-current (Neher 1971, Standen 1974). Recent investigation of this phenomenon in the *Aplysia* neuron, R_{15}, measuring calcium ionic and gating currents, however, suggests that the Ca channel itself may indeed be inactivated at hyperpolarized potentials (Adams & Gage 1979b, c).

Recovery from Ca-current inactivation, studied with double pulse techniques, exhibits two phases in *Aplysia* neurons (Tillotson & Horn 1978, Adams & Gage 1979b). The first phase has a time constant on the order of milliseconds and the second phase relaxes over several seconds. Only about 50% recovery of Ca current has occurred 1 sec after conditioning activation.

Differences between inward currents carried by Ba^{2+} and by Ca^{2+} ions bear on the mechanism of Ca-current inactivation. Barium appears to carry current through the same channels as calcium, but barium currents exhibit a much slower and less complete inactivation (Magura 1977, Gola et al 1977, Connor 1977, Adams & Gage 1979b). Since Ba^{2+} ions are known to suppress outward potassium currents (see Pharmacology of Outward Current), two interpretations of the Ba^{2+} substitution effect should be considered: (*a*) Ba^{2+} prevents a residual potassium activation, which is otherwise erroneously identified as Ca-current inactivation, or (*b*) substitution of Ba^{2+} for Ca^{2+} actually alters the inactivation characteristics of the calcium conductance system. Both interpretations may be partially correct.

Connor (1979) has shown that Ca^{2+} inward currents after intracellular EGTA injection resemble currents after Ba^{2+} substitution. If one assumes that EGTA has this effect by its familiar action of calcium chelation, this observation implies that a chemically specific effect of intracellular Ca^{2+} accumulation may account for the observed differences between Ca^{2+} and Ba^{2+} inward currents. One such specific effect might be the activation of potassium current. Connor (1979) has suggested that the Ba^{2+} and the internal EGTA effects are evidence in favor of interpretation (*a*) above. Alternatively, intracellular calcium may have a specific blocking or inactivating action on Ca channels not shared by barium, as suggested by Tillotson (1979).

The Slow Inward Current, I_B

A component of inward current characterized by extremely slow decay after depolarizing pulses was originally observed in bursting pacemaker neurons by Gola (1974) and by Eckert & Lux (1975). This slow current may represent an additional conductance system, distinct from I_{Na} and I_{Ca}, which is characteristic of this cell type alone (Arvanitaki & Chalazónitis 1961). Because of its occurrence in bursting cells and the role it is thought to play in producing bursting activity, we will refer to this slow inward current as *B*-current (I_B).

B-current is active at subthreshold voltages within the pacemaker voltage range. For small depolarizations, I_B activates over a time course of seconds and there is a seconds-long tail current at the end of the pulse (Gola 1974, T. Smith et al 1975, Thompson 1976). *B*-current is the smallest of the ionic currents described in molluscan cells. The largest amplitudes of I_B observed are less than one-hundredth the maximum amplitude of I_{Na} or I_{Ca}. It is, nonetheless, readily distinguished from the other inward currents by its slow kinetics of decay at negative holding potentials. Because of the simultaneous activation of other much larger ionic currents, I_B cannot be observed directly in the positive voltage range. The kinetics of activation during depolarization must be inferred from observations of slow tails at potentials near the reversal potential for outward current following depolarizing pulses of various dimensions. An analysis of the voltage dependence and kinetics of I_B based on tail current measurements was reported by Thompson (1976). Activation appears to be approximately exponential in time and the current does not inactivate with prolonged depolarization. The activation time constant is about 2 sec at –60 mV and decreases with depolarization, reaching about 200 msec at 0 mV (Gola 1974, Eckert & Lux 1975, 1976, Thompson 1976).

