

THE ROLE OF CALCIUM IN THE ACTION POTENTIAL
OF TETRAETHYLAMMONIUM (TEA) - TREATED
TRACHEAL SMOOTH MUSCLE

A Thesis
Presented to the
University of Manitoba

In Partial Fulfillment
of the Requirements for the Degree of
Master of Science

by

Brian K.J. Simmons
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ABSTRACT

The tonic, multi - unit characteristics of the canine trachealis in vitro are changed by the agent TEA to those of a single - unit smooth muscle, that is, it exhibits spontaneous phasic electrical and mechanical activity and a myogenic response to quick stretch. The membrane is also partly depolarized and its resistance is increased. Since the mechanical responses were strongly calcium - dependent and blocked by the calcium antagonist D-600, these findings suggested an important role for calcium, perhaps in the action potential itself.

The present investigation was therefore undertaken in order to define 1) the voltage - dependence of the dynamic ion channels and 2) the ionic basis of the action potential produced in the presence of TEA. With reference to the first, the influence on action potential parameters of an independent increase in membrane resistance (effected by partial replacement of external chloride with the less permeant isethionate) was investigated to gain insight as to the recruitment of ionic channels by depolarization. Chloride deficiency increased 1) the membrane resistance (r_m) by 53% (as determined by measurement of hyperpolarizing electrotonic potentials (ETP's) which are directly proportional to membrane resistance), 2) the height of the action potential by 73% and 3) its maximum rate of rise by 157%. The greater increase in the maximum rate of rise of the action potential, which is determined by the inward ionic current, suggests that additional dynamic ion channels are being activated as a result of greater depolarization produced by a constant current across an increased resistance.

With reference to the second aim, the possibility that the inward current is carried by calcium was investigated by examining the effect of D-600 and altered external calcium concentration on the electrical and mechanical responses of muscle. Elevation of calcium from 2.0 to 6.0 mM in the presence of chloride - deficient TEA solution 1) increased the height of the action potential by 47%, 2) increased its maximum rate of rise by 222%, 3) decreased membrane resistance by 30% and 4) produced an alternating or phasic pattern in the resistance. Application of D-600 (bath concentration 10^{-5} M) in the presence of elevated calcium 1) reduced the

height of the action potential by 84%, 2) reduced its maximum rate of rise by 94%, 3) increased membrane resistance by 45% and 4) stabilized its value.

Corresponding mechanical activity was increased in elevated external calcium and inhibited by D-600. Calculations of calcium influx suggest that a sufficient quantity may enter the fibers during an action potential in the presence of TEA to directly activate the contractile myofilaments. The phasic nature of the membrane resistance observed in the presence of high calcium is indicative of a relationship between resistance and local intracellular calcium gradients.

The findings indicate that the dynamic inward current of the action potential produced in the presence of TEA 1) is controlled by the conductance of voltage - dependent ion channels, 2) is carried mainly by calcium ions and 3) in turn indirectly regulates membrane resistance by altering intracellular calcium gradients.

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INTRODUCTION

A. GENERAL INTRODUCTION

Based on functional characteristics, smooth muscles have been placed into either of two broad classifications known as single unit and multiunit (Bozler, 1948; Burnstock and Prosser, 1960 b). Smooth muscle (SM) cells generally completely surround the lumen of hollow organs and may possess both sensory and effector functions (Bülbring, 1955). Single unit muscles are known to respond with bursts of activity to changes in the degree of distension of the organ. Muscles with such responsiveness exhibit cyclic depolarization, action potentials (AP's) and phasic mechanical activity spontaneously, or in response to stimulatory agonists (Bülbring, 1955; Burnstock et. al., 1963; Burnstock and Holman, 1966 b). These muscles also display good cell to cell conduction of regenerative electrical activity (Bülbring et. al., 1958; Prosser, 1962; Burnstock and Prosser, 1960 b) and a myogenic response (MR) to stretch (Burnstock and Prosser, 1960 a; Folkow, 1964; Olsen et. al., 1967; Biamino and Kruckenberg, 1969). Some SM's may be classified as intermediate in that spontaneity can be induced by agents such as high potassium (K), acetylcholine (ACh) and adrenergic amines which stimulate the muscle directly (Biamino and Kruckenberg, 1969; Keatinge, 1966). Kroeger and Stephens (1975) suggested that the depolarization of these muscles caused by such agents leads to firing of normally latent pacemakers. A third group, multiunit SM's, have a lower membrane resistance (r_m), do not produce AP's, are stimulated by dense innervation and are thought to lack low resistance intercellular gap junctions and nexuses (Prosser et. al., 1960; Su et. al., 1964).

Until very recently, the function and regulation of tracheal smooth muscle (TSM) or "trachealis" have been poorly understood in spite of considerable early work (Miller, 1947; Widdicombe, 1963, 1966). In the cervical trachea this muscle only spans the dorsal space formed by the incomplete cartilaginous rings of a relatively non-compliant tube. Rather than being just part of a passive conduit, however, the trachealis is quite active and responsive (Widdicombe, 1966; Olsen et. al., 1967; Stephens et. al., 1968). Neural input consists of numerous vagal (cholinergic) neurones (Loofbourrow et. al., 1957; Widdicombe, 1966) and sympathetic dilator nerves (Miller, 1947). Stephens and Kroeger have

shown recently (1970) that canine TSM is a tonic muscle activated almost exclusively by cholinergic neural elements in response to electrical stimulation in normal solution. A tonic contraction is also elicited by agents and conditions such as ACh, potassium chloride (KCl), barium (Ba^{2+}), (Stephens et al., 1974), serotonin (Daly and Hebb, 1966) and histamine (Hawkins and Paton, 1958) while epinephrine and isoproterenol relax (Nissel, 1950; Hawkins and Paton, 1958). Though norepinephrine and high K had been reported (Biamino and Kruckenberg, 1969; Keatinge, 1966) to convert "intermediate" (Burnstock and Prosser, 1960 b) arterial SM into functionally single unit preparations, neither these nor any of the aforementioned agents could facilitate the production of phasic activity or a MR to stretch in trachealis (Stephens et al., 1974). Such characteristics are clearly consistent with those of multiunit muscles and would seem to indicate a predominant neural control of airway calibre. TSM does, however, possess a capability for supercontraction whereby this muscle can contract down to 10 to 15% of its optimal length (Stephens et al., 1969). This is unusual even for SM (Csapo and Goodall, 1954) and suggests that the muscle is capable of intimate control of airway calibre. This postulate is strengthened by the finding that TSM is able to prevent airway collapse due to sudden, high transmural pressures (which may reach 300 mm Hg - Ross et al., 1955) when it is contracted (Olsen et al., 1967; Coburn et al., 1972).

Although tonic contractions in response to stimulation by neural elements and bronchoactive agents may be quite vigorous (TSM has a relatively high force constant - Stephens et al., 1969), they may not be capable of counteracting airway closure due to the elevated transmural pressures encountered in pathological conditions. Several recent findings have indicated that trachealis may possess a potential for spontaneous activity. Kirkpatrick (1975) has reported that histamine produces depolarization of bovine trachea with slow oscillations in membrane potential (E_m) which are correlated with rhythmic fluctuations in contractile activity. In the K contracture an initial phasic component is also evident. Furthermore, phasic activity has been observed in vivo (Loofbourrow et al., 1957), and in vitro immediately after removal from the dog (Stephens and Kroeger, unpublished observations). Akasaka et al. (1975) have succeeded in recording

electrical activity from bronchial SM of normal healthy patients and from those with bronchial asthma. In the healthy patients they observed low amplitude (25 to 100 μ V) potentials with widely variable discharge frequency. Both amplitude and frequency of AP's were increased during spontaneous and Mecholyl-induced asthmatic attacks. These changes corresponded properly to the clinical features of the asthmatic attack, including marked disturbance in breathing pattern and marked increase in wheezing sound. These authors considered these bronchial SM spasms to be one of the major etiological factors responsible for airway narrowing during asthmatic attack. Finally, additional evidence for the possibility that spontaniety might occur in trachealis was provided by pharmacological studies on a different preparation. Mekata (1971) showed that membrane depolarization and spontaneous phasic electrical and mechanical activity is produced in rabbit carotid artery, an "intermediate" SM, by tetraethylammonium (TEA). These findings were tested and confirmed for trachealis (Kroeger and Moorhouse, 1973). This drug caused a depolarization of the membrane and an increase in r_m , elicited trains of small, decrementally conducted AP's and facilitated a MR to stretch (Stephens et. al., 1974; Kroeger and Stephens, 1975). These effects were attributed to a reduction in P_K by TEA (Stanfield, 1970; Ito et. al., 1970), which functionally converts TSM into a single unit preparation. As a result, it was considered that TEA might provide us with an excellent model for studying the changes in biophysical properties which might occur in vivo.

1. Definition of the Problem

In studying the way in which TEA affects TSM physiology, investigations should be conducted in terms of the muscle's function and the mechanism by which the control of that function is normally effected. The function of muscle is 1) to stiffen and bear weight and 2) to shorten and move a load through a distance, and relative parameters for TSM in these terms have been measured (Stephens et. al., 1969; Stephens et. al., 1974). Since TEA increases r_m , produces AP's and facilitates a Ca - dependent MR to stretch, it effects a change in the control mechanism probably at the levels of membrane P_K and the ionic requirements of electric

electrical activity and E-C coupling. The present study focuses on the latter.

The above changes in membrane phenomena induced by TEA are of special interest since in most cases (Somlyo and Somlyo, 1968; Hinke, 1965) excitation of SM cells is mediated by membrane electrical activity which in turn may be affected by neurotransmitter release or circulating vasoactive agents (Bülbring, 1957; Bülbring and Kuriyama, 1963 b; Burnstock et. al., 1963; Burnstock and Holman, 1966 b; Marshall, 1962). Also, multiunit SM contains a relatively large store of sequestered Ca which is resistant to depletion by Ca-free media (Kroeger and Stephens, 1971) and can be mobilized by drugs for contraction (Devine et. al., 1973). Consequently, we considered it important to provide further information regarding the Ca pool (s) utilized for electrical activity and E-C coupling in the presence of TEA. Specifically, our experiments were designed to demonstrate whether the Ca mobilized by TEA might result from 1) a non-specific increase in membrane permeability which results in depolarization and Ca influx in parallel, 2) a specific activation of Ca channels by depolarization which provides activator Ca for contraction (Brading et. al., 1969) or 3) a release of trigger Ca which mobilizes sequestered Ca (Imai and Takeda, 1967). Since the effects of TEA on TSM had been shown to be abolished by Ca-deficient solution and a Ca-antagonist (Stephens et. al., 1974) (D-600, a potent inhibitor of dynamic, depolarization - activated Ca channels, Kohlhardt et. al., 1972), it appears likely that activated Ca currents may play an important role in the electrophysiological (as a current-carrier) and biophysical (as "activator" or "trigger" Ca) effects mediated by TEA. Therefore, this study was undertaken in order to:

1. Determine the voltage dependence of dynamic Ca channels by effecting an independent increase in r_m and measuring resultant changes in AP parameters.
2. Determine the role of Ca_o in electrical activity and in E-C coupling by measuring the effect of elevated external Ca and a Ca-antagonist, D-600, on AP parameters and mechanical responses.
3. Determine the sensitivity of membrane conductance to external Ca levels by measuring the amplitude of the hyperpolarizing electrotonic potential

(ETP) (which is directly proportional to r_m) simultaneously with AP's during treatment with high Ca and D-600.

B. THE MEMBRANE CONCEPT AND THE IONIC BASIS OF THE MEMBRANE POTENTIAL

1. Nerve and Striated Muscle

a) Nernst equation, Bernstein hypothesis and Gibbs - Donnan equilibrium

That the integrity of the cell is maintained by a specialized structural arrangement on the cell surface was appreciated by the turn of the century due to observations of osmotic and electrical phenomena. Long before the molecular structure and composition was deduced (Davson and Danielli, 1943), Overton (1902 a) had studied the osmotic behaviour of frog striated muscle and Bernstein had used a ballistic galvanometer to determine the time course of the AP. Though it was known that the cell interior was negative with reference to the outside (i.e. that there was a voltage or potential difference across the membrane) and contained a much higher concentration of K^+ ions, it was not known how these phenomena were accomplished by the cell. In 1902, Bernstein proposed a theory which applied the physicochemical concepts of Nernst and Ostwald to bioelectric phenomena. He suggested that the resting cell surface behaved as a membrane which was selectively permeable only to K^+ ions, which meant that the resting membrane potential (RMP) was defined by the K equilibrium distribution. The relationship of the K equilibrium potential (E_K) to the K distribution is best described by deriving the Nernst equation (Woodbury, 1965).

Electric potential differences, in general, appear at the boundaries between two electrolyte solutions if there are ions of different mobility or concentration (properly expressed as activity) on either side. In the absence of a membrane one obtains a small, transient liquid - junction potential or diffusion potential until the concentrations are equalized by diffusion. However, if two such solutions are separated by a membrane having differential permeabilities to ions of the same charge (e.g. Na^+ and K^+), a greater transient, transmembrane diffusion potential will be established until the transmembrane concentration

gradient of each ion species is abolished. The behaviour of ions in such a system is governed by the following factors: 1) that the transmembrane flux of diffusible ions will continue until their individual concentrations are equal on both sides of the membrane if transmembrane electrical potentials do not prevent this; 2) that the diffusion potential will transiently impede ion fluxes; 3) that the bathing fluids do not accumulate any net charge (i.e. retain bulk neutrality); and 4) that the rate of flux of any ion is limited by the permeability of the membrane to that ion.

In the case of Bernstein's theory, which states that the membrane is permeable only to K^+ ions, the K concentration gradient is maintained by the presence of large, intracellular organic anions (A^-) to which the membrane is impermeable. The electrical neutrality of the two (intra - and extracellular) solutions is thus established even though the K^+ ions are out of equilibrium with reference to concentration. As a result, opposite charges build up in the vicinity of the membrane creating a transmembrane potential.

The relationship between potential and ion gradient can be quantified in terms of 1) an equilibrium distribution of a given substance (in which no work is needed to transfer a small amount of that substance across the membrane), 2) a transmembrane potential capable of equalizing fluxes regardless of concentration gradients or membrane permeabilities, and 3) electrochemical laws. The amount of work necessary to move a given number of ions (e.g. 1 mole) across a membrane consists of the sum of the work necessary to overcome concentration differences (W_c) and electric forces (W_e). In a system, their effect will be additive (i.e. total work, the electrochemical potential difference $\mu = W_c + W_e$) and each can be considered separately by keeping the other factor constant.

Using the K^+ ion as an example, the chemical potential difference (W_c) is proportional to the difference between the logarithms of the internal and external K^+ concentrations. The universal gas constant, R , and absolute temperature, T , apply in changing the proportionality to an equation. Thus, for one mole of ions,

$$W_c = RT (\log_e [K^+]_i - \log_e [K^+]_o) \\ = RT \log_e \frac{[K^+]_i}{[K^+]_o}$$

The electrical potential energy difference (W_e) of one mole of ions is equal to the product of the transmembrane voltage, E_m , the Faraday, F and the valence, z , of the ion involved, so that

$$W_e = z_K F E_m$$

As a result, the electrochemical potential difference ($\Delta \mu$) of that ion is expressed as:

$$\mu = W_c = W_e = RT \log_e \frac{[K^+]_i}{[K^+]_o} + z_K F E_m$$

Ionic equilibrium obtains when $\Delta \mu$, and therefore, net flux, is zero. At this point E_m will equal the equilibrium potential (E_x) for a given ion (e.g. E_K) (the potential which reduces net transmembrane flux to zero). This potential can be calculated by setting $\Delta \mu$ to zero, substituting E_K for E_m and solving for E_K , yielding the Nernst equation:

$$E_K = \frac{RT \log_e [K^+]_o}{z_K F \frac{[K^+]_i}{[K^+]_o}} \\ = \frac{RT \log_e [K^+]_o}{z_K F \frac{[K^+]_i}{[K^+]_o}} = (1.99) (310) \cdot 2.3 \log_{10} \frac{[K^+]_o}{[K^+]_i} \\ = \frac{F}{23,050} \log_{10} \frac{[K^+]_o}{[K^+]_i} \text{ volts (V) (at } 37^\circ\text{C)} \\ = -61 \log_{10} \frac{[K^+]_o}{[K^+]_i} \text{ mV}$$

Thus, Bernstein believed that the RMP behaved as a K^+ electrode whose potential (E_K) is related to the transmembrane K^+ ion concentration gradient in the

above manner. Na^+ and Cl^- ions were not thought to play any role in this regard. The theory was attractive in that it provided an efficient mechanism for the maintenance of the ionic concentration gradients by the resting cell, since movement of K alone down its concentration gradient would create an opposing electrostatic force. Therefore, the existence, the electrical sign and the approximate magnitude of the RMP seemed to be accounted for. Membrane potentials were recorded which were 10 to 15 mV less negative than the equilibrium potential calculated in the above manner from known values for intra- and extracellular K concentrations. Such deviations, however, were expected, considering the unsophisticated experimental apparatus in use at that time, and were attributed to experimental error (i.e. liquid junction potentials). Although the theory was modified in the 1940's to include permeability to other ions, the close dependence of the RMP on the K distribution was later demonstrated by Adrian (1956) who plotted RMP and E_K as functions of $\log [K]_o$ and by Hodgkin and Keynes (1955 b) who showed that the RMP reflected the $[K]_o$ even more closely during the recently documented period of increased K conductance (g_K) which followed the AP.

The first alteration to the notion of exclusive K permeability was put forward by Boyle and Conway (1941), who showed that the frog sartorius muscle fiber also possesses a significant Cl^- ion permeability (P_{Cl}), since increments in $[K]_o$ resulted in a substantial influx of Cl^- . In addition, no appreciable change in cell volume was observed when external KCl was progressively increased to 210 m eq./l. Consequently, these authors extended the Bernstein hypothesis by proposing that the RMP, and the distribution of K^+ , Cl^- and H_2O , could be accounted for by the conditions required for a Gibbs - Donnan equilibrium, whereby the membrane is impermeable to large, intracellular organic anions and to extracellular sodium ions (Na^+). This meant that K^+ and Cl^- would distribute themselves according to $\frac{[K^+]_i}{[K^+]_o}$

$$= \frac{[Cl^-]_o}{[Cl^-]_i}, \text{ such that } E_m = -\frac{RT}{F} \ln \frac{[K^+]_i}{[K^+]_o} = -\frac{RT}{F} \ln \frac{[Cl^-]_o}{[Cl^-]_i}, \text{ and } E_m \text{ versus } \log$$

$[K]_o$ would have a slope equal to 61 mV per tenfold change in $[K]_o$.