Several studies have identified an inward current in the pacemaker voltage range from measurements of steady-state I-V curves (Wilson & Wachtel 1974, Eckert & Lux 1975, 1976, T. Smith et al 1975). The inward current studied in this way bears resemblance to the slow current studied by the tail current method, especially in voltage dependence of activation. A note of caution was suggested by Partridge et al (1979), however, who studied very similar steady-state I-V curves in nonbursting cells where I_B was not observed. The kinetics of inward current relaxation in these cells were very

much faster than those of I_B observed in bursters. Smith et al (1975) found that the slow inward current was not blocked by TTX in *Otala* but its amplitude depended on external Na^+ concentration. Eckert & Lux (1976) found that the current amplitude was reduced in low Ca^{2+} in *Helix*. It is not presently clear whether I_B represents the sum of different Na^+ and Ca^{2+} currents or a single mechanism with mixed selectivity. Because of its very low amplitude, I_B is difficult to examine free of contamination by other currents. Neither direct measures of reversal potential nor measures of instantaneous current voltage relations seem feasible. Although the voltage dependence of its amplitude and kinetics suggest a voltage sensitive gating mechanism, the characterization of its activation remains incomplete.

B-current is thought to play a significant role in the generation of bursting activity (Gola 1974, T. Smith et al 1975, Thompson 1976). Because of its slow kinetics in the pacemaker voltage range, I_B gives rise to a prominent depolarizing afterpotential after individual spikes and similarly provides a lasting depolarizing drive that leads to the aggregation of spikes into sustained bursts (Thompson & Smith 1976). There have been suggestions that control of neuronal bursting by neurotransmitters or neurohumoral agents may be affected via modulation of the slow inward current (Barker & Smith 1979, Wilson & Wachtel 1978, see also Pellmar & Carpenter 1979).

OUTWARD IONIC CURRENTS

The outward currents seen during voltage clamp steps are thought to be the sum of three separate potassium conductance systems that can occur in varying ratios in different cells. The three potassium ionic currents are designated I_A, I_K, and I_C. I_A is a transient potassium current first studied by Hagiwara & Saito (1959) and subsequently by Connor & Stevens (1971b) and Neher (1971). I_K is a late outward current controlled by a voltage dependent gating mechanism. I_C is a late outward current activated by increases in internal ionized Ca^{2+} concentration (for review see Meech 1978). I_A can readily be separated from the late outward currents, and from inward sodium and calcium currents (at least over part of the range of activation potentials) because it activates at more negative voltages. It can also be readily identified on kinetic grounds since it activates and inactivates more rapidly than late outward current (Kostyuk et al 1975a, Neher 1971, Connor & Stevens 1971c). Furthermore, I_A appears to have no dependence on intracellular calcium ions (Connor 1979). These considerations and the results of pharmacological experiments described below leave little doubt that *A*-current represents a distinct and separable component of potassium conductance. It is possible to eliminate it from current records simply by

studying membrane currents on depolarization from a holding voltage that results in complete inactivation (Connor & Stevens 1971b). Separation of the late outward current into its components (I_K, I_C) is not so straightforward. Because both components activate over similar voltage ranges and there is overlap in kinetics, independent methods must be used to separate them. Pharmacological techniques allow such a separation.

Pharmacology of Potassium Currents

The potassium channel blockers 4-aminopyridine (4-AP) and tetraethylammonium (TEA), and blockers of Ca^{2+} current can be used either alone or in combination to separate and identify I_K and I_C.

Activation of I_C is blocked by procedures that prevent the concentration of intracellular ionized Ca^{2+} from increasing during depolarization. Connor (1979) outlined several effective procedures: (*a*) replacement of external Ca^{2+} with Mg^{2+}, (*b*) blockage of Ca^{2+} influx, (*c*) internal buffering of Ca^{2+} by EGTA injection, (*d*) replacement of Ca^{2+} by Ba^{2+}.

Method (*a*) is the least effective, probably because residual Ca^{2+} in membrane infoldings cannot readily exchange with the bathing medium, but the effectiveness of the approach can be improved by addition of a Ca^{2+} blocking ion. Injection of EGTA into cytoplasm blocks the activation of I_C but does not block Ca^{2+} influx. This suppression of I_C is probably due to prevention of the increase in intracellular Ca^{2+} concentration by the buffering action of EGTA. Connor (1979) has shown that 1–2 mM internal EGTA is sufficient to block the activation of I_C in nudibranch neurons.

The potassium current blocker 4-aminophyridine (4-AP) is a particularly effective antagonist of I_A. One-half block occurs at an external concentration of 1.5 mM in nudibranchs (Thompson 1977). The block by external 4-AP is voltage dependent, increasing with time during hyperpolarizing conditioning steps and decreasing with time during depolarization. In contrast to its effects on axonal membrane, 4-AP does not affect the late outward currents at concentrations that completely block I_A (Thompson 1977, Adams & Gage 1979a).

Tetraethylammonium ion also has differential effects on the components of potassium current. Furthermore, its action and effective concentration differ depending on whether TEA is applied externally or internally. Internal TEA blocks I_A and I_K approximately equally (Neher & Lux 1972). External TEA dramatically reduces late outward current but is much less effective in blocking I_A (Connor & Stevens 1971a). A 50% reduction of I_K occurs at 5–12 mM external TEA in *Helix* and at about 8 mM in marine species (Neher & Lux 1972, Thompson 1977). *A*-current is 50% blocked at a concentration of 20–80 mM in *Helix* and 100 mM in nudibranchs. Even at high concentrations, however, some of the late outward current remains

TEA insensitive (Hagiwara & Saito 1959, Heyer & Lux 1976, Thompson 1977). This relatively TEA-insensitive component has been identified as the calcium-activated potassium current. Even though TEA is not totally selective in its action, the three components of potassium conductance differ in their sensitivity to it. TEA, in fact, has been used to block I_K in order to measure I_C during depolarization (Aldrich et al 1979a). Hermann & Gorman (1979) have reported that potassium currents due to Ca^{2+} microinjection are more sensitive to external TEA than is I_K.

The Transient Potassium Current, I_A

The activation of I_A results in a transient potassium current that peaks and then inactivates at a much slower rate. Inactivation is removed by a conditioning hyperpolarization. Unlike the late outward currents, I_A is activated at subthreshold voltages near the resting potential, and exerts a major influence on excitability in that voltage range: slowing the rate of depolarization in response to a stimulus thereby delaying the action potential, and slowing the rate of repetitive firing in response to maintained stimuli, especially for the first few spikes in a train (Connor & Stevens 1971c). Gola (1974) has suggested there may be a residual level of A-current activation even at voltages negative to the reversal potential for the process (about –65 mV) and that the inactivation gating of I_A may therefore contribute to the inward rectification that is prominent in some cells.

The activation of A-current on depolarization follows a sigmoid rise to a maximum in 10 to 50 ms, which is followed by an exponential inactivation (Connor & Stevens 1971b, Neher 1971). Both activation and inactivation time constants exhibit shallow voltage dependencies, decreasing with depolarization. The inactivation time constant characteristically differs among various identified cells in a species although it is fairly constant for any single identified cell. To ascertain the role played by I_A as a determinant of the voltage trajectory near threshold, it would be interesting to know how differences in kinetics are correlated with differences in repetitive firing to constant current stimulation.

Several detailed analyses of the voltage dependence of I_A have appeared (Connor & Stevens 1971b, c, Neher 1971, Gola 1974, Partridge & Connor 1978, Thompson 1977, Connor 1978). Connor & Stevens (1971c) described the kinetics of I_A with an equation resembling that used by Hodgkin & Huxley (1952) to describe the Na current in squid axon. The conductance of A-current channels was described by the product of activation and inactivation terms whose values are determined by first-order rate equations employing voltage dependent activation and inactivation time constants. The value of the activation term was raised to the fourth power to account for the delay in the rise of I_A in nudibranchs. Neher (1971) used a third power relation to describe the kinetics in *Helix*.

A-current is first seen with depolarization ranging from –60 to –45 mV in the nudibranchs and to –70 mV in *Helix*. Measurement of the voltage where the conductance activates fully is complicated by activation of other currents and has not been clearly determined. Steady-state inactivation is complete near the resting potential and is removed completely at about –100 mV.