The assumption of a Na^+ - impermeable membrane had to be abandoned, however, after publication of data from radioactive tracer studies (Levi and Ussing, 1948; Harris and Burn, 1949; Keynes, 1954) on frog skeletal muscle. These and later radioisotope experiments have shown that both Na^+ and K^+ are in a steady-state of flux across the membrane and that the net passive fluxes are almost of the same magnitude (Hodgkin and Horowicz, 1959; Keynes and Swan, 1959). The Gibbs - Donnan distribution has also become untenable because, in the absence of ion pumping, the presence of large, impermeant, intracellular organic anions would present the cell with an osmotic imbalance such that K^+ , Cl^- and water would flow down their gradients. This flux would continue until the cell burst, unless prevented by an outward - directed water pump or a rigid cell membrane to maintain cell volume; in view of the fluid chemical nature of the plasma membrane, the latter possibility is highly unlikely.

b) Constant - Field theory

A new explanation had to be derived which could account for the additional influence of P_{Na} and the strong inward electrochemical potential gradient for Na on the RMP. The ratios of the K and Cl concentrations in muscle agreed satisfactorily only for $[K]_o > 10$ mM, below which E_m deviated somewhat from E_K (i.e. slope of E_m vs. $\log [K]_o < 61$ mV per tenfold change in $[K]_o$). A suitable set of equations and assumptions that are still in use today were derived by Goldman (1943) and modified by Hodgkin and Katz (1949).

Goldman studied the mechanisms (i.e. conductance changes) underlying net ion movements due to diffusion and electrical forces, using artificial protein-lipid membranes. From these studies he derived the constant-field theory which assumes that 1) the ions move under the influence of diffusion and the electric field as they would if in free solution, 2) the electric field is constant throughout the membrane, 3) the concentrations of ions at the edges of the membrane are directly proportional to those in the intra- and extracellular solutions and 4) the membrane is homogeneous. This theory led to the following equation for the K current (I_K) as a function of E_m and K distribution:

$$I_K = P_K \frac{F^2 E_m}{RT} \frac{[K^+]_o - [K^+]_i \exp\left(-\frac{FE_m}{RT}\right)}{1 - \exp\left(-\frac{FE_m}{RT}\right)} \quad (2)$$

where $P_K = \mu_K B_K \left(\frac{RT}{aF}\right)$ and μ_K = ionic mobility in the membrane, B_K = partition

coefficient between the aqueous solution and membrane phase, and a = membrane thickness. The factor $\frac{FE/RT}{1 - \exp\left(-\frac{FE}{RT}\right)}$ represents the effect of the electric field

field in lowering or raising the chances of passage of individual ions (Katz, 1966).

Though the constant - field equations have proved to be very useful in the development of the theory of excitable membranes (Hodgkin and Huxley, 1952 b; Hodgkin, 1951, 1958), they do present some difficulty. Current available information predicts that the membrane is far from being a homogenous structure: the proteins appear to be fixed to and imbedded to varying degrees in the lipid bilayer (Fox, 1972). With such variation obviating a very non-homogenous fixed - charge distribution, and the fact that estimates of the membrane thickness still conflict, it is doubtful that the electric field is constant throughout the membrane.

The modification that Hodgkin and Katz (1949) made was to solve for E_m in terms of the ion permeabilities and concentrations. If there is no ionic current flowing across the membrane, the net movement of K, Na and Cl ions must be such that there is no net charge movement, so $I_K + I_{Na} + I_{Cl} = 0$. Under these conditions the membrane potential is given by

$$E_m = \frac{RT}{F} \ln \frac{P_K [K]_o + P_{Na} [Na]_o + P_{Cl} [Cl]_i}{P_K [K]_i + P_{Na} [Na]_i + P_{Cl} [Cl]_o} \quad (3)$$

in which P_K , P_{Na} and P_{Cl} are permeability coefficients (usually expressed in units of 10^{-6} cm/sec). These investigators calculated the ratio of $P_K : P_{Na} : P_{Cl}$ for the squid axon at rest by a process of trial and error: they chose values for the

internal concentrations of K, Na and Cl (Steinbach, 1941; Steinbach and Spiegelman, 1943) that yielded the best agreement with the changes in E_m obtained on exposure to K - free, K - rich, low Na and Cl and high Na and Cl solutions.

Under resting conditions, this ratio was 1 : 0.04 : 0.45.

This valuable equation (3) was simplified (Hodgkin and Katz, 1949; Hodgkin, 1958) in order to calculate the ratio $\frac{P_{Na}}{P_K}$. The Cl terms can be eliminated by assuming that Cl

assuming that Cl^- ions are in equilibrium with the RMP (Conway, 1957; Hodgkin, 1958) or that $[Cl]_i$ is negligible in Cl - free solution (Hodgkin and Horowicz, 1959 b; Mullins and Noda, 1963), yielding

$$E_m = \frac{RT}{F} \ln \frac{[K]_o + b [Na]_o}{[K]_i + b [Na]_i} \quad (4)$$

where $b = \frac{P_{Na}}{P_K}$. Thus, when $b = 0$, $E_m = E_K$, and when b is very large it defines

E_{Na} . According to the Goldman hypothesis then, E_K and E_{Na} are limiting values, and intermediate values of b correspond to intermediate membrane potentials. Values of b for nerve and skeletal muscle have been calculated as about 0.01 (Hodgkin, 1958; Hodgkin and Horowicz, 1959 a). Hodgkin and Katz (1949) then calculated the permeability coefficients in two ways: 1) by dividing the flux value by the product of the greater concentration and the "electric field factor" $\left(\frac{EF/RT}{1 - \exp(-EF/RT)} \right)$

such that

$$\begin{aligned} P_K &= \frac{\text{efflux}}{[K]_i \times \text{EFF}} = \frac{2.0 \times 10^{-6} \text{ cm/sec}}{[K]_i \times \text{EFF}} \\ P_{Na} &= \frac{\text{influx}}{[Na]_o \times \text{EFF}} = \frac{2.0 \times 10^{-8} \text{ cm/sec}}{[Na]_o \times \text{EFF}} \end{aligned} \quad (5)$$

and 2) from the ratio b and the cell membrane resistance (r_m) (to which P_{Na} makes

a negligible contribution), from which they calculated $P_K = 1.8 \times 10^{-6}$ cm/sec. Hodgkin and Horowicz (1959 b) found a similar value for P_K in frog skeletal muscle. In summary, then, the ionic currents are dependent only on the electrochemical potential gradients and the ease with which the ions may cross the cell membrane (Hodgkin, 1951, 1958). The nerve and muscle cell membrane is much more (about 75 times) permeable to K than to Na, and it is this property which is the major determinant of E_m .

c) Sodium pump

As stated earlier, any deviation of the recorded RMP from the calculated ion equilibrium potentials signifies a disequilibrium which must be maintained by an active ion transport mechanism. The deviation is accounted for by the small but significant permeability to external Na^+ ions, which means that Na would be constantly moving into the cell under the influence of the high driving force of its electrochemical gradient ($E_m - E_{Na} \approx -90 - 60 = -150$ mV). This, along with the passive K loss due to the membrane's relatively high P_K , would eventually abolish the Na and K concentration gradients, and consequently, E_m . It is evident that there must be a mechanism to maintain the observed high $[K]_i$ and low $[Na]_i$. Three types of Na pumps, or alternatively, one pump working in different ways under different conditions, have been proposed: 1) an electrogenic Na pump which extrudes Na ions as fast as they diffuse into the cytoplasm (i.e. net Na flux = 0), with K and Cl ions distributing themselves asymmetrically as in a Donnan equilibrium; 2) an electroneutral Na - K exchange pump which moves one K inward for every Na expelled; and 3) an electrogenic Na - K pump which transfers more Na than K, thus producing a net transfer of outward charge which contributes to the RMP.

Ussing (1949) proposed the first type of pump to account for the uneven distribution of K, which would be drawn into the cell due to the high internal negativity maintained by the Na pump. If this was the case however, E_m would equal E_K which, of course, does not occur. The constant - field theory, however, agrees fairly well with observed values of E_m and can be derived assuming the presence of an electroneutral, 1 : 1 pump (Hodgkin, 1958). Originally proposed by Dean (1941),

by Dean (1941), Hodgkin and Keynes (1955 a) provided evidence for this type of coupling in squid axon along with the characteristics and requirements of pumps in general. Since it had been elegantly demonstrated 1) that Na and K ions moved passively down their electrochemical potential gradients at rest, and during excitation in response to increases in permeability (Hodgkin and Huxley, 1952 a,b), and 2) that membrane permeability was intimately related to E_m (Goldman, 1943; Cole and Curtis, 1941; Hodgkin and Huxley, 1952 b), changes in pump activity should have no effect on RMP or spike activity and, conversely, alterations in E_m should not affect ion pumping. Furthermore, movement of ions "uphill" against their electrochemical potential gradients must require metabolic energy. Thus, it was shown that both Na efflux and K influx 1) were inhibited by metabolic inhibitors and cooling, 2) were unaltered by membrane hyperpolarization and 3) had little effect, when inhibited, on E_m and AP's. They also observed that changes in Na extrusion were very similar to the changes in K uptake due to alterations in energy supply and external Na and K levels. As a result, they proposed a tightly-coupled, 1 : 1 exchange pump, but cautioned that the techniques employed might not detect small electrogenic effects. Also, the coupling ratio might not be strictly 1 : 1 since K - free solution inhibited Na extrusion to a lesser extent than inhibition of energy production. A small K accumulation outside the membrane, though, might be sufficient to maintain a tight coupling ratio even in K - free solution.

Coupling of K influx to the Na extrusion has been demonstrated many times, but the majority of evidence supports the notion of an electrogenic pump. Lundberg and Oscarson (1952) showed that the postanoxic overshoot of the demarcation potential of cat spinal roots was 10 mV higher than that in normoxic medium and did not occur in Na - free solution. Ritchie and Straub (1957) attributed the posttetanic hyperpolarization (PTHP) of nonmedullated mammalian C fibers to the Na pump. Although they believed the PTHP to be caused by the pump's increased K^+ uptake leading to a local reduction in external K^+ , Adrian and Slayman (1966) later demonstrated an additional, reversible hyperpolarization in frog skeletal muscle when K_o was replaced with rubidium. It was further shown that the PTHP

produced by the electrogenic Na pump in nerve is dependent upon the presence of K^+_o and Na^+_i , can be reduced by the short-circuiting effect of internal Cl^- ions and by metabolic inhibitors, and is enhanced by conditioning hyperpolarization that shows no reversal potential (Rang and Ritchie, 1968; Nakajima and Takahashi, 1966). It must be noted that any treatment which inhibits the Na pump will lead to a loss of K^+_i , which will decrease E_K and E_m depending on passive K movement (Thomas, 1972). Though results from such interventions can also be interpreted as being due to an augmentation of an electrically neutral pump, a rapid change in pump activity and E_m is supportive evidence for an electrogenic contribution.

The most convincing evidence is the immediate membrane hyperpolarization produced by intracellular injection of Na into nerve fibers (Coombs et al., 1955; Hodgkin and Keynes, 1956) and by warming of nerve and skeletal muscle (Adrian and Slayman, 1966; Senft, 1967). This subject has been extensively examined and reviewed (Koketsu, 1971; Thomas, 1972), and there is a good deal of evidence to suggest that the Na pump in nerve and skeletal muscle might be electrogenic, thus contributing only a few mV to the RMP, probably through a 3 Na : 2 K coupling ratio (Mullins and Brinley, 1969).

d) Adsorption theory

The foregoing evidence in support of the presence of a highly - organized, semipermeable membrane has met with some opposition, however. The majority of investigators subscribe to the membrane theory, but a small group believes 1) that the functions ascribed to the membrane are subserved by the cytoplasm, 2) that the greater part of the internal negative charges are provided by the polyelectrolyte proteins, 3) that K^+ ions possess selective affinity and are chemically bound to the proteins, and 4) that the internal water behaves in a physically anomolous fashion and is called "structured water" (Ling, 1962; Ling and Walton, 1975; Troshin, 1966). According to this theory, osmotic equilibration to different solute concentrations should be attributed to the swelling and shrinking of the polyelectrolyte gel rather than to the presence of a semipermeable membrane. Conway (1950) however, supports the view that the structural proteins with their fixed electric

charges and associated counterions make only a small contribution to the osmotic balance. He calculated that the negative charges in muscle are largely supplied by organic phosphates and amino acids and only about 10% are fixed to structural proteins.

There is considerable evidence at variance with this theory, showing that the cell surface possesses a low ion permeability (high resistance) which can be altered by ionic interventions, electric fields and drugs, and that the inner cytoplasm is very conductive (which the adsorption theory denies). The axoplasm of squid giant axons can be squeezed out and replaced by artificial solutions or a glass capillary with little harm to conduction, while local injury to the surface results in reversible loss of E_m and impulse conduction (Baker *et al.*, 1962). Electrical measurements of cable properties (Cole and Hodgkin, 1939; Cole and Baker, 1941; Hodgkin and Rushton, 1946; Katz, 1948; Falk and Fatt, 1964) have shown that the cytoplasm behaves as a good electrolyte conductor (low resistance) which is separated from the medium by a high resistance, high capacitance surface layer. Finally, the half time for entry of labelled K^+ ions into 200 μ diameter axons was approximately 10 hours while values for intracellular selfdiffusion and ionic mobility were only slightly less than corresponding values in sea water (Hodgkin and Keynes, 1953).

2. Smooth Muscle

a) Membrane potential and permeability

There is reasonable agreement that the general ionic distribution and permeability properties of SM resemble those of other excitable cells. Interpretation of data from studies on ionic distribution and ion fluxes, though, reveal a system that is quantitatively more complex and obscure.

The early work of Meigs (1912), repeating Overton's studies on osmotic behaviour, led him to state that the SM cell did not behave as if bounded by a semipermeable membrane but that the skeletal muscle cell did. Bozler (1959, 1962) also concluded that the cell cytoplasm was more important than the membrane in determining the distribution of water and electrolytes in SM. Electrophysiological studies, on the other hand, have shown that the taenia coli and vas deferens of the

guinea pig (Tomita, 1966a; Abe and Tomita, 1968), the rat myometrium (Abe, 1971) and dog trachealis (Kroeger and Stephens, 1974) exhibit cable properties similar to those described by Hodgkin and Rushton (1946). This is very strong evidence in support of the membrane theory.

An interesting feature of the SM cell RMP, which is difficult to measure because of the very small cell diameter, is its relatively low value and stability compared to that of nerve, striated muscle and cardiac muscle (Bülbring, 1962; Marshall, 1962). Average values for RMP's range from - 40 mV for rabbit bladder (Ursillo, 1961) to - 64 mV for progesterone - dominated rat myometrium (Marshall, 1959). Estimates for E_K range from - 75 mV for rat and guinea pig myometrium (Casteels and Kuriyama, 1966; Inomata, and Kao, 1976) to - 90 to - 95 mV for taenia coli (Casteels and Kuriyama, 1966; Bülbring et. al., 1968; Vassort, 1975). Present evidence suggests that the RMP is mainly determined by the K distribution and that the RMP and ion distribution can be accounted for by the constant - field theory. The membrane potential, however, deviates much more markedly from the K equilibrium distribution described by the Boyle-Conway theory (Gibbs - Donnan distribution) than it does in other excitable tissues. Thus, the slope of the line relating E_m to $\log [K]_o$ is non-linear for taenia coli and has a maximum slope of 35 - 38 mV per tenfold change in $[K]_o$ for concentrations above 29.5 mM K^+ (Holman, 1958; Kuriyama, 1963 a; Casteels and Kuriyama, 1966). Below this concentration the slope is only 26 - 30 mV per tenfold change (Burnstock and Straub, 1958). Substantial evidence is available that the Na and Cl distributions play a much greater role in determining E_m in SM's, as discussed below.

Casteels (1969 a, 1970) has calculated the permeability coefficients for taenia coli from measurements of intracellular ion concentrations (Casteels and Kuriyama, 1965, 1966), flux values (Goodford and Hermansen 1961; Durbin and Monson, 1961) and RMP using the constant-field assumptions. It was also assumed that the passive fluxes were due only to their own electrochemical forces and that all ion fluxes were independent of each other (Hodgkin and Huxley, 1952 a).

Multiplying the electric field factor $\left(\frac{EF/RT}{1 - \exp(-EF/RT)} \right)$ at $E_m = - 55$ mV by the

internal ion concentration and dividing into the flux value, he obtained $P_K = 8.3 \times 10^{-8}$ cm/sec, $P_{Na} = 1.6 \times 10^{-8}$ cm/sec and $P_{Cl} = 5.45 \times 10^{-8}$ cm/sec. Compared to values obtained for frog skeletal muscle fibers (Hodgkin and Horowicz, 1959 b), P_K and P_{Cl} are much smaller while P_{Na} is quite similar, resulting in a much greater $\frac{P_{Na}}{P_K}$ ratio in SM. For the taenia coli, calculated ratios have

included 0.23 (Schatzmann, 1964a), 0.19 (Casteels, 1970) and 0.16 (Inomata and Kao, 1976). Thus, although K^+ ions are the main factor determining E_m (Casteels and Kuriyama, 1966), P_{Na} is relatively high in SM, as evidenced by 1) the low P_K (see above), 2) the effect of Na in producing nonlinearity in the relation between E_m and $\log K_o$ for K_o up to 30 mM (Kuriyama, 1963a), and 3) the high rate of exchange of radioactive ^{24}Na ($t_{1/2} = 1$ minute, Goodford and Hermansen, 1961; Durbin and Monson, 1961). With respect to the last observation, the extremely high Na efflux rates can not be reconciled with the contribution of active processes, as SM does not contain sufficient energy stores to support all this movement (Brading, 1976). Consequently, an energy - sparing Na - Na exchange mechanism has been postulated (Brading, 1976). The observations and calculations discussed above are consistent with the relatively low RMP and high membrane resistance (Tomita, 1966b; Abe and Tomita, 1968).

b) Chloride distribution

This is a very interesting aspect to ion distribution in SM cells. As mentioned before, the RMP is 25 to 35 less negative than E_K and the maximum slope of the relation between E_m and $\log [K]_o$ is only 38 mV per tenfold change in $[K]_o$ (Kuriyama, 1963 a; Casteels and Kuriyama, 1966). A RMP governed by a membrane permeable only to K and Cl, and by a passive Gibbs - Donnan distribution, would follow the equilibrium potential for K as specified by the Nernst equation. Therefore, there clearly exists, in SM, a disequilibrium, since the Nernst predicts a slope for E_m vs. $\log [K]_o$ of 61 mV per tenfold change in $[K]_o$. As already pointed out above, this may partially be attributed to the influence of Na with its high electrochemical gradient. Recent work has shown that Cl plays a major

role in determining the low RMP.

According to Casteels (1970) and Casteels and Kuriyama (1966), E_{Cl} is 25 to 35 mV less negative than the RMP of the taenia coli of the guinea - pig. The major reason for this, of course, would be the presence of an inward - directed Cl pump which increased $[Cl^-]_i$, thereby decreasing E_{Cl} $\left(E_{Cl} = - 61 \log \frac{[Cl]_o}{[Cl]_i} \right)$,

and consequently, the RMP toward zero potential (Casteels, 1965; Kao, 1967). Supporting this notion is the high $[Cl]_i$ (Kao and Nishiyama, 1964), which does not have to be explained by compartmentalization or binding, since the major component of Cl efflux behaves as a single fraction (Durbin and Monson, 1961; Casteels, 1965, 1970). Droogmans and Casteels (1976) have calculated that Cl - pumping in taenia coli contributes approximately 15 mV to the RMP, since in Cl - free solution the electrogenic Na pump contributed only 6 mV. The increase in Cl - efflux observed after exposure to ouabain or high - K solution indicated that Cl uptake may be coupled to the Na - K pump (Casteels, 1971).