The Voltage-Activated Late Potassium Current, I_K

A number of studies describing the properties of total late outward current have appeared (Connor & Stevens 1971a, Neher 1971, Meech & Standen 1975, Adams & Gage 1979a, Leicht et al 1971). Such studies are important because they relate directly to the normal operation of the cell, but they do not permit description of the properties of I_K in isolation. As in other sections of this review, we restrict ourselves to those studies that have attempted to fully isolate I_K from I_C by any of the methods mentioned and we consider data where I_K has been clearly separated from I_A either by subtraction or by using a holding voltage at which A-current is fully inactivated (Heyer & Lux 1976, Kostyuk et al 1975a, Meech & Standen 1975, Eckert & Lux 1977, Thompson 1977, Aldrich et al 1979a).

During prolonged depolarization from the resting potential, K-current rises with a delay to a peak and then declines over several seconds to a nonzero steady-state value. The decline in I_K appears to be due to inactivation of potassium conductance and not due to extracellular accumulation of potassium near the membrane. Although potassium accumulation can occur (Alving 1969, Eaton 1972, Neher & Lux 1973), inactivation is observed under conditions that do not result in a shift in reversal potential (Gola 1974, Heyer & Lux 1976, Aldrich et al 1979a).

Slow inactivation of late outward current has been noted by several authors (Hagiwara et al 1961, Hagiwara & Saito 1959, Connor & Stevens 1971a, Eckert & Lux 1977, Gola 1974, Heyer & Lux 1976, Aldrich et al 1979a, Leicht et al 1971, Kostyuk et al 1975a, Barker & Smith, 1979). Furthermore, progressive frequency dependent decline in outward current amplitude occurs during repetitive depolarization. When voltage pulses are presented at low frequencies (e.g. 1 Hz) the maximum outward current during the second pulse is often less than the current at the end of the preceding pulse, which gives the impression that inactivation continues at about the same rate even during the repolarized interval (Kostyuk et al 1975a, Neher & Lux 1972). Also characteristic of this process is a slowing of the rate of rise of I_K as inactivation progresses toward saturation during repetitive depolarizations. This distinctive inactivation process is seen in

many molluscan neural somata but it differs qualitatively from that observed in axonal preparations and from the inactivation of I_A. The process has been called cumulative inactivation (Aldrich et al 1979a).

K-current begins to activate with depolarization to –30 mV and activation increases with depolarization up to positive voltages. The rising phase is sigmoidal, begins after a substantial delay, and is best described by a sum of exponentials rather than by a power of a single exponential (H. Reuter & C. F. Stevens, personal communication). Measurements of the voltage dependence and kinetics of activation of I_K have not been accurately conducted because of complications introduced by the inactivation process.

Inactivation during prolonged depolarization is incomplete and the degree of inactivation depends on voltage and differs among cells. I_K is about one-half inactivated at rest and inactivation is maximal between +10 and +20 mV. With further depolarization, inactivation begins to decrease again. The steady-state inactivation curve is, therefore, a U-shaped function of voltage (Magura et al 1971, Kostyuk et al 1975a, Heyer & Lux 1976, Eckert & Lux 1977, Aldrich et al 1979a). The time course of inactivation measured from the decline of outward current during a prolonged depolarization can be reasonably fitted by a single exponential function. The time constant is on the order of seconds for *Helix* (Heyer & Lux 1976) and nudibranchs (Aldrich et al 1979a) and is a bell-shaped function of voltage, first decreasing then increasing with depolarization. A more complete picture is obtained when the time course of inactivation is measured with a method that compares the effect of pre-pulses of various durations on the peak amplitude of I_K during a test pulse. When measured by this method the time course is best fitted by the sum of two exponential functions. The slower relaxation corresponds to the decline in current during a long depolarization to the same voltage, while the more rapid relaxation is up to ten times faster and occurs on a time scale similar to the rise time of I_K on depolarization. In dorid neurons, as much as 90% of inactivation occurs by the fast process (Aldrich et al 1979a). Significant inactivation occurs before the time of peak current during depolarization so that in the absence of inactivation the peak current would be much larger.