The second factor leading to a more positive E_m is the relatively high P_{Cl} . This is suggested by the rapid Cl efflux (Barr, 1959; Durbin and Monson, 1961) and the effects of replacing external Cl with less permeant anions. Substitution with sulphate, ethane sulphonate or isethionate results in 1) a transient depolarization (Holman, 1958), 2) a reduction in the difference between the slopes of E_m and E_K vs $\log K_o$ (Kuriyama, 1963 a; Burnstock and Straub, 1958), 3) a 50% increase in r_m (Ohashi, 1970) 4) a decrease in K efflux (Casteels and Meuwissen, 1968), and 5) suppression of the secondary depolarization of the control potentials (slow waves) of rabbit jejunal SM (El Sharkawy and Daniel, 1975). The effect of Cl - substitution on E_m is time-dependent. During the initial period for anion substitution, E_{Cl} becomes more positive due to decreased extracellular Cl

$\left(E_{Cl} = - 61 \log \frac{[Cl]_o}{[Cl]_i} \right)$. At the same time, active Cl uptake is reduced due

to lack of substrate, so intracellular Cl leaks out under the influence of its new electrochemical gradient. Consequently, E_{Cl} becomes progressively more negative

(thus hyperpolarizing E_m) and becomes established at a new value determined by the nature of the replacement anion. E_{Cl} becomes more negative with a more permeant anion (eg. nitrate) than with a less permeant anion; in the latter case the final RMP is more positive than it was in Cl solution (Holman, 1958; Kuriyama, 1963 a). The overall effect, therefore, of replacing Cl with a less permeant anion is a decrease in Cl fluxes, resulting in membrane depolarization and an increase in r_m .

c) Sodium pump

From the values calculated above, the RMP for taenia coli calculated from the modified Goldman equation (#3 - Hodgkin and Katz, 1949) equals - 35 mV (Casteels, 1970) while the measured value is - 50 to - 55 mV. This discrepancy may indicate 1) that the assumption of independent ion movement is invalid or 2) that the difference is maintained by an electrogenic Na pump. It is possible that K^+ ions move in a single file along a chain of sites (Hodgkin and Keynes, 1955 b), so the calculated g_K would be n times lower than the real g_K :

$$g_K = n \frac{P_K F^3 E_{K^+}}{R T^2} \frac{1}{1 - \exp(-EF/RT)} \quad (6)$$

There is considerable evidence, however, to suggest the existence in SM of an electrogenic Na pump. Ouabain and metabolic inhibitors cause a rapid depolarization of rat uterine SM and taenia coli (Daniel et al., 1962; Casteels, 1966) which is abolished after removal of ouabain and reversed to 10 mV higher than control. Removal of external K caused about a 5 to 10 mV depolarization of taenia coli which was unaffected by tetrodotoxin, atropine or low Cl solution, but after ouabain or cooling the removal of K caused a hyperpolarization (Tomita and Yamamoto, 1971; Casteels et al., 1971 a). Kuriyama and co-workers (1971) found that ouabain, Na - free solution or cold reduced E_m of guinea - pig portal vein by 20 to 26 mV and estimated that the normal electrogenic - Na pump contribution to E_m was 10 to 20 mV. Several workers have shown a correlation between the slow electrical waves of intestinal SM and oscillatory activity of an electrogenic Na pump (Daniel, 1965; Job, 1969; Liu et al., 1969), though the

contribution is shared with changes in g_{Na} and g_{Cl} (El Sharkawy and Daniel, 1975). Relaxation of rat myometrium by adrenergic beta receptor agonists has been correlated with an increase in E_m (Marshall, 1967) and attributed to stimulation of an electrogenic Na pump (Somlyo et. al., 1970). However, ouabain and lithium chloride do not antagonize the isoproterenol - induced relaxation (Bose and Innes, 1972) and Na - free solution does not alter the hyperpolarization (Marshall, 1968). The KCl - induced relaxation of Na - enriched cat carotid artery (Bose and Innes, 1972) and rabbit main pulmonary artery (Somlyo et. al., 1970) is consistent with stimulation of an electrogenic Na pump. There appears to be a component of the RMP attributable to the Na pump in rat myometrium but the contribution to E_m is unknown (Taylor et. al., 1969, 1970). In summary, therefore, there is supportive evidence that a component of the RMP of SM's is attributable to an electrogenic Na - K pump. It has been concluded that the taenia coli pumps Na and K at a ratio of 3 : 2 (Brading, 1973; Casteels et. al., 1973) and contributes 15 to 20 mV to the RMP (Casteels et. al., 1971; 1973).

C. EXCITATION AND CONDUCTION

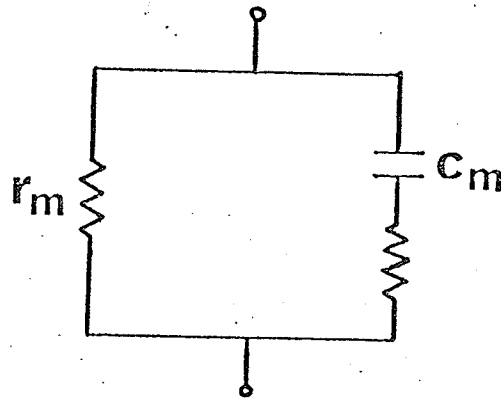
1. Cable Properties

a) Cable constants

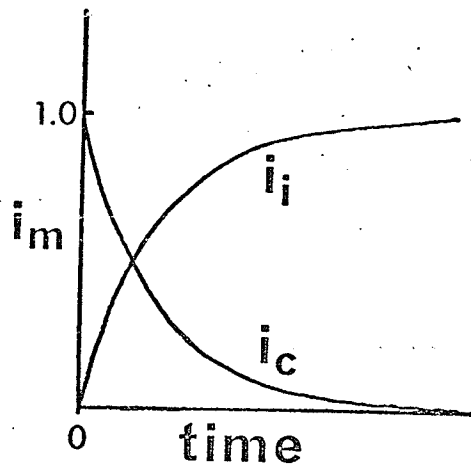
As was previously stated, nerve and muscle fibers are cylindrical conductors surrounded by surface membranes which insulate them from the external electrolyte solution. These fibers consequently possess properties analogous to those of an electrical coaxial cable. In attempting to relate the structure and function of these excitable (and non - excitable) preparations to this model, it is essential to derive a simple equivalent circuit. From this one may predict how changes in different cable parameters may effect current spread, and how these parameters may be calculated from measurement of downstream transmembrane voltage changes.

A simple electrical model or equivalent circuit (Figure 1a) was proposed by Cole (1937) and Hodgkin (1938) to explain the subthreshold voltage responses of a unit length of squid axon to brief square wave pulses of inward and outward current. The gradual decay of the extracellularly - recorded potential change

a)



b)



c)

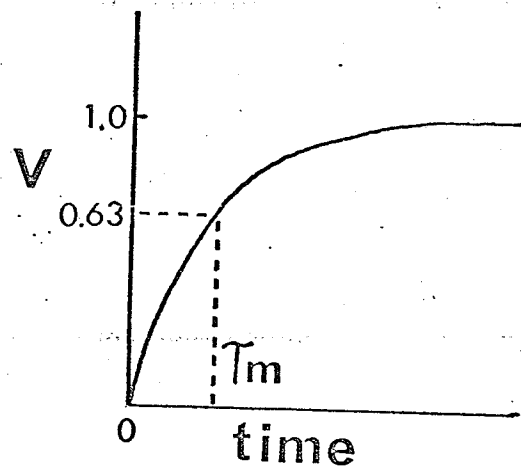


Figure 1. Panel a - Simple equivalent circuit model of unit nerve membrane; membrane resistance and capacitance, respectively. Panel b - Time course of membrane current flow through equivalent circuit; ionic current and capacitive current after onset of square - wave pulse. Panel c - Time course of ETP produced by membrane current flow; determination of membrane time constant (τ_m).

indicates that the membrane behaves electrically like a series resistance (r_m) with a capacitance (C_m) in parallel. The membrane resistance is a measure of its permeability to ions and is the inverse of the membrane conductance, g_m . The membrane capacitance is correlated with the large membrane surface area, the extremely small ($70 - 100 \text{ \AA}$) thickness of the membrane and the high dielectric constant of the insulating lipid-protein material. Functionally therefore, the membrane separates charge across a very small distance but the small ion permeability allows a small loss of charge, so the net effect is that of a high "leak" resistance in parallel with a capacitance. The capacitive component implies that the electrical energy supplied to the membrane (by ion gradients or stimulating current) can be stored in an electrostatic field.

Figure 1 also shows the time course of membrane current (i_m) and the resultant transmembrane voltage change (V), or electrotonic potential (ETP). Inward (anodal) stimulating current initially flows into the capacitance at a very high rate (capacitive current, i_c) causing it to be charged. As charges of opposite sign build up on the "plates" of the capacitor, the voltage across the plates increases, progressively opposing further capacitive current but increasing ionic current according to Ohm's Law. The net result of the current flow in this circuit is the exponentially-rising ETP. The temporal decay of the ETP when the stimulus is turned off is a mirror image of the rise and represents the discharging of the capacitance.

It can be seen from the preceding considerations that the total amount of current passing through the membrane at any instant is the algebraic sum of the ionic and capacitive current, given by

$$i_m = i_i + i_c$$

If the values of r_m and c_m are independent of voltage and time,

$$i_i = \frac{V}{r_m} \quad \text{and} \quad i_c = c_m \frac{dV}{dt}$$

and therefore,

$$i_m = \frac{V}{r_m} + c_m \frac{dV}{dt} \quad (2)$$

Thus, the value of r_m can be found from the relation between the steady state

amplitude of the ETP ($V_{t=\infty}$) in mV and the current intensity (m amp/cm²).

The value of c_m can be determined from the time course of V , which rises exponentially to 63% $\left(1 - \frac{1}{e}\right)$ of $V_{t=\infty}$, in a time " τ " after the current has been turned

on (and falls to $\frac{1}{e} = 37\%$ of $V_{t=\infty}$ in time τ after the current has been turned

off). Thus the time course is determined by the value of τ_m , the membrane time constant which is defined by

$$\tau_m = r_m c_m \quad (3)$$

and is the time taken for the change in total charge on the membrane to reach $\frac{1}{e}$

of completion (Aidley, 1971). The values of r_m and c_m for a unit length of membrane can be converted to values per unit area of membrane, the specific R_m and C_m by the equations

$$R_m = \frac{2 \pi r_m}{a} \quad C_m = \frac{c_m}{2 \pi a}$$

where a = cell radius. The voltage at the stimulating electrode at a time t is given by

$$\begin{aligned} V_{x=0,t} &= V_{x=0,t=\infty} e^{-t/\tau_m} \text{ for discharging} \\ &= V_{x=0,t=\infty} (1 - e^{-t/\tau_m}) \text{ for charging} \end{aligned} \quad (4)$$

On the basis of the above we can now predict how changes in the magnitudes of r_m and c_m would affect the ETP. A higher c_m would increase the time ($\tau_m = r_m c_m$) for the same current to charge it to a given voltage, though the final voltage across it would be the same since more charge is accumulated ($E = \frac{q}{c_m}$). A

high r_m would result in a greater IR drop across itself, i.e. an ETP of greater steady state ($V_{t=\infty}$) magnitude. Since the time course of the ETP is determined by the product of r_m and c_m , the time course would also be increased (i.e. decreased rate of rise, $\frac{dV}{dt}$) with a higher r_m . Therefore, alterations in membrane ion permeability

will have profound effects on the time course and amplitude of "passive" potential

changes, but to predict the spatial decay of the ETP one must account for the effect of the intra - and extracellular electrolyte solutions.

The model given above is somewhat oversimplified in that it does not account for the electrical resistances of the internal and external fluids, cell geometry or distance. If the cell has a uniform diameter that is very small compared with its length, the values of r_m and c_m can be determined from a consideration of the linear cable-like or "core-conductor" properties of the cell. Figure 2 a shows the equivalent circuit of the nerve axon as a coaxial cable which was proposed by Hodgkin and Rushton (1946) to account for "electrotonus" or electrotonic spread of potential changes (provided r_m and c_m are independent of voltage and time). All parameters in the circuit are referred to unit length of membrane, and r_o can be neglected if the cell is placed in a large volume of physiological saline.

If a current is passed into an axon at some point, the current will tend to flow along the axis of the conductor through r_i (i.e. path of least resistance). Since the membrane has a finite permeability to ions, it behaves as a current shunt to the exterior. Thus r_i and r_m have a voltage divider effect and forward (longitudinal) current spread (and hence, the ETP) will depend on the relative magnitudes of r_i and r_m . This qualitative relationship can be quantified. The current flowing in an axial direction along the inner conductor (i_i) at a distance x from the current source is given by

$$i_i = - \frac{1}{r_i} \frac{dV}{dx} \quad (5)$$

Taking the second derivative,

$$\frac{di_i}{dx} = \frac{1}{r_i} \frac{d^2V}{dx^2} \quad (6)$$

For all regions of the axon except those close to the stimulating electrode,

$$i_m = \frac{di_i}{dx}$$

Therefore, the second derivative of the change in V is proportional to the membrane current (Katz, 1966):

$$i_m = \frac{1}{r_i} \frac{d^2V}{dx^2} \quad (7)$$

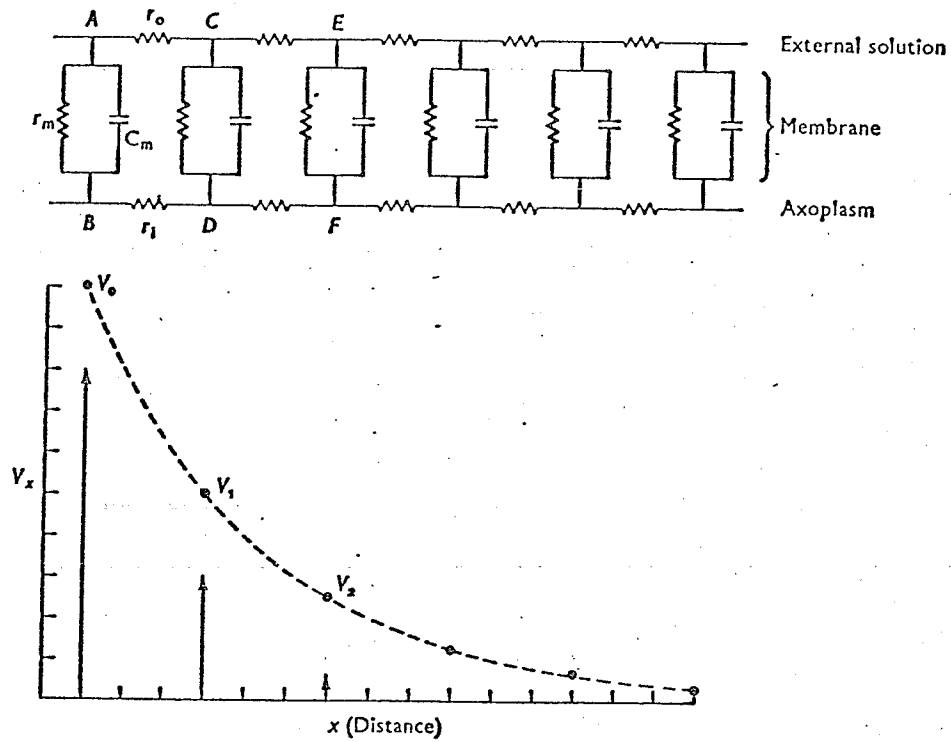


Figure 2. Panel a - Equivalent circuit of a length of core - conductor (e.g. nerve axon); membrane resistance and capacitance, external fluid resistance and internal fluid resistance given for unit lengths. Panel b - Spatial decay of the steady - state ETP ($V_{x,t=\infty}$).

Since

$$\begin{aligned} i_m &= i_i + i_c \\ &= \frac{V}{r_m} + C_m \frac{dV}{dt} \end{aligned}$$

then

$$\frac{V}{r_m} + C_m \frac{dV}{dt} = \frac{1}{r_i} \frac{d^2 V}{dx^2} \quad (8)$$

and

$$\begin{aligned} V &= \frac{r_m}{r_i} \frac{d^2 V}{dx^2} - r_m C_m \frac{dV}{dt} \\ &= \lambda^2 \frac{d^2 V}{dx^2} - \tau_m \frac{dV}{dt} \end{aligned} \quad (9)$$

$$(10)$$

Thus, the space or length constant and time constant are given by

$$\lambda = \sqrt{r_m/r_i} \quad (11) \text{ and } \tau_m = r_m C_m$$

This is the well known cable equation which describes the time course and spatial distribution of the ETP in long, thin cells (Hodgkin and Rushton, 1946).

For a constant current flowing for an effectively infinite time, the steady state voltage at distance x is given by

$$\begin{aligned} V_{x,t=\infty} &= \lambda^2 \frac{d^2 V}{dx^2} \\ &= V_{x=0, t=\infty} e^{-x/\lambda} \end{aligned} \quad (12)$$

This means that the voltage across the membrane falls off exponentially with distance from the point at which the current is applied (figure 2 b). If we make $x = \lambda$,

$$V_{x=\lambda, t=\infty} = e^{-1} \quad (13)$$

and λ can thus be defined as the length over which the transmembrane voltage falls to $\frac{1}{e}$ of its original value.

The net effect of the cable properties is 1) attenuation of the signal with distance, since current is constantly leaked off through the membrane with a

resultant increase in the effect of r_i with distance, and 2) slowing of the signal with time since progressively less current is available to discharge the adjacent c_m . It can easily be seen from $\lambda = \sqrt{\frac{r_m}{r_i}}$ that, assuming r_i remains

constant (which it does in the absence of volume changes), an increase in r_m will increase λ by reducing the shunting of internal current flow. The axial current will consequently spread a greater distance, as will the ETP which will therefore decay at a slower rate. In other words, the amplitude of the ETP is directly proportional to r_m . This relationship makes for a simple method of obtaining reliable estimates of changes in r_m , because ETP's can be recorded at various distances from the stimulating electrode before and after experimental manipulations.

b) Nerve

The similarity between excitable cells and electrical cables is most apparent in the geometry and ultrastructure of the nerve fiber. In studying the cable properties of such cells, the giant axon in the hindmost stellar nerve of the squid has proved to be an ideal preparation. The cells are extremely large (up to 1 mm in diameter), making it relatively easy to impale the membrane with a large capillary microelectrode (Hodgkin and Huxley, 1939; Curtis and Cole, 1940) for E_m measurements. In addition, the axoplasm can be carefully squeezed out of the cell for analysis of ion concentrations (Hodgkin and Keynes, 1953). A glass capillary or metal electrode can then be inserted down the fiber core and used for injecting substances or for recording electrical changes, with little impairment of impulse conduction (Curtis and Cole, 1942; Hodgkin and Huxley, 1945). Furthermore, compared to muscle, the nerve cell membrane is easier to penetrate with a glass microelectrode and, of course, the cell does not respond mechanically when stimulated as muscle does. Also, the lack of surface invaginations (T - tubule system in striated muscle) and cell junctions makes the measurement of resistance and capacitance much less complicated. Therefore, the bulk of the classical electrophysiological investigations regarding excitable cells have employed the squid giant axon.

Cole and Hodgkin (1939) first obtained a measurement of the resting R_m of

squid giant axon by passing a known square wave (DC) pulse down the axon and recording the steady state ETP extracellularly. They obtained a mean of 700 ohm cm^2 but assumed that the value should be approximately 1000 ohm cm^2 for a fresh tissue. Lobster and crab nerve have values of 2000 and 5000 ohm cm^2 , respectively (Katz, 1966). Cole and Curtis (1938, 1939) measured specific R_m and C_m by passing sinusoidal (alternating) current of various frequencies through stimulating electrodes in a bridge circuit and balancing the bridge with known values of variable resistance and capacitance. They and others (Cole, 1949; Hodgkin, 1939; Weidmann, 1951) found that C_m is 1.0 to $1.2 \mu\text{F/cm}^2$. These values are consistent with a bimolecular layer of lipid $50 \text{ \AA}$ thick, a dielectric constant of 5 or 6 and an electrical resistivity of $2 \times 10^9 \text{ ohm cm}$ (Hodgkin, 1958). These measurements have provided strong support for the membrane theory as the axoplasm resistance is only 30 to 60 ohm cm (Hodgkin, 1939; Weidmann, 1951).

Values for λ and τ_m for crustacean nerves (see Table 1) range from 2.5 to 5.0 mm and 0.7 to 5.0 msec, respectively (Katz, 1966). It will be shown in later sections that compared to different types of muscle, C_m is relatively small, and consequently has a direct bearing on the comparatively fast impulse conduction velocity of nerve.

c) Skeletal muscle

Structurally, single striated muscle fibers are long slender cylinders which are up to tens of cm in length and 10 to several hundred μ in diameter. Exceptions to this are crustacean muscles which are of similar diameter (100 - 600 μ) but much shorter, only 2 mm (Fatt and Katz, 1953). All of the fibers extend the length of the muscle and there are no low resistance electrical connections between fibers (Katz, 1966). Two major types of muscles, fast twitch and tonic, are differentiated according to their relative excitability (ability to produce all - or - none propagated AP's) and their innervation. In spite of real functional differences however, all striated muscle fibers have cable properties (Katz, 1948; Fatt and Katz, 1951, 1953 a; Burke and Ginsborg, 1956; Tasaki and Hagiwara, 1957; Falk and Fatt, 1964; Adrian and Peachey, 1965).

Fast twitch fibers closely resemble nonmyelinated nerve axons: each cell is a

Electrical Membrane Parameters of Nerve
and Striated Muscle

Preparation	R_m (ohm-cm ²)	C_m (μF/cm ²)	R_i (ohm cm)	λ (mm)	T_m (msec)	Ref.
A. Nerve:						
Loligo axon	1000	1.1	30	5	-	1
Sepia axon	-	1.2	60	-	-	2
Lobster axon	-	-	-	2.5	2	3
Crab axon	-	-	-	2.5	5	3
B. Skeletal Muscle:						
Frog sartorius	-	2.6	230 ^{d)}	2	24.5	4 & 5
	1900	6.1	-	-	12	6
	4100	8.0	-	-	34.5	7
Iliofibularis						
1) Twitch	3100	6.8	-	-	21.5	8
2) Slow	29000	1.6 - 2.5	-	-	46	8
Crab	116	42	-	-	4.6	9
Crayfish	1000	20	-	-	20	10

1) Hodgkin, 1939; 2) Weidmann, 1951; 3) Katz, 1966; 4) Falk and Fatt, 1964; 5) Katz, 1948; 6) Ishiko and Sato, 1960; 7) Fatt and Katz, 1951; 8) Adrian and Peachey, 1965; 9) Fatt and Katz, 1953; 10) Fatt and Ginsborg, 1958

functional unit no current flows to other cells, and ETP's decay exponentially along the length of the fiber until they are blocked by the high resistance of the membrane at the end of the fiber. A significant radial current flow exists, however, in short, wide crustacean fibers (Fatt and Katz, 1953; Orkand, 1962). In such cells, the ETP may decay in amplitude more steeply than would an exponential function in the immediate vicinity of the polarizing electrode but decay more gradually (i.e. smaller dV/dx) over the rest of the fiber (Orkand, 1962). Burke and Ginsborg (1958) reported that in the tonic skeletal muscle fiber which receives distributed innervation, stimulation of the small nerves produces a uniform depolarization which decays more gradually than a focally (intracellularly) produced ETP.

In attempting to measure the cable parameters of striated muscle or changes in their values, fiber geometry must be taken into account. When current flows uniformly across the whole membrane of unit length, the current - voltage (I-V) relation shows the membrane resistance directly (i.e. $\frac{V}{I} = \text{input } r = r_m$). This can be achieved for squid axon by inserting a long stimulating electrode into the fiber (Hodgkin and Huxley, 1952 a). If current is applied at a point of a cable - like fiber (e.g. striated muscle) with a microelectrode, the current density decreases exponentially along the fiber. From the cable equation (Hodgkin and Rushton, 1946), the I-V relation at the point of current injection is now proportional to the square root of r_m (i.e. $\frac{V}{I} \approx \sqrt{r_m}$). Using the unmodified Ohm's Law ($r_m = \frac{V}{I}$), Fatt and Katz (1953) obtained a value of only 116 ohm cm² for R_m of crab claw muscle. On the other hand, Fatt and Ginsborg applied the correction accounting for the cable decay and found that R_m of crayfish muscle was 1000 ohm cm² (see Table 1) which is very similar to that of squid nerve. Frog twitch muscles have R_m of the same order of magnitude as that of squid axon but the R_m of tonic (slow) muscles is 29,000 ohm cm² (Adrian and Peachey, 1965). The possible explanation for these differences is discussed below.

Tasaki and Hagiwara (1957) modified the cable equations to account for responses to applied alternating current in order to analyze c_m of toad sartorius.

The amplitude of the potential changes at $x = 0$ varied inversely and linearly with the square root of the frequency (i.e. V versus $\frac{1}{\sqrt{f}}$ is a straight line), which

indicated that for frequencies between 25 and 1500 Hz, c_m was independent of frequency. They also determined the c_m from the time constant (τ_f) of potential rise in the foot of a propagated AP. This region is the electrotonic component and is determined by 1) the cable properties at rest and 2) the impulse conduction velocity (Hodgkin, 1954). By measuring the conduction velocity and the rate of rise (dV/dt) of the AP foot, they calculated the conduction velocity as 1.8 to 2.5 m/sec and τ_f as 0.10 to 0.27 m sec. As stated in section C, part 1 a, the time constant is given by

$$\tau_f = \frac{1}{r_i c_m \Theta^2} \quad (14)$$

and

$$C_m R_i = \frac{a}{2 \Theta^2 \tau_f} \quad (15)$$

The data obtained by both methods were in close agreement and gave values for C_m of 5 to 9 $\mu F/cm^2$. The space constant can be calculated from the equation

$$\lambda = \sqrt{\frac{a}{2}} \sqrt{\frac{r_m}{r_i}} \quad (16)$$

or from the conduction velocity and τ of the foot of the conducted spike by

$$\lambda = \sqrt{\tau_m \Theta^2 \tau_f} \quad (17)$$

By analyzing data from experiments in which square-wave DC pulses were passed down the muscle, Tasaki and Hagiwara calculated $\tau_m \approx 30$ msec and $\lambda \approx 3$ mm.

The foregoing studies failed to take into consideration the extensive triad or T - tubular system of striated muscle fibers when measuring R_m and C_m . This structure, found by Porter and Palade (1957) by electron microscopy, consists of membranous invaginations of the cell membrane into the cell interior and situated between the terminal cisternae of the sarcoplasmic reticulum. Depolarizing current from an external electrode passes via these tubules into the interior and activates contraction (Huxley and Taylor, 1958). This tubular system is well developed in vertebrate twitch and many invertebrate muscles, and it has been

shown that the presence of this system is associated with a high resting P_K (Adrian and Freygang, 1962; Adrian and Peachey, 1965). This observation is consistent with the low R_m of twitch fibers and high R_m of tonic fibers which have very little tubular system (Peachey and Huxley, 1962).

The sarcotubular system also makes a significant contribution to the total cell membrane capacitance. The high values of 5 to 8 $\mu F/cm^2$ (Katz, 1948; Fatt and Katz, 1951) and 20 to 40 $\mu F/cm^2$ (Fatt and Katz, 1953; Fatt and Ginsborg, 1958) for c_m of frog and crustacean muscle fibers respectively are attributable to a high capacitance (C_e) in the tubular system in parallel with the surface membrane capacitance (C_m) (Falk and Fatt, 1964). There is also a small resistance (R_e) in series with c_e . The values which they measured for frog sartorius were $R_m = 3100 \text{ ohm cm}^2$, $c_m = 2.6 \mu F/cm^2$, $R_e = 330 \text{ ohm cm}^2$ and $C_e = 4.1 \mu F/cm^2$. The equivalent circuit which they proposed is shown in figure 3 superimposed on a schematic diagram of the ultrastructure (from Freygang *et al.*, 1967). Briefly, they proposed that current flow through a fiber with this system would be described by two time constants, $\tau_m = (C_e + C_m) R_m$ and $\tau_e = C_e R_e$, for which they calculated values of 21.0 and 1.3 msec, respectively. In general, therefore, the T - system alters axial current flow along the core conductor and its cable properties determine the speed and extent of current spread from the surface into the fiber interior.

The findings of Falk and Fatt have since been confirmed (Gage and Isenberg, 1969; Schneider, 1970; Freygang *et al.*, 1967) but there is some controversy as to the structural correlate of R_e , as to whether or not the tubules are open - mouthed (Adrian *et al.*, 1969; Peachey and Adrian, 1972), and as to the extent of radial current flow (Schneider, 1970). The findings that deeper myofibrils are activated later (Gonzalez - Serratos, 1966) and require a larger surface membrane depolarization for activation (Adrian *et al.*, 1969) indicate that radial currents are likely present. These currents require an equivalent circuit with R and C spatially - distributed throughout the cross - section (Schneider, 1970). It appears that the tubule may offer some resistance (R_T) to the outside since $R_T \approx 3 R_o$ (Schneider, 1970), only 1/3 of the T - system is accessible to ferritin

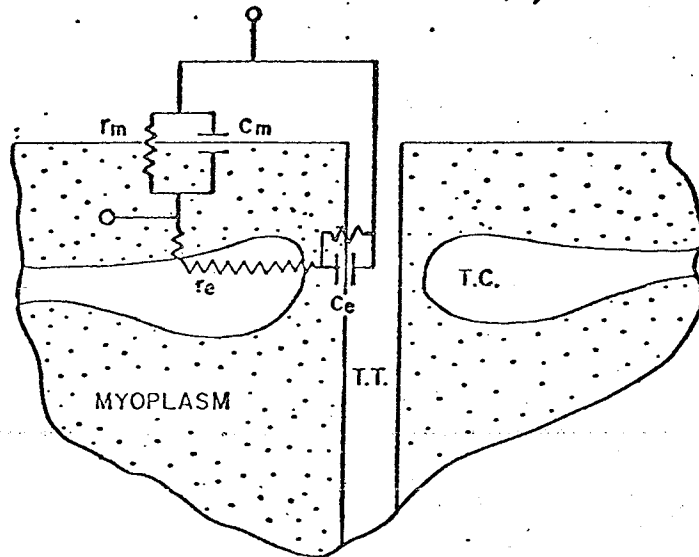


Figure 3. Equivalent circuit for striated muscle fiber membrane proposed by Falk and Fatt (1964) superimposed on a schematic diagram of the ultrastructure. Location of r_e tentative. Resistances and capacitances for unit length of fiber. T.T.^e refers to the transverse tubular system and T.C. refers to the terminal cisternae (from Freygang et al., 1967).

(Peachey and Schild, 1968) and the effective T - system space constant is shorter than it would be if $R_T = R_o$ (Adrian et. al., 1969). The general conclusions which appear to be reasonably independent of the specific model adapted are that the surface membrane has a capacity of $1 \mu\text{F}/\text{cm}^2$ and that the tubular system makes a substantial contribution to the low frequency capacity.

d) Cardiac muscle

Most cardiac cells are not of uniform diameter and infinite length but fuse with one another at each end, forming low resistance intercalated discs. The cells average 150μ in length and 50μ in diameter, and behave electrically as a functional syncytium (Fozzard and Beeler, 1975). This, of course, is very important as the contractions of myogenic hearts must be synchronized by pacemaker cells which have the most rapid spontaneous contraction frequency.

Electrical membrane constants for several cardiac cell types are shown in Table 2. The R_i (120 ohm cm) and C_m ($2.4 \mu\text{F}/\text{cm}^2$) appear to be very similar for the various cell types while R_m ranges from 1200 to 2000 ohm cm^2 , and τ_m from 13 to 19.5 msec . The high value of $12 \mu\text{F}/\text{cm}^2$ obtained by Weidmann (1952) has been suggested to consist of a surface membrane capacitance which is rapidly discharged during the initial spike of the AP, and a sarcotubular capacitance which is slowly discharged during the beginning of the plateau (Noble, 1962a). Fozzard (1964) showed that the "high frequency" capacitance obtained from the foot of a propagating AP was 2 to $3 \mu\text{F}/\text{cm}^2$ while the "low frequency" capacitance obtained by square - wave current analysis was 10 to $12 \mu\text{F}/\text{cm}^2$. Noble (1962a) indicated that a C_m of $3 \mu\text{F}/\text{cm}^2$ is required in order to obtain appropriate values of depolarization rate and conduction velocity in computed responses. He later (1966) suggested that the cable equation should be modified along the lines suggested by Falk and Fatt (1964) to account for the "internal" membrane capacitance. It has been demonstrated though, that the time course of the foot of the propagated AP in the frog ventricle fits the predictions made from the cable theory (Van der Kloot and Dane, 1964).

The fibers of the atria are complex in that they form an extensive branching network of cables. Consequently, cable equations and experimental protocol have had to be modified (Noble, 1966). Woodbury and Crill (1961) developed a model

Table 2

Electrical Membrane Parameters of
Cardiac Muscle Fibers

Prep.	R_m (ohm-cm ²)	C_m (μF/cm ²)	R_i (ohm-cm)	τ_m (msec)	Ref.
Purkinje fiber	1200-2000	11-12.4	105	13-19.5	1 & 2
	-	2.4	120	-	3
Terminal purkinje	-	2.4	120	-	4
Ventricular fiber	-	2.4	120	-	4
Atrium	-	2.4	120	-	5 & 6

1) Weidmann, 1952; 2) Coraboeuf & Weidmann, 1954; 3) Fozzard, 1966;
4) Matsuda, 1960; 5) Vaughan Williams, 1958a; 6) Vaughan Williams, 1958b.

to account for the effect of branching on the time course and spatial distribution of V and i_m . In cable - like cells the membrane area increases linearly with distance from a polarizing electrode. In extensively branching preparations, the area increases more rapidly with distance. If the preparation is relatively thin and the branching frequent (e.g. mouse atrium), the relation between $V_t = \infty$ and x is adequately described if represented by a single planar cell of infinite dimension, bounded by two parallel, sheet - like membranes. Assuming this geometry, the area increases as the square of the distance (x^2) and the current density in the vicinity of an intracellular current - passing microelectrode decreases rapidly with distance. In other words, the membrane is shunted by a relatively low electrical resistance due to the large membrane area surrounding the electrode. The equivalent cable equation for a sheet membrane separating a layer of intracellular fluid from the extracellular fluid is given by

$$\frac{d^2V}{dx^2} = {}^3R_i C_m \left(\frac{dV}{dt} + I_i - \frac{1}{x} \frac{d^2V}{dx^2} \right) \quad (18)$$

where 3R_i = resistance of unit length and breadth of intracellular fluid.

In conclusion, cardiac muscle cells form an extensively branching network with low resistance junctions such that it functions as a syncytium. The innervation is therefore sparse and concentrated to certain pacemaker sites, and the functional unit is the whole muscle.

e) Smooth muscle

Perhaps the only generalization concerning the size, shape and arrangement of SM cells in different preparations is that their diversity defies generalization; thus various models of cell interrelationships have been put forward to relate to electrophysiological data (Burnstock and Prosser, 1960b; Prosser, 1962; Nagai and Prosser, 1963a; Barr, 1963; Vayo, 1965; Bennett and Merrillees, 1966; Tomita, 1966b; Bennett and Rodgers, 1967; Abe and Tomita, 1968).

With reference to the ultrastructure of SM cells, their contour is uneven and they range in size from 2 to 6 μ in diameter and from 90 to 600 μ in length (Burnstock, 1970). The separation of neighbouring muscle cells is generally between 500 and 800 Å (Merrillees, 1968). In the taenia coli and vas deferens,

the cells have a staggered, weaving arrangement with adjacent cells overlapping, such that each cell is surrounded by 10 to 12 others (Merrillees, 1968; Bennett and Rodgers, 1967). Many ultrastructural features which are thought to be specialized for intercellular communication have been described. These include "bridges" (areas of close apposition), "intrusions" (peg and socket arrangement), "desmosomes" (attachment plaques) and "tight junctions" or "nexuses" (representing low resistance areas of adjacent plasma membranes) (Rhodin, 1962; Lane and Rhodin, 1964a; Dewey and Barr, 1962, 1964; Burnstock and Merrillees, 1964; Merrillees, 1968; Bennett and Rodgers, 1967). Such formations can be observed on electron micrographs but the integrity of the structures depend on both the physiological and fixation conditions. Thus it is difficult to differentiate between a labile structural arrangement and an artifact of the fixation process (Dewey and Barr, 1964; Burnstock, 1970). Consequently, electron micrographs are at best only a supplement to electrophysiological studies. Although true fusion has not been demonstrated, bridges and nexuses may form up to 6% of the total surface area of visceral SM cells (Lane and Rhodin, 1964a; Taxi, 1965), thus providing a reasonable ultrastructural basis for intercellular electrical communication. The cells of most SM's are arranged in branching bundles or fasciae, about 100 μ in diameter and surrounded by connective tissue sheaths (Burnstock and Prosser, 1960b; Bennett and Burnstock, 1968). With regard to intracellular structures, the sarcoplasmic reticulum and sarcoplasmic tubules are present but poorly - developed in SM (Burnstock and Merrillees, 1964; Rogers, 1964; Carsten, 1969). On the basis of these considerations, therefore, one might expect current to cross from cell to cell (depending on the resistance of the close contacts) and the cell capacitance to be less than it is in striated muscle cells (due to the paucity of intracellular membranes).

It has been demonstrated that the cable equations originally derived for nerve and skeletal muscle (Hodgkin and Rushton, 1946; Tasaki and Hagiwara, 1957) can be used to analyze the passive electrophysiological characteristics of the guinea-pig taenia coli, even though the resistance of the junctional membrane between cells contributes substantially to the longitudinal tissue resistance, r_l (Tomita, 1966 a,b; Jones and Tomita, 1967; Abe and Tomita, 1968). The results

obtained by external polarization strongly suggest that the muscle fibers are electrically interconnected and aggregated as a functional bundle (Tomita, 1966a). The exponential decay of the ETP indicated that the fibers could be represented electrically by a series of independent cables connected by junctional resistances (r_j) and capacitances (c_j) located between cells (Abe and Tomita, 1968) as shown in figure 4a). Each cell is represented by four unit membrane equivalent circuits and three series myoplasmic resistance while the interior of each cell is connected by one unit junctional membrane to the interior of another cell. Although interconnections also exist in the transverse direction, they can be disregarded in external polarization since all cells in a plane parallel to the polarizing electrode are thought to be at equi-potential (Tomita, 1966 b).