Recovery from inactivation is extremely slow near the resting potential; it requires 20–30 seconds in *Helix* and as much as 1 min. in *Archidoris* (Kostyuk et al 1975a, Heyer & Lux 1976, Aldrich et al 1979a). The recovery time course is usually measured with a two-pulse procedure, where the persistence of inactivation accrued during the first pulse is measured at different intervals by its effect on a test pulse. For interpulse intervals less than about one second, inactivation increased with interval duration while for greater intervals gradual recovery is observed. The overall recovery time course is, therefore, U-shaped (Heyer & Lux 1976, Eckert & Lux 1977, Aldrich et al 1979a). Final recovery from inactivation is very slow, at least an order of magnitude slower than the onset of inactivation at the same voltage. Very slow recovery necessitates the use of long (30–60 sec) interpulse intervals during voltage clamp experiments to insure return to control conditions. Kostyuk et al (1975a) and Aldrich et al (1979a) have presented models for I_K inactivation. Both models assume a single population of channels that are subject to two kinds of inactivation processes.

Heyer & Lux (1976) and Eckert & Lux (1977) reported voltage dependent inactivation of I_K, but also reported that the distinguishing properties of cumulative inactivation (frequency dependence, greater inactivation for the second pulse in a pair, prolonged recovery) were absent in *Helix* neurons when Co^{2+} was substituted for Ca^{2+}. Concluding that cumulative inactivation was a property of the Ca^{2+} dependent potassium current (I_C), they suggested that I_C could be blocked by intracellular Ca^{2+} ions that accumulate during depolarization (see Plant 1978). Presumably the recovery rate from inactivation would then be determined by the rate of clearance of free Ca^{2+} in the cell. In nudibranch cells, however, cumulative inactivation occurs in Co^{2+} substituted saline (Aldrich et al 1979a). Furthermore, *Kostyuk & Krishtal (1977b)* observed cumulative inactivation in *Helix* cells dialyzed internally against EGTA solutions, conditions where accumulation of Ca^{2+} should be prevented. Differences between these results seem to be more procedural than actual, and more studies on specifically identified cells will be needed to clarify this difference.

The reversal potential for I_K measured from tail currents is about –65 mV and is similar to I_A reversal. This value contrasts with the ionic equilibrium potential for potassium in *Aplysia* (–75 mV) measured with ion selective electrodes (Kunze, Walker & Brown 1971). The discrepancy can be explained if the channel has a finite permeability to other ions. Ionic selectivity of the channel has been studied in *Helix* neurons from reversal potential measurements and found to be $Tl^+ > K^+ > Rb^+ > Cs^+ > Li^+ > Na^+$. Single channel conductance measured for *K*-current in the same study using dialyzing voltage clamp and fluctuation analysis was 2–3 pS (H. Reuter & C. F. Stevens, personal communication).

Consideration of its rate of activation suggests that the main function of I_K is to provide outward current to repolarize the membrane during a spike. Because of this, cumulative inactivation can have a significant effect on spike shape, contributing to broadening of the action potential during repetitive firing (*Barker & Smith 1979, Aldrich et al 1979b*). It is interesting that unlike the curves for the other currents, the voltage dependence of cumulative inactivation is steep at the resting potential so that small changes in subthreshold voltage can lead to significant changes in the level of inactivation of I_K.

The Calcium-Activated Late Potassium Current, I_C

The properties of I_C, the calcium-activated potassium current, in molluscan neurons and in other cell types were reviewed recently (Meech 1978). It seems clear that activation of I_C occurs subsequent to an increase in cytoplasmic free calcium concentration whether the calcium enters through the plasma membrane during depolarization, is released from internal storage sites by photosimulation, or is injected into the cell as a Ca^{2+} salt or EGTA-Ca buffer (Eckert & Tillotson 1978, Thomas & Gorman 1977, Andressen & Brown 1979, Meech 1974, Ahmed & Connor 1979). Calcium influx can be uncoupled from potassium flux by appropriate buffering of internal Ca^{2+} (Connor 1979). The exact relationship between internal free calcium concentration and potassium conductance is not known for neurons (see Simons 1976, for studies on red blood cells), but experiments using injected EGTA-Ca buffers indicate a liminal effective Ca^{2+} concentration of $1–9 \times 10^{-7}$ M (Meech 1974).