On the application of square current pulses "intracellularly" to this model using reasonable but arbitrarily chosen values of resistances and capacitances similar to those of SM cells, the decay of the ETP with distance was found to be exponential (Abe and Tomita, 1968). The decay was sharper at the junctions since r_j was 3.3 times total r_i of a cell, but the decay in the taenia coli was suggested to be smoothly exponential as r_j (≈ 200 ohm cm) is only 1 or 2 times r_o ($\approx 100 - 200$ ohm cm) in this tissue (Jones and Tomita, 1967; Tomita, 1969) and the cell length ($\approx 100 \mu$) is much shorter than the tissue space constant (≈ 1.5 mM, Tomita, 1966a; Abe and Tomita, 1968). The λ of the decay along the model could be calculated from the ordinary cable equation ($\lambda = \sqrt{\frac{r_m}{r_i}}$) and was found to be 2.4 cell lengths.

The time course of the ETP at any point also fitted the theoretical time course (Hodgkin and Rushton, 1946) and was minimally affected by the c_j provided the time constant ($\tau_j = c_j r_j$) was less than 5% of τ_m . As is discussed below, the model accounts quite well for the electrophysiological data from many smooth muscles.

An electrical model has also been constructed in order to explain why the spatial decay of the ETP produced by intracellular polarization is much sharper than that produced by external stimulation (Tomita, 1966b). The model (figure 4b) consists of five columns of longitudinally connected fibers, each represented by 12

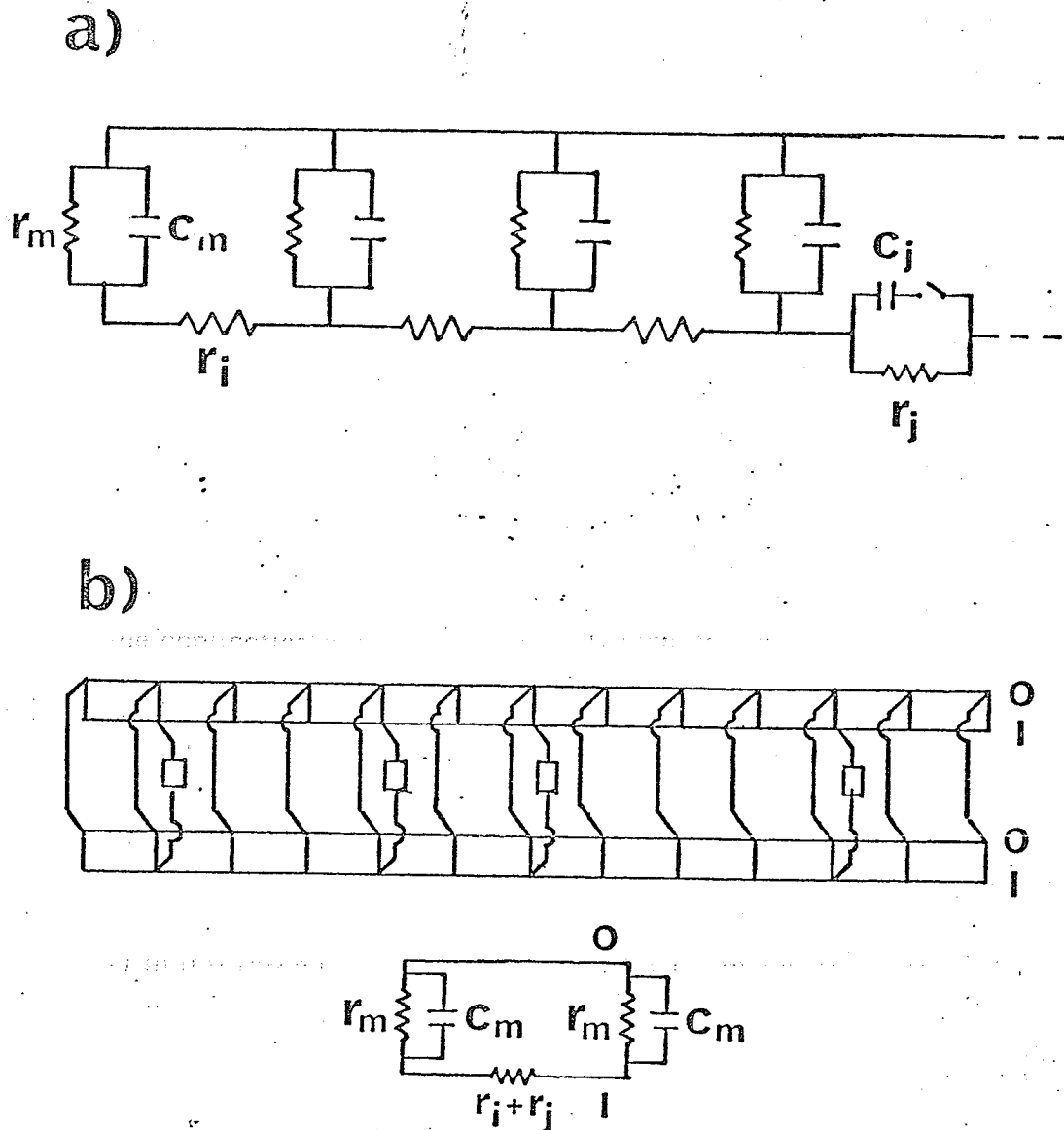


Figure 4. Panel a - Equivalent circuit of smooth muscle fiber; membrane resistance and capacitance, internal fluid resistance and junctional resistance and capacitance given for unit lengths. Panel b - Electrical model to simulate interfiber connections of smooth muscle. Model represents two columns of muscle fibers connected in series, the interior of one connected with the interior of the other at intervals through unit shown beneath (from Tomita, 1966 b).

repeating unit membrane circuits and connected to the inside of the other fibers by high and low resistance connections, values arbitrarily chosen. The ETP produced by external polarization (applied to end of 5 fibers simultaneously) decays less sharply with distance and its time course can be expressed by an error function, while that elicited by intracellular stimulation has a time course which can be expressed neither by an error function nor by an exponential function. Also, the effective membrane resistance (R_e) when external polarization is used is the same when calculated either by $\frac{Vx = 0}{I}$ or by the cable equation ($R_e = \frac{\sqrt{r_m r_i}}{2}$), and is greater than that calculated using intracellular polarization. Therefore, the model, although a simplification of reality clearly shows a difference between the two methods.

In order to predict the actual spatial decay and time course of the ETP in a cable - like preparation, the relation of current spread to tissue geometry must be defined (Woodbury and Crill, 1961; Noble, 1962a, 1966). In cable - like preparations the membrane area increases linearly with the distance from a given point. In a thick preparation with three - dimensional interconnections between cells, like those of SM, the area increases rapidly with distance, probably as the cube (x^3) as discussed above. Therefore, the current density in the immediate vicinity of an intracellular current - passing microelectrode spreads in three dimensions and rapidly becomes very low. In other words, the membrane area near the microelectrode is shunted by the low electrical resistance due to a large membrane area surrounding the electrode. With external polarization, on the other hand, electrotonic current in the transverse direction is minimized while that in the longitudinal direction is maximized, since all cells lying in parallel at the same distance from the polarizing electrode are thought to be polarized to the same magnitude (Tomita, 1966 b). The inference from these considerations is that intracellular stimulation produces only a very local polarization which is severely space - limited while extracellular stimulation polarizes a whole plane of cells so that the current can spread along the muscle bundle as if it were a single cable.

The method of intracellular polarization has been employed in earlier studies to detect the presence of , and determine the degree of electrical coupling via low resistance junctions between cells. By applying square wave pulses intracellularly, Barr (1961) measured the resistance between the cell interior and the solution and between two neighbouring cell interiors. Finding that the resistance across two cell membranes was double that across one, he concluded that there were no low resistance cell contacts. On the other hand, Nagai and Prosser (1963 b) found the resistance between two cell interiors to be 1/2 to 1/3 of that across one membrane, and claimed to be able to record an ETP with negligible decay in amplitude in an adjacent cell. By the resistance measurements they give however, at least a partial reduction in ETP amplitude would be expected. In subsequent studies the amplitude of the ETP produced intracellularly decayed very rapidly with distance (Sperelakis and Tarr, 1965; Bennett, 1967 b; Kobayashi et. al., 1967; Kuriyama and Tomita, 1965). These results were evidenced by the low values obtained for λ and for τ_m (1.5 to 31 msec, most under 10 msec, see Table 3). The latter values indicate a very fast time course which can not be fitted by either an error function (according to cable theory) or an exponential function (Kuriyama and Tomita, 1965). These results are consistent with the three - dimensional current spread discussed above. In addition, these investigators neglected these interconnections when calculating the passive membrane cable parameters. The specific R_m was calculated by multiplying the input resistance ($\frac{V_{x=0}}{I}$) with the cell surface area, assuming homogenous current spread over the surface membrane (Barr, 1961a; Nagai and Prosser, 1963 b; Kobayashi et. al., 1967 a, b). These assumptions, however, are prone to error due to 1) the high length/diameter ratio ($\approx 100 \mu/5\mu$), 2) high internal resistance ($\approx 5 \text{ M ohm}/100 \mu$) of single cells and 3) the three-dimensional current spread (Tomita, 1966 b; 1970). As it was pointed out by Tomita (1970), input resistance equals r_m for a uniformly - depolarized squid axon, but $\frac{V}{I} \approx (r_m)^{1/2}$ for a cable - like preparation polarized at one end (e.g. striated muscle fiber), $(r_m)^{1/4}$ for a closed syncytium (e.g. cardiac muscle fiber) and even less for a three-dimensional syncytium or "volume conductor" like smooth muscle. Based on the data of Nagai and Prosser (1963 b) but applying appropriate

Table 3

Electrical Membrane Parameters of Smooth
Muscle fibers; Intracellular Polarization

Prep.	R_m (ohm cm ²)	C_m (μF/cm ²)	τ_m (msec)	Ref.
Cat intestinal SM				
a) Circular	560	56	31	1
	1050	21	21	2
	377	40	5-30	3
	980	-	-	4
b) Longitudinal	780	13	10	2
Guinea - pig				
a) Taenia coli	320	10	2-4 (exp)	5
	-	-	10 (erf)	5
	600	5	-	6
b) Vas deferens	-	-	1.8	7
	-	-	1.5 -3 (exp)	8
	-	-	3.5 -5 (erf)	8
c) Longitudinal intestinal	600 - 650	4.5 - 5	3-4	6
d) Ureter	160 - 300	8 - 15	2 - 3	9

1) Nagai & Prosser, 1963b; 2) Kobayashi, Prosser & Nagai, 1967;
3) Sperelakis & Tarr, 1965; 4) Barr, 1961; 5) Kuriyama & Tomita, 1965;
6) Kuriyama, Osa & Toida, 1967b 7) Bennett, 1967b 8) Tomita, 1967a 9)
Kuriyama, Osa and Toida, 1967a

corrections, Vayo (1965) calculated total cell resistance to be 2000 M ohms and the resistance of a single bridge about 40 M ohms. He thus concluded that some of the current from an intracellular stimulating electrode would pass through bridges. Even so, the ETP falls as the cube of the distance, indicating that little current is left by the time it reaches another cell to produce an ETP. Thus it is difficult to analyze cable properties by the use of intracellular polarization.

On the other hand, extracellular stimulation lends itself quite well to the measurement of cable properties of SM fibers because all of the cells in a transverse plane are polarized to the same extent and consequently, each muscle bundle behaves as if it were a single cable. Electrotonic spread is easily measured several mm from the electrode, such that λ is up to 2.1 mm and T_m up to 330 msec (Table 4). This is convincing evidence for the presence of low resistance intercellular connections since the ETP is recorded many cell lengths away.

With this in mind, the membrane of the muscle bundle is represented by a series of parallel R-C units in series with the longitudinal intracellular resistance, and the amplitude of the ETP must be directly proportional to R_m . A novel apparatus for the extracellular stimulation of ETP's was designed by Tomita (1966 a) who placed two platinum wire ring electrodes at one end of the taenia coli muscle strip for stimulation and employed a glass capillary microelectrode for intracellular voltage measurements. In order to prevent 1) the occurrence of a stimulus artifact due to the electrical field in the bath and 2) sharp spatial decay of the ETP due to loss of considerable current to the solution, a celluloid insulating partition was placed immediately adjacent to the stimulating electrode nearest the recording electrode. This partition surrounded the muscle and divided the bath into stimulating and recording chambers. This method allowed the measurement of 1) the space constant 2) time constant, 3) changes in r_m (to which the ETP is directly proportional) and basal isometric tension simultaneously.

Abe and Tomita (1968) utilized the same principle but improved the geometry of the apparatus by replacing the celluloid and coating the side of the stimulating electrode exposed to the recording chamber with an insulating epoxy resin (Araldite). The muscle was passed through a small hole in this same electrode.

Table 4

Electrical Membrane Parameters of Smooth
Muscle Fibers; Extracellular Polarization

Preparation	R_m (ohm cm ²)	C_m (μF/cm ²)	τ_m (msec)	λ (mm)	Ref.
Cat intestine					
a) Circular	-	-	133	-	1
b) Longitudinal	-	-	92	-	2
Guinea pig					
a) Taenia coli	30k-50k	2-3	107	1.45	10
			60-100	1.68	11
b) Vas deferens	-	-	100	1.5-2.6	8
c) Longitudinal intestinal	-	-	14-100	-	6
d) Rectum	20k	4.3	84	0.81	12
e) Portal vein	-	-	330	0.52	13
f) Biliary system	-	-	<100	0.7-0.8	14
Rabbit aorta	-	-	-	2.1	15

- 1) Nagai & Prosser, 1963 b; 2) Kobayashi, Prosser & Nagai, 1967;
6) Kuriyama, Osa & Toida, 1967b; 8) Tomita, 1967a; 10) Abe & Tomita, 1968;
11) Tomita, 1966a; 12) Kuriyama & Mekata, 1971; 13) Ito & Kuriyama, 1971;
14) Creed & Kuriyama, 1971; 15) Mekata, 1976.

Current loss to the recording chamber was thus reduced. The λ was measured by plotting the steady - state ETP semilogarithmically as a function of x and finding the distance at which the ETP decayed to 37% ($\frac{1}{e}$); λ was found to be 1.45 mm for the taenia coli. The τ_m was calculated by inserting the experimentally - determined λ into the theoretical time course equation (Hodgkin and Rushton, 1946) and by plotting the time to half-maximum of the ETP versus distance (also linear). The values calculated were 106.7 and 103.4 msec, respectively. The specific C_m was calculated from the responses to applied alternating current and R_m was calculated from the equation $\tau_m = R_m C_m$. The analysis indicated that the smooth muscle of the guinea - pig taenia coli has a C_m of 2 - 3 $\mu\text{F}/\text{cm}^2$ and R_m of 30 - 50 k ohm cm^2 . These results were later confirmed by the double - sucrose gap method (Bülbring and Tomita, 1969 a). This method has received wide acceptance as the method of choice for the determination of the cable properties of smooth muscle (Abe, 1971; Ito and Kuriyama, 1971; Kuriyama and Mekata, 1971; Kroeger and Marshall, 1973; Creed and Kuriyama, 1971; Kroeger and Stephens, 1974; Mekata, 1971, 1976; Bolton, 1972).

It should be noted however, that the apparatus has limitations which must be kept in mind when designing experiments and analyzing data. Basal isometric tension is continuously measured by attachment of one end of the muscle via surgical silk to a force gauge. Muscle length must therefore be kept constant (once equilibrated) in order to eliminate stretch-induced changes in tension(myogenic response) when analyzing the effects of drugs and ionic interventions (Stephens and Kromer, 1971; Stephens and Kroeger, 1972). In addition, a considerable amount of stimulating current is lost to the bath, so that a relatively high voltage must be applied in order to produce electrical and mechanical responses comparable to those of previous studies. Kroeger and Marshall (unpublished) reduced this shunt loss considerably, by attaching a celluloid partition to the opposite side of the partition electrode, leaving a small space between it and the Ag/Ag Cl electrode. Thus, current loss to the recording and stimulating chambers is prevented by the epoxy coating and celluloid respectively, and current is focused on the muscle

surface. Consequently, the stimulus intensity required to produce a given ETP is reduced by about a factor of 10. Finally, to facilitate simultaneous recording of electrical and mechanical responses, a floating recording electrode assembly must be employed. Few investigators, however, have been able to record at distances less than 1 mm from the partition due to surface tension effects.

In addition, there are two theoretical limitations which must be considered for correct analysis of results. The evaluation of changes in r_m relies on the assumption that junctional resistances remain unaltered during the action of agents and ionic interventions. The resistance of the junctional membrane contributes substantially to r_j (Tomita, 1966 a, b, 1970) so that concomitant alterations in r_j would significantly affect axial current flow, and consequently, the ETP. Since r_j can not be determined in SM by present techniques and since membrane properties are analyzed in terms of r_m , it must be assumed for the present that r_j remains constant. The effect of Ca on r_j will be discussed below (section 4).

A much more important source of error may be encountered in relating a change in amplitude of the ETP to a change in r_m . Some investigators (Abe and Tomita, 1968; Ohashi, 1970) have been able to record at very small distances (i.e. < 1 mm) from the partition (stimulating) electrode, and have therefore assumed that $x \ll \lambda$. Based on this assumption, they have simplified the cable equation (Hodgkin and Rushton, 1946; Fatt and Katz, 1951)

$$\left(\frac{V'}{V}\right)^2 = \frac{r'_m r'_i}{r_m r_i} \frac{\exp(-x/\lambda)}{\exp(-x/\lambda)} \quad (19)$$

to

$$\left(\frac{V'}{V}\right)^2 = \frac{r'_m}{r_m} \quad (20)$$

by neglecting the last term (assuming $-x/\lambda \approx 0$) and assuming that r_i is relatively constant (Bulbring and Tomita, 1969a). These equations represent the relationship between the change in ETP amplitude and the change in r_m during agonist action ("prime" symbol for altered values). However, with an increase in x or a decrease in λ , the term $\frac{-x}{\lambda}$ becomes great enough to significantly alter equation 20. Thus

at distances of $x > 0$, a change in r_m results in alterations both in V and in λ .

The error which occurs with a decrease in r_m (and therefore, λ) is greater than that with an increase in r_m . As a result, the value of $\left(\frac{V'}{V}\right)^2$ will progressively

overestimate the change in r_m as x becomes greater (Bolton, 1972). As it is very difficult to accurately impale cells at a desired distance of less than 1 mm with the floating electrode assembly, it is advisable to use the cable equation in its original form (Kroeger and Marshall, 1973). These authors devised a graphical solution for $\frac{r'_m}{r_m}$ which is described in the methods section of this manuscript. It should be also

noted that a small error may be made in the assumption (Abe and Tomita, 1968) that the current passing through the tissue 1) is directly proportional to the voltage applied to the external electrodes and 2) is unaffected by large changes in r_m (Bolton, 1972). Again, these are variables which would make quantitative evaluation of resistance changes very difficult unless assumed as stated above. Although this type of error is very small, it can be removed by plotting the steady state amplitude of the ETP as a function of distance on semilogarithmic paper, thus eliminating the current factor.