C-current has an apparent voltage dependence because of its requirement for internal Ca^{2+} and the fact that I_{Ca} is voltage dependent. The apparent voltage dependence is bell-shaped, rising to a peak with increasing depolarization and then falling off as the reversal potential for Ca current is approached (Meech 1974, Thompson 1977). The more direct question of an intrinsic voltage dependence of I_C gating, which is additional to or synergistic with the Ca^{2+} requirement, has not been addressed.

Meech (1974) reported that Ca^{2+}, Sr^{2+}, and Ba^{2+} ions could activate potassium conductance when injected into *Helix* neurons but Mg^{2+} was not effective. When activated by depolarization the amplitude of I_C is greatly reduced if Ba^{2+} or Sr^{2+} replace external Ca^{2+} (Connor 1979, Eckert & Lux 1977). Sr^{2+} is not as effective in suppressing I_C as Ba^{2+}. From experiments on cells bathed in both Ba^{2+} and Ca^{2+}, Connor (1979) concluded that Ba^{2+} does not act like a blocker of I_C, but more like an ion which fails to activate the conductance mechanism as well as Ca^{2+} does.

The selectivity of the channel for various ionic species is not yet known, but measures of reversal potential suggest that I_C may be somewhat less selective for K^+ ions than I_A or I_K (Meech & Standen 1975). Reversal potentials for I_C, measured from tail currents, are somewhat more positive than those for I_K or I_A. This could reflect a less than perfect potassium selectivity, but the measures are almost certainly contaminated by relaxations of other currents, and because of the long clamp pulses needed to activate significant current there may be shifts due to potassium accumulation. Studies on the ion selectivity of I_C will be important in helping to identify it as a unique component of potassium conductance.

The time course of I_C during depolarization can be observed in cells bathed in saline-containing TEA. *C*-current increases much more slowly than I_K on depolarization and it continues to increase throughout prolonged pulses (Aldrich et al 1979a). Similar results were reported by Heyer & Lux (1976) and Eckert & Lux (1977) for *Helix* neurons. During repetitive pulses, Heyer & Lux (1976) and Eckert & Lux (1977) reported frequency dependent depression of I_C. This effect was not observed in nudibranch cells (Aldrich et al 1979a). In the protocol of Heyer & Lux (1976) the contribution of open I_C channels is neglected and the incremental increase in I_C during repetitive pulses was noted to decrease. The decrease could result from a number of causes other than I_C inactivation: (*a*) partial inactivation of the Ca^{2+} current (see above), (*b*) approach to saturation of the conductance governing I_C, (*c*) a nonlinear relation between intracellular Ca^{2+} and I_C. Further work is needed before one can decide between the alternatives.

The simplest model for *C*-current activation would be to assume that the onset of I_C on depolarization and its relaxation on repolarization are proportional to the rate of change of internal Ca^{2+} concentration near the membrane. Relaxation of I_C after a pulse can last for more than a minute in nudibranch cells after a brief activating pulse (Thompson 1976, S. Smith 1978, Partridge et al 1979). The relaxation of I_C follows a time course quite similar to the absorbance change of the Ca^{2+} indicator, arsenazo III (Gorman & Thomas 1978). The correspondence is not exact, especially at short times after the end of a pulse, but it does argue for the general validity of the simple model. Models describing the time course of intracellular Ca^{2+} concentration transients have been presented for the case of Ca^{2+} entry through the membrane (Thompson 1977, S. Smith 1978) and for the release of Ca^{2+} from bound stores on photostimulation (Andresson, Brown & Yasui 1979). It is not yet clear whether Ca^{2+} is a direct activator of the channel or if a cofactor is required for activation as in muscle contraction or various enzyme activation processes.