In conclusion, the method of extracellular stimulation and intracellular recording of ETP's is suitable for measuring the passive electrophysiological characteristics of smooth muscle. It is especially useful for evaluating changes in r_m due to agonist action providing limitations are taken into account and suitable corrections are applied.

2. Ionic Basis of the Action Potential

a) Nerve

It is clear from the analysis of cable properties that the axial spread of the ETP (due to current flow along the core-conductor of a cable-like structure) decays much too rapidly to transfer electrical "messages" over the long distances encountered in higher organisms. There is, however, an additional mechanism which makes use of the cable properties in its initiation, but thereafter becomes regenerative and propagating. This mechanism is the action potential which is the result of a

threshold amount of depolarization causing a regenerative increase in membrane permeability to Na^+ ions. Consequently, Na rushes inward down its electrochemical gradient depolarizing the membrane further, causing a spike potential with a very fast rate of rise. The ionic processes underlying the depolarization and subsequent repolarization of the spike are dealt with in detail below.

Historically, it is of interest that this AP was first thought to represent a breakdown of the membrane selectivity for K, causing E_m to fall to zero potential (Bernstein, 1912). It was later shown (Hodgkin and Huxley, 1939, 1945; Curtis and Cole, 1942) that the membrane potential underwent a transient reversal (i.e. interior became positive to exterior) during passage of an impulse. It was also known that membrane conductance (g_m) increased transiently during passage of an impulse (Cole and Curtis, 1939), and that local potentials and AP's were produced with increasing cathodal (depolarizing) current intensity (Hodgkin, 1938). Hodgkin and Katz (1949) put forward the hypothesis that a large reversal of E_m could be obtained if it was assumed that the active (depolarized) membrane reversed the resting conditions by becoming highly and specifically permeable to Na. Since $[\text{Na}]_o \gg [\text{Na}]_i$ (Steinbach and Spiegelman, 1943), the sodium equilibrium potential would be positive (about 60 mV) and the electrochemical gradient would be overwhelmingly inward. These authors demonstrated a clear relationship between AP height, rate of rise and conduction velocity and the Na concentration outside the fiber (Hodgkin and Katz, 1949). Quantitatively the P_K/P_{Na} ratio was reversed from 1:0.04 at rest to 1:20 during an AP.

The "sodium theory" was supported by the data of Keynes (1951) and Keynes and Lewis (1951) who demonstrated a net entry of labelled Na during an AP, which was quantitatively sufficient to account for the observed change in E_m . However, it was also reported that the negative after - potential (underswing) of the AP was affected by changes in $[\text{K}]_o$ (Hodgkin and Katz, 1949) and that an amount of K equivalent to the net Na entry was lost during the AP (Keynes, 1951). Hence, it was reasonable to conclude that the Na influx caused the rising phase of the AP and K efflux the falling phase. The time course of these events had to be measured though, by a more sensitive and accurate

technique.

The ionic basis of the AP is difficult to analyze because the measured voltage transients are produced by several ionic currents which vary with voltage and time. This problem was solved by Hodgkin and Huxley (1952 a-e) by 1) measuring the ionic currents of the squid nerve membrane with transmembrane voltage "clamped" to desired values and 2) comparing the reversal potential of each current to the ion equilibrium potentials. Thus, ionic currents are measured as a function of voltage and time.

The voltage clamp technique involves a sudden simultaneous displacement of E_m over the whole of the cell membrane from its resting value to a predetermined fixed potential by a feedback - controlled current source. The current which flows through a definite area of membrane under the influence of the applied voltage change is measured with a separate amplifier (Cole, 1949). The important point is that the amplitude and direction of the ionic current (after a rapid capacitive charge or discharge) during this period varies greatly with 1) the amplitude of the voltage step, 2) with the concentration of ions in the external medium, and 3) with time.

The total membrane ionic current consists of a rapid (1 msec), transient inward current which changes more gradually to a steady maintained outward current. That the initial inward current was carried by Na (I_{Na}) was evident in that 1) the current density decreased with greater depolarization, 2) the current reversed direction at $E_m = E_{Na}$ and 3) it was abolished by replacement of Na with choline. The outward current remaining after Na substitution was assumed to be carried by K as it was only slightly affected by changes in $[Na]_o$. This interpretation was supported by tracer studies (Hodgkin and Huxley, 1953) which showed that depolarization caused a large increase in K efflux which was equivalent to the total electric charge passing through the same area of membrane in the same time. The next step, the determination of the Na component of total ionic current was accomplished by subtracting I_K , produced by a moderate depolarization, from the total ionic current, I_t . The height and the extremely fast rate of rise of the spike due to the Na current was later shown (Hodgkin, 1958) to be explained by the voltage - and time - dependence of the Na activation and inactivation processes.

The variation of g_{Na} with E_m is very steep (e - fold change per 5 mV) and the depolarization and increase in g_{Na} are mutually reinforcing. As a result, the spike approaches very close to E_{Na} . However, as soon as active Na channels are opened (activation), the inactivation process begins. Different channels are likely activated at slightly different times, so that at the peak of the spike, some Na channels are being inactivated and thus prevent the AP from reaching E_{Na} . These differences in the onset of activation also account for the latent period between the onset of a stimulus and the peak of the AP. The latency decreases with increasing stimulus intensity, which therefore improves the synchronization of activation. The minimum stimulus duration required to excite is the utilization time. The Na inactivation process would slowly repolarize the membrane by itself but it is aided by the voltage - dependent and more slowly responding outward K current. In summary, therefore, the AP is produced by 1) a rapid depolarization - activated increase in g_{Na} which permits a regenerative flow of Na down its electrochemical gradient, 2) a rapid Na inactivation process and 3) a delayed, voltage - dependent increase in g_K which accelerates the membrane repolarization.

An attempt was made to describe these observations by an electrical model (Figure 5) and a set of equations. Briefly, the theory is based on the findings that the movements of Na and K during the AP are determined 1) by their respective electrochemical gradients, which can be expressed as the difference between the RMP and the ion equilibrium potential (battery symbol), and 2) by the Na and K conductances ($g_{Na} = \frac{1}{R_{Na}}$). For sudden changes in potential, the instantaneous ionic currents are linear functions of their electrochemical potential gradients and are given by

$$I_{Na} = g_{Na} (E_m - E_{Na}) \quad (21)$$

$$I_K = g_K (E_m - E_K)$$

$$I_{Cl} = g_{Cl} (E_m - E_{Cl})$$

where I_{Cl} is the main contributor to the "leak" current (I_l) in the Hodgkin - Huxley model. The conductances are thus calculated from the experimentally - obtained

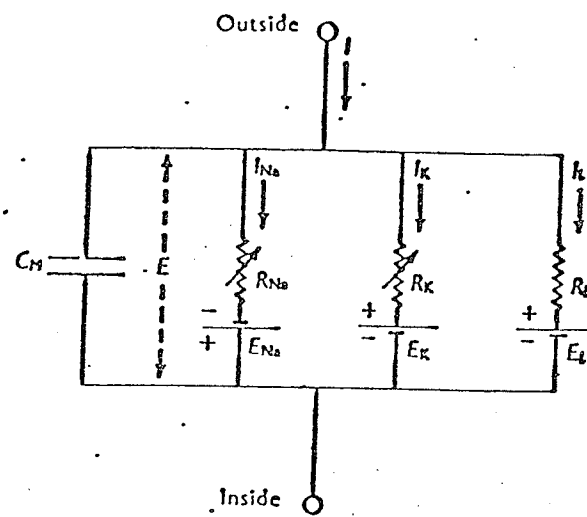


Figure 5. Electrical model of unit excitable nerve membrane; equilibrium potentials (E), conductances ($g = \frac{1}{R}$) and currents carried by Na and K ions and "leak" ions, membrane capacitance and membrane potential (from Hodgkin and Huxley, 1952e).

values for the respective ion equilibrium potentials and currents at peak current flow, and are termed chord conductances. The authors finally derived a set of complex equations describing the ionic currents, and by the use of computer programming, were able to predict the form of the AP. The models which they derived, it must be noted, contain components with no physiological correlates.

Several additional features of the AP which were also explained by delineation of the voltage - and time - dependence of the ionic currents are 1) the absolute and relative refractory periods, 2) accommodation, 3) anode - break excitation and 4) rectification. The refractory periods occur when Na inactivation is well developed and g_K increasing, making the membrane temporarily, and then partially, inexcitable. Accommodation is the result of a slowly - rising depolarization allowing sufficient time for the Na inactivation and K activation processes to suppress the increase in g_{Na} , thereby inhibiting the production of an AP. In other words, the threshold level of E_m (i.e. E_{Th}) is gradually increased. On the other hand, anode - break excitation is due to a fall in E_{Th} with hyperpolarizing (anodal) stimulation, such that E_{Th} may be more negative than RMP when the current is terminated. This leads, therefore, to an AP.

The mechanism underlying rectification eluded several investigators (Cole and Curtis, 1939; 1941; Goldman, 1943) until the Hodgkin - Huxley studies. Cole and Curtis (1939) first showed that in the steady - state, the squid nerve membrane has a much lower resistance to depolarizing current than to hyperpolarizing current, thus behaving as an electrical rectifier. However, resistance to depolarizing current also decreases with time so is called delayed rectification. This underlying process is now known to be the gradual (delayed) increase in g_K with depolarization. Thus, the ionic currents are linear functions of the electrochemical potential gradient (i.e. $I - E$ plot given by straight line) only for instantaneous changes in E_m . This effect is best observed by superimposing depolarizing and hyperpolarizing ETP's: due to rectification caused by an increase in resting g_K , depolarizing ETP's are lower in amplitude for a given stimulus current intensity (Hodgkin, 1938).

b) Skeletal and cardiac muscle

The AP of striated muscle fibers is similar to that of nonmyelinated axons in

that it is produced by a specific, rapid, regenerative increase in g_{Na} (Narahashi et al., 1960; Nastuk and Hodgkin, 1950) which is rapidly inactivated (Adrian et al., 1968). Although the height (60 - 70 mV), rate of rise (450 V/sec) and duration (1 - 2 msec) of the spike are very similar to those of nerve (Katz, 1948), the time course of repolarization is longer and E_m transiently "levels off" at a potential less negative than RMP (positive after - potential). With reference to the time course of repolarization, this is thought to be prolonged as the result of a slower, smaller increase in g_K in comparison to that of nerve which is inactivated by repolarization (Adrian et al., 1970). With reference to the positive after - potential, there are two hypotheses regarding the underlying mechanism, the first of which is that g_K transiently decreases to less than its resting value during repolarization (Hodgkin and Horowicz, 1959a). The second proposal is that E_K transiently decreases as a result of local K accumulating in the T - tubule system (Gage and Eisenberg, 1969b) or sarcoplasmic reticulum, thereby increasing local $[K]_o$ (Adrian et al., 1970).

As well as delayed rectification, Adrian and Freygang (1962) demonstrated anomalous rectification in skeletal twitch fibers, in which g_m is greater for inward current than for outward especially in high K solution. This phenomenon is the opposite of delayed rectification and is not found in tonic skeletal fibers (which lack a T - tubule system) (Stefani and Steinbach, 1969) or in twitch fibers whose transverse tubules have been disrupted by glycerol (Gage and Eisenberg, 1969 b). The results have been interpreted as being due to changes in g_K in the sarcotubular and/or T - tubular systems, but exact locations and underlying mechanisms are unknown.

With reference to cardiac electrophysiology, the AP is also very similar to that of nerve in its height (80 - 100 mV), rate of rise (up to 570 V/sec) and dependence of these parameters on $[Na]_o$ (Brady and Woodbury, 1960; Weidmann, 1955). However, the overshoot and repolarization phases of ventricular and Purkinje fiber AP's are characterized by a plateau and slow repolarization which may last several hundred milliseconds (Draper and Weidmann, 1951). The initial rapid Na current is rapidly inactivated (Noble, 1962) but there simultaneously

begins 1) a slow inward Ca current (Reuter, 1968; Vitek and Trautwein, 1971) which may generate a notch or secondary depolarization and is then inactivated, and 2) a slow outward K current that underlies the slow delayed rectification which repolarizes the membrane (Noble and Tsien, 1968). These "plateau" currents are both voltage - and time - dependent, and both delayed and anomalous rectification have been demonstrated (Noble and Tsien, 1968). According to their analysis the inward (anomalous) rectifier is great enough to reduce the repolarizing current progressively supplied by the delayed, outward rectifier, thus slowing repolarization.

In order to differentiate between the Na and Ca components of the initial inward current, Kohlhardt et al. (1972) serially voltage - clamped the ventricular trabeculae of cats at two different levels of depolarization and preferentially suppressed I_{Na} and I_{Ca} with inhibitors. The first step (30-35 mV) elicited the immediate rapid inward I_{Na} which was abolished by complete replacement of Na with choline chloride and reduced by the local anaesthetics, xylocaine and procaine. The second step further decreased E_m by 20 mV and elicited another inward current which varied in magnitude with $[Ca]_o$. This current was selectively and almost completely blocked by the agents verapamil and D-600 (a methoxy derivative of verapamil) and restored by increasing $[Ca]_o$ in the bath. This study, therefore, elegantly demonstrated a means of blocking selectively the influx of external Ca activated by depolarization and that the inward Na and Ca currents are independent of each other.

An important characteristic of cardiac muscle fibers is their ability to spontaneously depolarize, i.e. produce pacemaker potentials. The spike subsequently produced is preceded by a slow depolarization of up to 15 mV in the frog sinus venosus (Hutter and Trautwein, 1956) and 18 mV in the rabbit heart (Paes De Carvalho, Mello and Hoffman, 1959). It has been shown that the RMP of this region (-61 mV) in rabbit atrium is lower than that (-73 mV) in non-pacemaker regions of the atrium (Marshall, 1957) which is consistent with a relatively high P_{Na} or, more likely (Noble, 1962 a), a relatively low P_K . The voltage - clamp studies of Vassalle (1966) confirmed that during the slow depolarization preceding the AP (diastole), r_m steadily increases, which is consistent with earlier

studies showing that pacemaker activity is probably due to a time - and voltage - dependent decrease in g_K and finally, a voltage - dependent increase in g_{Na} as the threshold is approached (Noble, 1962).

c) Smooth muscle

The technical and theoretical difficulties posed by the ultrastructure of SM cells with reference to a suitable method of stimulation for the measurement of cable properties has been discussed above. Additional problems due to structural arrangements are met when attempting to record potential transients and ionic currents. The fibers of most SM's are branching and intertwining with each other and interwoven to various degrees with connective tissue, making it difficult to interpret current spread and mechanical responses. The cells are very small so that microelectrodes must be employed which have a very small tip diameter ($< 0.5 \mu$); moreover these are easily dislodged by mechanical activity. Also, the thickness of the tissue and the presence of substantial amounts of connective tissue result in diffusion limitation to substrates, O_2 and chemical agents. The taenia coli of the guinea - pig, however, a longitudinal intestinal SM, has proved to be a suitable model for electrophysiological work because the muscle is relatively thin and the fibers are parallel and relatively free from connective tissue (Burnstock, 1970).

The AP's of spontaneously active SM's such as the taenia coli are characterized by a relatively low amplitude, maximum rate of rise and conduction velocity when compared to those of nerve and striated muscle (Burnstock and Prosser, 1960b). The fact that, they are fully propagated however, is consistent with the muscle's behaving as a single cable (Abe and Tomita, 1968). The spikes arise from pacemakers which may be localized (e.g. ureter) or distributed throughout the muscle (e.g. gastrointestinal tract) (Burnstock et al., 1963). Each SM cell of the gastrointestinal tract possesses an inherent rhythmicity, and the net result of many different pacemaker foci is varying degrees of synchrony depending on excitability and cell interaction (Bozler, 1948; Tomita, 1970). Spikes are found to vary considerably as to 1) frequency, occurring singly, in small groups or in rapid bursts, and 2) waveform, being simple spikes which may be followed by a plateau or a positive after -

potential (Burnstock et al., 1963). Due to the small size and three - dimensional interconnections of SM cells, it has been extremely difficult until very recently to analyze the ionic currents of the AP by voltage - clamping (Anderson et al., 1971). Studies have therefore been limited to measurement of voltage transients either intracellularly (microelectrode) or extracellularly (sucrose gap) in the presence of different external concentrations of physiological cations and anions (Bülbring and Kuriyama, 1963a; Brading et al. 1969b; Kuriyama, 1963a; Holman, 1958; Marshall, 1968). The results have been very difficult to interpret due 1) to the unknown effects of the replacement ion (discussed below), 2) to the lack of accurate simultaneous measurements of changes in r_m during microelectrode measurements, and to the lack of understanding regarding the overall roles of Na and Ca.

As in other excitable tissues, spontaneously active SM's (visceral, uterine and ureter) upon depolarization, develop a transient inward current followed by a late repolarizing current as observed by voltage clamp technique (Kumamoto and Horn, 1970; Mironneau, 1974; Kao and McCullough, 1975; Vassort, 1975). The current - voltage relations of these currents also resemble those of other tissues, and, the results of Na - replacement suggest that Na may be the main current - carrying ion for the AP. The amplitude of the spontaneous spike of the guinea - pig taenia coli is reduced when $[Na]_o$ is reduced below 20 mM and abolished in the presence of 2 mM Na (tris replacement) or 10 mM Na (sucrose replacement) (Holman, 1957, 1958). The rate of rise however, was confirmed to be more dependent on $[Na]_o$ than the amplitude (Bülbring and Kuriyama, 1963a). In pregnant rat myometrium, partial substitution of Na Cl with sucrose causes a reduction in the spontaneous spike height of 45 mV per tenfold change in $[Na]_o$ (Goto and Woodbury, 1958). The importance of Na for the pregnant uterus was supported by the similar data of Marshall (1963) for the rat and by that of Kao (1967) for the rabbit. The findings of Keatinge (1968) indicate that the AP of the sheep carotid artery has a prominent Na component. This contention is also supported by voltage - clamp studies (Anderson, 1969; Anderson et al., 1971) who observed a great reduction of the initial inward current (I_a) in Na - free solution alongwith a shift of the reversal potential of this current (E_a) and

disappearance of spikes. It might be noted in this regard that a Ca current was also observed, but $[Ca]_o$ and inhibitors of Ca currents had greater effects in controlling the voltage - dependence of the conductance channels; they therefore concluded that Na plays a more prominent role in the spike regenerative mechanism. These observations and conclusions were also supported by the similar findings of Kao and McCullough (1975). The Na channels in some cases appear to lack specificity and thus, in Na - free solution, Ca appears to be capable of supporting the AP. In the ureter, I_{Na} has a slower time course, producing a plateau (Kobayashi, 1965; Kuriyama et. al., 1967a; Kuriyama and Tomita, 1970). Interpretation of ionic processes underlying spontaneous spikes is difficult on the basis of data obtained from Na - deficiency experiments since the pacemaker potential and spike conduction may also be suppressed (Tomita, 1970). Thus, in Na-free solution (hydrazine substitution) tension responses can still be recorded in response to electrical stimulation, indicating that the cells are not inexcitable but that the pacemaker mechanism (i.e. spontaneity) has failed (Axelsson, 1961 b). It is apparent from the results treated above that the presence of Na is essential for the production of electrical activity but the bulk of the available evidence suggests that Ca may be more important in this regard.