As noted above, the relaxation of I_C after depolarizations or spikes can be extremely prolonged. *C*-current is evidently responsible for several very slow events such as post-burst hyperpolarization and spike frequency adaptation, which have a powerful effect on the integrative function of the neuron. Furthermore, because of the dependence on internal Ca^{2+} concentration, *C*-current is potentially subject to influence by factors such as hormones or synaptic modulators, which modify cellular metabolism.

CONCLUSION

Curves depicting the steady-state voltage dependencies of all of the ionic currents have been collected from the literature and presented in Figure 1. The information comes from a number of sources but an effort has been made to select representative data in order to give the best impression of the alignment of the curves. Inspection of the figure shows the relationship

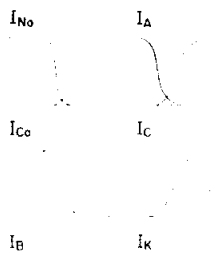

Figure 1 Steady-state voltage dependence of six ionic current components showing the relative magnitude of activation and inactivation variables plotted against a millivolt scale. Note that the voltage scale for I_C is shifted 50 mV to the left. The data used in plotting this figure come from several sources; I_{Na}, Adams & Gage 1979b; I_A, Thompson 1977; I_{Ca}, Adams & Gage 1979b (solid); Akaike et al 1978b (dashed); I_C, Thompson 1977, Smith 1978; I_B, Smith 1978; I_K, Aldrich et al 1979a.

of the currents to each other and to the resting potential and spike threshold. It provides some basis for understanding the range of excitability phenomena in these central neurons when combined with information on the kinetics and relative magnitudes of the currents. Several of the components are activated by depolarizations from rest into the threshold voltage range (I_{Na}, I_{Ca}, I_K). These components have rapid kinetics, relatively large maximum conductances, and are responsible for action potential firing. Other components (I_A, I_B, I_C) are active at more negative voltages, influence the subthreshold voltage behavior of the cell directly and are, therefore, important to the frequency encoding function of the neuron.

The importance of particular properties of the ionic current to electrical excitability can be investigated by mathematical reconstruction procedures. This approach was first used by Hodgkin & Huxley (1952) to demonstrate that the sodium and potassium currents they measured in axons could account for the firing and propagation of action potentials. Reconstruction of excitability behavior is most meaningful when all necessary parameters are determined strictly from voltage clamp data, following the precedent of Hodgkin and Huxley. The first study to account for distinctive features of soma excitability in this way was the analysis by Connor & Stevens (1971c) of repetitive firing in dorid neurons (see also Connor 1978). Subsequent investigations have applied similar procedures to analyze spike frequency

adaptation (Partridge & Stevens 1976), bursting pacemaker activity (Smith & Thompson 1975, Gola 1976, Thompson 1976, S. Smith 1978), and effects of drugs or temperature on firing behavior (Williamson & Crill 1976, Partridge & Connor 1978).

A striking feature of the central neurons in molluscan ganglia is the distinctive differences in membrane current patterns encountered in different identified cells. For example, bursting pacemaker cells have *B*-current, whereas other cell types do not (Thompson & Smith 1976). Aldrich et al (1979a) have documented another source of variability that involves differences in the relative contribution of I_K and I_C to the total outward current among cells. It seems likely that this kind of finding will be extended to the other ionic currents as well, allowing an explanation of the distinguishing features of different cells in terms of the relative contributions of the possible ionic conductance systems.

The calcium current in gastropod somata appears to possess many of the same properties as the calcium current involved in transmitter release at presynaptic terminals of the squid and of frog motoneurons (Katz 1969, Llinás, Steinberg & Walton 1976). The function of calcium entry at the soma, however, is not as well understood. Though the calcium current does contribute to the ability to fire action potentials, it is probably secondary to sodium current in this role. The localization of calcium channels to soma as opposed to axonal regions suggests the likelihood of a specific somatic function (Kado 1973, Junge & Miller 1974, but see Horn 1978). The importance of calcium currents to slow conductance changes involved in pacemaking and long-term variations in excitability has already been noted, but it seems likely that the major significance of the calcium influx may lie in the ability of calcium concentration changes to transmit information within the cytoplasm (see Kretsinger 1977).

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