Calcium apparently plays a major role in carrying the current of the AP. Ca - deficiency certainly inhibits electrical activity but its effects are nonspecific due to the simultaneous alterations in ion conductances which are thought to be regulated by the presence of Ca. In the taenia coli, Ca - deficiency causes depolarization and decreased amplitude and rate of rise of spontaneous spikes (Holman, 1958; Bülbring and Kuriyama, 1963a) and evoked spikes (Brading et. al., 1969b). Excess $[Ca]_o$ causes opposite effects and increases threshold while reducing the latency from the onset of the stimulus to the peak of the AP (Brading et. al., 1969b). In the presence of normal $[Na]_o$ complete removal of Ca causes depolarization, abolishes all activity and reduces r_m considerably (Brading et. al., 1969 b). Reduction or removal of Na_o restores membrane resistance and spontaneous spikes for a short time (Holman, 1958; Bülbring and Kuriyama, 1963a). However, nearly normal spikes can be evoked and spontaneous activity returns after addition of only 1/10 normal $[Ca]_o$ (Brading et. al., 1969b). These effects are

more than can be explained by the shifts in E_m in low Ca and low Na media. Similar evidence is available for the myometrium whose AP parameters are similar to those of the taenia coli (amplitude up to 70 mV, $dV/dt_{\max}$ about 10 V/sec and duration 10 - 50 msec) but vary with species and hormonal condition. Both the spontaneous and evoked spike are followed by a negative after - potential. The height and $dV/dt_{\max}$ are reduced only for $[Na]_o < 1/10$ normal (Csapo and Kuriyama, 1963; Marshall, 1965). Kao (1967) has argued that this small effect was due to very slow diffusion of Na_i from a thick preparation, but this suggestion seems unlikely in the light of Na flux studies (Durbin and Manson, 1961; Goodford and Hermansen, 1961). The evoked spike is very dependent upon $[Ca]_o$ and Ca - deficiency has a greater effect in normal $[Na]_o$ than in low Na solution (Marshall, 1965; Abe, 1968, 1971). As the foregoing results are difficult to interpret with regard to the actual contribution of I_{Ca} and/or I_{Na} , a more specific method is required.

The use of antagonists of Na and Ca channels is a more selective method of determining the identity of the active ionic currents. The AP of the taenia coli is 1) sensitive to manganese ion (Mn^{2+}), an inhibitor of Ca influx (Nonomura et al., 1966; Brading et al., 1969b; Bülbring and Tomita, 1969c), 2) unaffected by tetrodotoxin (TTX) (Nonomura et al., 1966; Kuriyama et al., 1966) which blocks nerve stimulation (Bülbring and Tomita, 1967) and 3) maintained in Ca - free, Na - free solution by barium ion (Ba^{2+}) which apparently replaces Ca in carrying the current of the AP (Hotta and Tsukiu, 1968; Bülbring and Tomita, 1968b, 1969c). Similarly, the AP of myometrium is suppressed by Mn^{2+} but not by TTX (Abe, 1970).

An even more direct but technically more difficult method is the voltage - clamp technique to measure the ionic currents. In the taenia coli, Inomata and Kao (1976) observed that reduction of $[Na]_o$ to 50% of normal or total replacement with dimethyldiethanol ammonium ion or choline ion fails to cause a significant change in the reversal potential (E_a) of the early inward current (I_a) or reduce the magnitude of I_a . Reduction of $[Ca]_o$, on the other hand, to 1/4 or 1/10 of normal shifts E_a toward negative values and reduces I_a without affecting the maximum chord conductance $\left(\frac{I_a}{V}\right)$ of I_a . Also, 5 times normal $[Ca]_o$ increases I_a and $\frac{I_a}{V}$.

although E_a is not shifted unless treated with TEA to reduce the strong outward rectifying current. Anderson et al. (1971) showed that in the rat myometrium I_a is greatly reduced by the Ca influx inhibitors Mn^{2+} , cobalt (Co^{2+}) and lanthanum (La^{2+}) but is unaffected by TTX, although they concluded that I_{Na} was more important than I_{Ca} in the capacity of carrying the inward current. Vassort (1975), however, showed that in the guinea - pig myometrium, I_a 1) has a low reversal potential (E_a) of about +20 mV, 2) is unaffected by alterations in $[Na]_o$ (substitution with lithium or sucrose) but dependent on $[Ca]_o$, and 3) is insensitive to TTX but inhibited by Mn^{2+} or D-600 (a specific antagonist of depolarization - activated Ca influx). From these studies it has been concluded that Ca is responsible for AP generation in the taenia coli and myometrium although a slow inward current dependent on $[Na]_o$ has been identified in the myometrium (Anderson et al., 1971; Vassort, 1975).

Similar evidence obtained by other workers has implicated Ca as the major AP - generating cation in the cat ureter (Kobayashi, 1965), rat ureter (Kuriyama et al., 1967a; Kuriyama and Tomita, 1970), vas deferens (Bennett, 1967a; Tomita, 1967a), guinea pig stomach (Sakamoto and Kuriyama, 1970) and rat portal vein (Daemers - Lambert, 1976).

The late outward current is primarily carried by K since it becomes inward for $[K]_o > 120$ mM with the potential held at RMP (Kao and McCullough, 1975; Inomata and Kao, 1976) and is markedly reduced by TEA (Mekata, 1971; Inomata and Kao, 1976). Vassort (1975) and Kao (1971) have differentiated between 1) a fast early outward current, that is blocked by TEA and has a reversal potential of about -95 mV, and 2) a late outward current that causes delayed rectification. The fast outward current appears to account for the low amplitude of the AP and its fast repolarization. This interpretation is supported by the finding that the amplitude and duration are increased by TEA and conditioning hyperpolarization which suppress the increase in g_K (Vassort, 1975). Although it will be discussed at greater length in section C, part 4, Vassort (1975) has proposed that the fast outward current is a result of an initial fast Ca influx, which transiently increases the local $[Ca]$ at the inner membrane surface, thereby momentarily increasing P_K .

This concept has important implications in the control of firing behaviour by the nervous system, circulating neurohumors and the ionic environment. It should be also noted that delayed rectification is the only type which occurs in SM. Anomalous rectification is not observed (Mekata, 1976) even in Na - free, high K_2SO_4 solution as seen in striated muscle fibers (Adrian and Freygang, 1972). This is consistent with the lack of tubular invaginations of the surface membrane and the small amount of sarcoplasmic reticulum in SM (Carsten, 1969).

In conclusion, the spike of most, if not all, smooth muscles is based on a transient, rapid inward Ca current followed by a delayed outward K current which repolarizes the membrane. There may also be a slower inward Na current giving a plateau effect and/or a rapid outward K current which limits spike height. It also appears that 1) Ca bound or in association with the cell membrane stabilizes membrane conductance by altering g_{Na} and g_K and activating the regenerative process for spike generation (Frankenhaeuser and Hodgkin, 1957; Bülbring and Tomita, 1970) and that 2) Na may compete with Ca in carrying inward current and in controlling g_m . Thus the effect of reducing $[Na]_o$ on E_m and on the spike is similar to that of increasing $[Ca]_o$, indicating a competitive interaction as proposed by Lüttgau and Niedergierke (1958) for frog heart.

Another indication that Ca serves as the major current - carrying cation is based on the distribution and permeability of Ca. Since the maximum dV/dt of the AP is determined only by the ionic current (average dV/dt is also affected by conduction velocity of the AP and several other factors, Kao, 1967), it is a function of the external concentration of the ion and thus indicative of the nature of the ionic current. Consistent with the low dV/dt_{max} (up to 10 V/sec) of the AP of SM cells is the low $[Ca]_o$ and the low membrane permeability to Ca. In addition, the high inward electrochemical gradient for Ca $\left(\frac{[Ca]_o}{[Ca]_i} \approx \frac{2.5 \times 10^{-3} M}{10^{-7} M} \right)$

(Goodford, 1970) puts Ca in a position to carry inward positive current upon depolarization - activated increase in Ca conductance.

It must be stated however, that there is a serious problem in interpreting the effects of the various agents and ionic alterations discussed above.

Substitution of various substances for part of the external Na is a drastic intervention since Na is a major determinant of the osmotic balance of the cell. Some of the agents which have been used are 1) sucrose, which is nonionic and therefore reduces the total ion concentration, 2) tris (hydroxymethyl aminomethane) which has an amino group and is generally found to have toxic effects, 3) lithium which is able to substitute for Na in the Na pump (Friedman, 1975), and 4) choline which may have a stimulatory effect similar to that of ACh. According to the Nernst equation, a decrease in $[Na]_o$ should make E_{Na} less positive and therefore hyperpolarize the membrane slightly. After a transient hyperpolarization tris depolarizes the taenia coli, indicating a toxic effect (Bülbring and Kuriyama, 1963a). When sucrose is substituted, an equivalent amount of Na Cl is reduced so that the effects of Cl-deficiency and decreased ionic strength must be taken into account. Thus the hyperpolarization seen with sucrose substitution is likely due to the shift in E_{Na} , but the reduction in $[Cl]_o$ would decrease E_{Cl} (since sucrose is nonionic and nonpermeant) as well as g_{Cl} . This, in turn, would tend to depolarize the membrane (or at least reduce the hyperpolarization) and increase R_m , possibly accounting for the greater spike amplitude (Kuriyama, 1963a, 1970). The lower ionic strength might 1) increase the number of fixed sites in the membrane for Ca which in turn would reduce P_K and P_{Na} discussed below, thereby increasing r_m (Frankenhaeuser and Hodgkin, 1957), or 2) reduce the activity of a Na - Ca pump (if present) so that more Ca is retained in the cell, thereby increasing r_m and mechanical responses. Furthermore, the lack of inhibition by TTX is not conclusive since tetrodotoxin - resistant Na influx has been demonstrated in newt and tetrodon fish muscle (Kao, 1966), barnacle muscle (Hagiwara et. al., 1969) and ovariectomized, estrogen - primed rat uterus (Anderson et. al., 1971). Mn ion has been shown to partially replace Ca in its membrane stabilizing action (i.e. shifts voltage - dependence of conductances) (Hagiwara and Takahashi, 1967) and to reduce the delayed outward K current in calf Purkinje fiber (Kass and Tsien, 1975). These additional effects, however, would not conflict with the interpretation of its primary effect on Ca influx. The apparent ability of Ba to substitute for Ca may be due to trace amounts of Na and/or Ca left in the extracellular space (Bülbring and Tomita, 1970) and/or to a greater

P_{Ca} due to Ba. Since Ba is known to decrease g_K , and increase r_m (Bülbring and Kuriyama, 1963a; Hermsmeyer, 1976), depolarization would be greater and might increase the number of dynamic Ca channels, depending on their voltage dependence. Finally, with regard to the voltage clamp studies (Inomata and Kao, 1976), the failure of high $[Ca]_o$ to shift the reversal potential (E_a) of the early inward current (I_a) toward a more positive value was corrected by reducing the outward I_K with TEA. Thus it was concluded that E_a is influenced as much by the voltage threshold for activation of I_K as by the electrochemical driving forces on I_a .

The possibility that Ca may replace Na in carrying the charge to generate the spike only in low $[Na]_o$ can be determined by the application of a specific inhibitor of dynamic Ca channels such as D-600 (Kohlhardt *et al.*, 1972). The observation that spike parameters, especially dV/dt_{max} , are increased in high $[Ca]_o$ and then abolished after addition of D-600 would be strong evidence for a dominant role for Ca in the AP. The role for Ca as a membrane stabilizer will be discussed at greater length in section C, part 4 below.

Indirect evidence is available that AP's are produced by canine tracheal smooth muscle (TSM) *in vitro* in the presence of certain agents, and that they are Ca - dependent (Stephens *et al.*, 1974; Kroeger and Stephens, 1975). This preparation does not normally produce any phasic electrical and mechanical activity *in vitro* either spontaneously or in response to agents and conditions such as ACh, high K, Ba^{2+} , acidosis, hypoxia or electrical stimulation (Stephens *et al.*, 1974). It responds only with a graded depolarization and a tonic contraction proportional to the strength of the stimulus, and a myogenic contraction (MR) in response to quick stretch can not be elicited (Stephens *et al.*, 1974). Histamine produces increased tone and slow oscillations of the electrical response which are dependent on $[Ca]_o$ (Kirkpatrick, 1975). However, spontaneous phasic electrical and mechanical activity and a MR to stretch are produced in the presence of tetraethylammonium (TEA) (Stephens *et al.*, 1974; Kroeger and Stephens, 1975) which blocks g_K (Mekata, 1971). The ability to produce a MR in TEA is absolutely dependent on $[Ca]_o$ within the physiological range of $[Ca]_o$ (Stephens *et al.*, 1974) and is

completely blocked by D-600. Thus it was considered that the MR in the presence of TEA is caused by stretch - induced depolarization which initiates AP's whose inward current is carried by Ca ions. The phasic mechanical activity in TEA is also abolished by Ca - free solution containing EGTA (a Ca chelator) and by lanthanum (which inhibits Ca fluxes and depletes the extracellular space of Ca, Mayer *et al.*, 1972) (Kirkpatrick, 1975). D-600, however, is the most specific Ca antagonist available (Kohlhardt *et al.*, 1972) and the validity of using it is strengthened by the findings that D-600 1) inhibits K - induced contractions to a greater extent than ACh - induced contractions, 2) does not inhibit Ca - sensitive actomyosin ATPase activity and 3) does not inhibit contractions induced by the Ca ionophore A23187 (Stephens *et al.*, 1974). Thus indirect evidence has been provided that the depolarization - activated Ca and/or Na currents produced in TSM are masked by a high resting g_K . When this is blocked by TEA, Ca - dependent AP's are seen.

3. Threshold Phenomena and Conduction

a) Nerve

As indicated in the previous section regarding the ionic basis of the AP, the cable properties are insufficient for information transfer. An AP must be generated and sustained for the entire length of the tissue (with the possible exception of muscles with extensive innervation). This function is critically dependent upon the condition that the current produced by a given stimulus is of sufficient intensity to depolarize the adjacent membrane to threshold. Current thus flows axially and then radially through the r_m and c_m and back to its point of origin, forming local circuit currents (Hermann, 1899). The achievement of threshold is therefore a function of the cable properties of the resting fiber and is thus current - and time - dependent. It might be noted that Hodgkin (1937) showed that the depolarization by electrotonic spread has exactly the same AP - generating effect as the depolarization produced by a current - passing electrode.

The probability that a given depolarizing current will produce threshold depolarization (E_{Th}) and excitation is related to the strength - duration curve (Hill, 1936). This is the relationship between the strength or intensity and the duration of a square - wave current pulse just sufficient to excite. For short durations

the quantity of charge ($i \times t$) required to excite is constant, and the relation is represented by

$$\frac{i}{i_{\infty}} = \frac{1}{1 - e^{-t/k}} \quad (22)$$

where i = pulse strength, i_{∞} = threshold strength when $t = \infty$ (rheobase) and k = a constant (Hill, 1936). The pulse duration when the intensity is twice rheobase is called the chronaxie and allows comparison between tissues. This equation, however, is simplified, in that it assumes a constant voltage threshold (i.e. unchanging r_m), but Hodgkin and Rushton (1946) showed that 1) E_{Th} increases as stimulation duration decreases and 2) intracellular depolarization leads to a falling voltage described by an error function, not exponential (Noble, 1966). Thus, as a result of the cable properties, strong currents of short duration produce depolarizations which fall off more rapidly with distance than depolarizations produced by weaker, longer currents (Aidley, 1971).

With reference to depolarization of regions adjacent to the active area by local circuit currents, these currents must first discharge c_m in order to depolarize the membrane to E_{Th} . Tasaki and Hagiwara (1957) have shown that the foot of a propagating AP rises exponentially with a time constant determined by the cable properties of the fiber at rest and by the conduction velocity of the AP. As depolarization proceeds, g_{Na} becomes progressively greater (voltage - and time - dependent), allowing an inward Na current which produces a subthreshold local potential over and above the ETP. Depolarization, however, also inactivates these Na channels (which repolarizes the membrane) and activates a gradual increase in g_K which accelerates the repolarization. A depolarization, which results in an inward Na current that is equal to or greater than the outward current, has achieved the threshold potential (E_{Th}) at which the ionic regenerative mechanism takes over to produce an AP (Hodgkin and Huxley, 1952b). The AP thus produced is independent of stimulus intensity (all - or - none law) and is fully - propagated while local potentials are conducted decrementally (Hodgkin, 1938). The AP's of nerve provide local circuit currents which depolarize the membrane in front of them, and the stimuli which produce these AP's (synaptic potentials) produce a depolarization which would be greater than E_{Th} had an AP not been generated. Such "suprathreshold" depolarization

provides the AP with a margin of safety which can compensate for any event (slight injury, fall in temperature) which could reduce the inward I_{Na} and thus the amplitude of the AP. Consequently, the production of a propagating AP is dependent upon a suprathreshold depolarization allowing the ionic regenerative mechanism to take over and generate local circuit currents.

The velocity of AP conduction is also determined by the passive cable properties in that it depends on the rate at which the c_m ahead of the impulse is discharged to the threshold level. This in turn depends on the absolute and relative magnitudes of c_m , r_i and r_o as well as the safety margin of the impulse and temperature. Since velocity varies inversely with the intracellular resistance ($\frac{1}{r_i}$), it is closely

related to fiber diameter. Theoretically, other factors being equal, propagation velocity increases with the square root of the axon diameter (Rushton, 1951; Hodgkin, 1954). These other factors include specific capacitances and resistances, time constants of the membrane and foot of the conducted AP and current density per unit area. If conduction is brought about by local circuit current in a fiber with cable properties, the conduction velocity (Θ) is proportional to a factor

$$\Theta = \frac{S'}{\sqrt{2 R'_m}} \cdot \frac{\sqrt{a}}{C_m \sqrt{R_i}} \quad (23)$$

(Katz, 1948) where S' = safety factor, R'_m = active R_m , and a = fiber radius (μ). Conduction velocity ranges from 1150 to 2500 cm/sec in squid axon (Hodgkin, 1939; Hodgkin and Katz, 1949).

These large fibers and high conduction velocities are far greater than the norm for invertebrate nerves. Specialization of nerve ultrastructure that accounts for a much higher conduction velocity is also found in vertebrate peripheral axons, but it is the effective membrane resistance rather than the diameter which is greater. These axons are very small in diameter (1 - 20 μ) but are surrounded at intervals by accessory cells called Schwann cells which consist of a fatty material called myelin. The Schwann cells are wrapped many times around the axon forming a tightly - packed sheath (Robertson, 1960). There are thus no extracellular channels between the axon and myelin except at the interruptions, termed the nodes of Ranvier.

The myelin acts as a chain of resistances and capacitances in series, and has a much higher transverse resistance (160 k ohm cm^2) and much lower transverse capacitance (2.5 p F/cm^2) than a normal cell membrane. Consequently, the r_m and c_m are effectively increased and decreased, respectively. The myelin sheath, therefore, permits a far greater electrotonic spread with the result that the ETP falls to $\frac{1}{e}$ of its value in 2 - 3 mm rather than 0.5 mm as occurs in nonmyelinated nerves (Davson, 1970). As a result, local circuit current leaves the core conductor only at the nodes which become depolarized to threshold. It has been shown that threshold stimulus intensity is lowest at the nodes (Tasaki, 1953) to which the depolarization - activated inward currents are restricted (Tasaki and Takeuchi, 1942; Huxley and Stampfli, 1949). Thus, AP's are produced only at nodes and are conducted much faster than AP's of nonmyelinated axons of the same diameter. The conduction velocity AP's of myelinated fibers is directly proportional to the diameter and may be as high as 100 m/sec in some vertebrates (c.f. Davson, 1970). Thus, in nerve axons, two mechanisms based on ultrastructural modifications are found which facilitate a high rate of propagation of impulses. With reference to myelinated axons, a large volume of signals can be integrated due to a great many axons of small diameter transmitting a large number of signals to higher centers.

b) Striated and cardiac muscle

The aspects of threshold and propagation considered above for nonmyelinated nerves generally apply to striated and cardiac muscle fibers. Striated fibers however, are normally activated by nerves, which terminate in end-plates or neuromuscular junctions (Birks et al., 1960). An AP will cause the release of ACh from a cholinergic nerve ending or noradrenaline from an adrenergic terminal. The transmitter in turn depolarizes the post-synaptic membrane, initiating an end-plate potential in twitch fibers that becomes an all-or-none, fully-propagated spike which activates the whole fiber (Fatt and Katz, 1951). Tonic fibers on the other hand, depend on simultaneous activation of numerous grape-like endings, no one of which is capable of inducing a propagated disturbance.

Cardiac fibers display spontaneous myogenic pacemaker activity as discussed

above. An AP is initiated when the slowly - rising pacemaker potential achieves threshold (Draper and Weidmann, 1951). Although the regions known as the sino-atrial (S-A) node, atrio - ventricular (A-V) node and Purkinje fiber system have their own rhythmicity, that of the S-A node has the highest frequency and thus acts as a pacemaker for the whole organ. Activity begins at the S-A node and spreads as a wave over both atria to the A-V node and finally along the Purkinje fibers which ramify in the septum separating the ventricles. Although the activity is transmitted across the intercalated discs, conduction becomes decremental at the A-V node, i.e. the AP is propagated but with diminishing velocity and amplitude (Paes De Carvalho, DeMello and Hoffman, 1959). However, the AP increases in velocity from 100 to 300 cm/sec as it reaches the Purkinje fibers (Hoffman, 1965).

Although the AP of cardiac muscle fibers is myogenic in origin, its rate and amplitude can be modified by the action of extrinsic nerves. Parasympathetic nerve fibers (cholinergic) cause hyperpolarization and cessation of spontaneous activity, due probably to an increase in g_K (Hutter, 1961). Sympathetic nerves (adrenergic) cause depolarization and an increase in AP frequency (Hutter and Trautwein, 1956). The electrical activity of the heart is therefore modified in different regions of the heart by differences in intercellular conduction and individual cell excitability and is under fine control by nervous influences.

c) Smooth muscle

Since SM's have cable - like properties (Abe and Tomita, 1968), it is natural to suppose that propagation of the spike is brought about by a local circuit current which flows between active and resting regions, as in nerve. If the relatively small spike normally seen in SM is initiated only in a single cable (a series of interconnected cells) which is surrounded by many interconnected cables as already discussed, propagation can not occur, because the local circuit current spreads in three - dimensional directions into surrounding cells, becoming too weak to excite (Kuriyama and Tomita, 1965; Tomita, 1966a, 1967a). However, spikes generated simultaneously in many cables can propagate because local circuit current flows only in the direction of the fiber axis (Tomita, 1966a; Abe and Tomita, 1968; Tomita, 1967a). Since the density of the local circuit current depends on the ratio of the active area to the surrounding passive area, it follows that the active response

shows quite different properties depending on the method of stimulation.

The threshold and spike mechanisms of SM cells are very similar to those of the other excitable tissues discussed above. The strength - duration curve for the taenia coli is a simple hyperbola, with a chronaxie of about 20 msec which agrees well with that calculated from cable theory (14 - 24 msec, Tomita, 1966a). Most of the intestinal SM's (especially longitudinal muscles) have spontaneous spikes which appear to be myogenic in origin. Thus spontaneous activity is observed after treatment with cholinergic blocking agents (atropine) or adrenergic blockers (hexamethonium or cocaine) (Bülbring, 1955; Bülbring et. al., 1958; Bülbring and Kuriyama, 1963a; Kuriyama et. al., 1967c) and also with TTX which blocks the nerve - mediated responses (Nonomura et. al., 1966; Kuriyama et. al., 1966, 1967c; Hashimoto et. al., 1967; Bulbring and Tomita, 1967; Tomita, 1967a; Ito and Kuriyama, 1971). Spike activity, however, is influenced by nervous activity since atropine and TTX modify the spontaneous spike activity by reducing the slow changes in E_m (Kuriyama et. al., 1967c).

The mechanism of automaticity in SM is still obscure and controversial, but it is based on slow oscillations of E_m which may be pacemaker potentials or local potentials (Holman, 1958; Bülbring and Kuriyama, 1963a; Bülbring, 1955; 1957a). The slow component in the taenia coli usually precedes a spontaneous spike, triggers it and may persist as a depolarization delaying full repolarization. However, the slow component may also appear on the falling phase as a notch or may follow a spike. Pacemaker activity is voltage - dependent since the slope of the pacemaker potential, and hence the frequency of the spike, is increased by depolarization and decreased by hyperpolarization (Bülbring, 1955, 1957a). The rate of rise is a function of the E_m from which they arise (Bolton, 1971). It is generally agreed that the slow potential is dependent on Na but several mechanisms have been put forward to explain its production. The finding that the electrical control of small intestine was sensitive to temperature, hypoxia, metabolic poisons and inhibitors of the Na pump led to the suggestion that this activity results from oscillations of an electrogenic Na pump (Job, 1969; Liu et. al., 1969; Daniel, 1965). However, slow waves produced by carbachol in longitudinal ileal SM persist in K - free solution (Bolton,

1972). In addition, inhibition of the electrogenic Na pump with ouabain or Na - free solution and stimulation by Na injection or admission of K to Na - rich tissues do not affect the magnitude and time course of the spontaneous slow waves to the extent expected on this basis (El-Sharkawy and Daniel, 1975). Na - free solution rapidly and reversibly abolishes slow waves but not spikes (Bolton, 1971, 1972; El-Sharkawy and Daniel, 1975; Bülbring and Kuriyama, 1963a). Since varying $[K]_o$, $[Cl]_o$ or $[Ca]_o$ had relatively minor effects on the slow waves produced by carbachol or acetylcholine, Bolton (1972) proposed that slow potential changes and the differences in configuration of the AP in various SM's may simply reflect differences in the relative time courses and current carrying capacities of two separate regenerative ion channels, one admitting mainly Na for the slow wave and the other mainly Ca (for the spike). The finding that spontaneous slow wave activity persisted after spikes and mechanical activity were stopped by verapamil but were abolished in nominally Ca - free solution indicates that the inward Na current suggested as responsible for slow wave generation may be dependent on the presence of Ca_o (El-Sharkawy and Daniel, 1975). These authors have concluded that the slow potential may consist of a depolarization phase with initial and secondary components which produce a rise and a plateau, respectively. They have demonstrated that the secondary depolarization is selectively depressed by impermeant anions and sucrose but not by lithium chloride and less permeant anions. Thus they have suggested that the slow wave results from a transient increase in g_{Na} and g_{Cl} , the latter with a slower time course. This proposal is consistent with the observations that replacement of Cl with a less permeant anion usually suppresses spontaneous electrical and mechanical activity of taenia coli (Axelsson, 1961a; Kuriyama, 1963a; Bülbring and Tomita, 1969a) and that many factors which reduce the spontaneous activity reduce $[Cl]_i$ (low $[K]_o$, low temperature and hyperosmolarity (Brading, c.f. Tomita, 1970)).

With reference to the origin of spontaneity of other SM preparations, the spontaneous contractions of pregnant rat uterus are caused, at least in part, by prostaglandins (PG's), since spontaneous and oxytocin - induced contractions and PG synthesis are blocked by indomethacin and restored by addition of PG's E_2 and

$F_{2\alpha}$ (Vane and Williams, 1973). Pacemaker - like activity is induced (or unmasked) in cat spleen capsule (Bose and Innes, 1974) and guinea - pig vas deferens (Cliff, 1968) by agents such as cocaine and other adrenergic blockers in the presence of α -adrenoceptor stimulants. Thus adrenergic nerve endings may inhibit such myogenic activity in the normal situation.

Every cell may have a tendency to produce a pacemaker potential at random, but since the AP produced in one cell is too weak to excite other cells and since the shape of the prepotential is variable, there are probably many cells involved in forming a pacemaker region (Tomita, 1970). These cells may be synchronized by 1) achievement of a threshold number or critical mass of cells producing a pacemaker potential and synchronizing the region, 2) stretch depolarizing a region and increasing spontaneous activity, or 3) a weak local circuit current stimulating a low threshold region (Tomita, 1970).

That AP's produced in SM are able to propagate from cell to cell via local circuit currents and without mediation of nerves was first proposed by Bozler (1938, 1948). His studies, based on data from spontaneously active SM's such as those found in the ureter, uterus and intestine, suggested that SM behaves as a functional syncytium since 1) large injury potentials as well as monophasic and diphasic AP's can be obtained (Bülbring et. al., 1958), 2) stimulation occurs only at the cathode, 3) cocaine does not abolish excitability or conduction, 4) chronaxie and refractory phase are much slower than in the slowest type of nerve fibers and 5) the rheobase is of the same order of magnitude as that of long striated muscle fibers. Further supporting evidence for the flow of local circuit currents in a functional syncytium is given by the findings that 1) conduction velocity is reduced (Nagai and Prosser, 1963b) or conduction reversibly blocked (Barr et. al., 1968) by increasing the electrical resistance of the external fluid, 2) conduction through a sucrose gap can be restored when the shunting resistance is reduced by connecting the chambers separated by the gap with a low electrical resistance (Barr et. al., 1968) 3) the time course of the foot of the propagated spike fits the prediction based on the cable theory (Tomita, 1966b; Abe and Tomita, 1968), and 4) the space constant of the spatial decay of the ETP is very similar (Tomita, 1966b; Abe and Tomita, 1968) to that of the skeletal muscle fiber (Fatt and Katz, 1951). Since it is assumed that

r_i is more or less similar (200 - 400 ohm cm) in all excitable tissues (Tomita, 1966a, 1969), the evidence presented above may partly be explained by a high r_m (Abe and Tomita, 1968) and partly by the suggestion that many fibers, connected in series and in parallel, are aggregated in bundles of large diameter (Bülbring, 1954) which may act as functional units (Tomita, 1966a, b).

The concept of a functional bundle is suggested by the observations that AP's are not fully propagated (i.e. become graded in amplitude and conduct decrementally) in bundles of less than 100 μ (Burnstock and Prosser, 1960b; Bennett and Burnstock, 1968) or if the tip of an external pressure electrode is less than 100 μ in diameter (Nagai and Prosser, 1963a). However, the bundle is not a simple cylinder but branches out and joins other bundles forming a mesh. Therefore, it is reasonable to assume that the geometrical factor of branching produces some complications of spike conduction (Noble, 1966; Tomita, 1967a). Thus, the local circuit current of a spike which propagates along a bundle must excite a larger area at the branching point. Therefore, the safety factor would be low and the spike more easily blocked (Tomita, 1969). Normal propagation in SM seems to require a critical amplitude for the spike. This amplitude depends upon the geometry and excitability of the region, but conduction appears to become decremental when the amplitude is reduced to about half the normal amplitude or the membrane is hyperpolarized by about 10 mV (Tomita, 1967b, 1969). The spike generation in the taenia coli appears to be both all - or none and graded. One explanation might be that spike size depends on the number of active cells participating, but the observation that spontaneous spike activity in the taenia coli is abolished in hypertonic sucrose solution while the evoked spike is only reduced without change in time course argues that individual cell excitability is the main factor determining AP generation (Tomita, 1966a). This is at variance with the report that hypertonic solution decreased nexal area and electrotonic coupling between the cells of the taenia coli and abolished propagated AP's (Barr et al., 1968). Increasing $[K]_o$ restores the spontaneous spikes in hypertonic solution (Tomita, 1966a), suggesting that the main factor is hyperpolarization rather than disruption of cell contacts. Graded excitability of a single cell would explain 1) the graded spike amplitude produced by intracellular

stimulation (Kuriyama and Tomita, 1965; Kuriyama et al., 1967a; Bennett, 1967b; Tomita 1967a; Hashimoto et al., 1966), 2) the increase in spike size produced by conditioning hyperpolarization 3) the graded responses produced by short current pulses and 4) the fluctuations in spike size during spontaneous activity in normal solution (Tomita, 1966a).

The conduction velocity of an AP may be slightly different in individual bundles and may be affected by membrane polarization (Tomita, 1966a). In SM which evidences fast conduction, the extracellular space is found to be relatively small, the fibers closely packed and the longitudinal internal resistance ($r_i + r_j$'s) of a strip in the sucrose gap is low compared to that of slow - conducting muscle (Burnstock and Prosser, 1960b; Prosser et al., 1960). Thus the conduction velocity may be related to the number of close intercellular contacts and the distance between them. The conduction velocity also depends on the rate at which c_m ahead of the spike is discharged and is proportional to the maximum rate of rise of the AP (Tomita, 1970). The ratio of $dV/dt_{\max}$ to conduction velocity is roughly constant in all muscle fibers (120 - 440 mV/mm) and represents the maximum potential gradient along the cable produced by the propagating spike (Tomita, 1970). The fastest conduction velocity in SM is seen in the taenia coli (6 - 9 cm/sec, Tomita, 1966a; Abe and Tomita, 1968) but this is only about 1/20 of that of striated muscle (Katz, 1948). Although SM fiber diameter is relatively small, the λ is comparable to that of skeletal muscle (≈ 1.6 mm). The slow velocity may be explained by 1) the c_m of a muscle bundle which may be large owing to the larger surface area of many aggregated interconnected fibers, 2) a low safety factor because of the low rate of rise of the Ca spike (Holman, 1958; Kuriyama and Tomita, 1965; Brading et al., 1969b) and 3) a high chord conductance (Tomita, 1966a).

From the foregoing considerations as well as those of ultrastructure and current spread in SM, the difficulty of initiating an AP by intracellular stimulation is evident. Due to the three - dimensional interconnections, intracellular polarization causes no frequency modulation of spontaneous discharge (Kuriyama and Tomita, 1965) and evokes a spike only occasionally. The spike thus triggered has 1) no slow component, 2) a graded height, 3) a high threshold, 4) a short latency

and 5) a very localized activity (Tomita, 1970). According to this idea therefore, a spike is more easily evoked in a cell with fewer low - resistance connections with surrounding cells.

The other explanation given above (spike generation in taenia coli, Tomita, 1966a) for graded responses is the possibility of individual cell excitability. Thus Bozler (1948) differentiated between SM's which display spontaneous phasic electrical and mechanical activity which he termed single - unit, and those which normally do not, known as multi-unit SM's. The first group (ureter, uterine and intestinal SM) also show 1) a myogenic response (MR) to stretch (Burnstock and Prosser, 1960a; Folkow, 1964; Olsen et al., 1967; Biamino and Kruckenberg, 1969) and 2) good cell - to - cell conduction of regenerative electrical activity (Bülbring et al., 1958; Prosser, 1962; Burnstock and Prosser, 1960b) which is consistent with their sparse nerve supply. Multiunit SM's are stimulated by dense innervation but the demonstration of a λ similar to that of single - unit SM's suggests that the absence of phasic electrical activity is not the result of a lack of close cell contacts. An intermediate group (vas deferens, arterial SM) can be stimulated to produce phasic activity by certain agents which stimulate the muscle directly, such as high K, ACh and adrenergic amines (Keatinge, 1966; Biamino and Kruckenberg, 1969).

The importance of K and g_K for excitability has been demonstrated in a variety of studies. It is interesting to note that nonspontaneously active SM's (e.g. sphincter pupillae, vas deferens) generally have higher RMP's (Burnstock et al., 1963), which would be consistent with a relatively high g_K/g_{Na} . Tomita (1966b) has suggested that a low g_K might be the reason for the low RMP and spontaneous activity observed in taenia coli (Kuriyama, 1963a; Bülbring et al., 1958; Bülbring, 1962). Low rate constants for K efflux have been obtained for dog carotid artery (Barr, 1961b) and rat uterus (Daniel et al., 1962) which are also spontaneously active. It has been demonstrated recently that high K increases g_K (decreases r_m), lowers E_m , and converts bursts of spikes and accompanying contractions to single spiking and greatly reduced contractions while Ba^{2+} reduces g_K and increases the number of spikes per burst and contraction amplitude (Hermsmeyer, 1976).

Therefore, it appears that a reduction in g_K/g_{Na} should produce depolarization, increased r_m and spontaneous activity (assuming the presence of dynamic, depolarization - activated ion channels).

TEA has been widely used as a tool to block g_K . The effects of TEA on various preparations fall into two categories: 1) blockage of the steady state K channel (time - and voltage - dependent block), characterized by decreased rectification and lengthening of the AP duration but no change in E_m (Tasaki and Hagiwara, 1957); 2) reduction of resting g_K , producing a decrease in E_m and an increase in r_m (Stanfield, 1970). Plateau - type AP's are produced in term - pregnant rat uterus (Kroeger, unpublished observations), rabbit carotid artery (Mekata, 1971) and sometimes bovine tracheal SM (Kirkpatrick, 1975). Rectification was reduced in all studies but r_m remained unchanged in guinea - pig antrum (Ito et. al., 1970) and fundus (Osa and Kuriyama, 1970), guinea - pig rectum (Ito and Kuriyama, 1971) and rabbit carotid artery (Mekata, 1971). In electrically excitable muscle fibers TEA increases AP amplitude and maximum dV/dt (Osa and Kuriyama, 1970; Ito and Kuriyama, 1971).

The most interesting feature of the actions of TEA is its ability to induce spontaneous phasic electrical and mechanical activity in multi-unit or intermediate SM's when other common stimulatory agents and conditions are ineffective in this regard (Stephens et. al., 1974). In rabbit carotid artery (intermediate), TEA treatment results in depolarization and greatly decreased rectification but no change in r_m (Mekata, 1971). This is of interest in that it indicates an effect of TEA on both resting and steady state g_K and perhaps on g_{Na} . Depolarization normally activates g_K which is reflected in a decline in r_m . Depolarization is also a result of a reduction in g_K (primary effect of TEA) in which case r_m is increased. The fortuitous absence of a change in r_m may represent an interaction of these two processes, and possibly an increase in g_{Na} which might contribute to the absence of a net increase in r_m . In canine trachealis there occurs a marked depolarization (12 mV), a small decrease in rectification and a substantial increase in r_m as evidenced by the increase in λ from 1.6 to 2.8 mm (Kroeger and Stephens, 1975). Similar results were obtained for bovine trachealis but the sucrose gap was unable to detect a change in r_m (Kirkpatrick, 1975). It is clear that simultaneous

measurement of r_m is essential to understanding the action of any agent.

The AP's produced in the canine trachealis by TEA are decremental and have a low amplitude, $dV/dt_{\max}$ and conduction velocity (Kroeger and Stephens, 1975). Thus, for stimulated AP's, 1) the amplitude and latency depend on the stimulus intensity and 2) the amplitude and $dV/dt_{\max}$ depend on the distance they are recorded from the stimulus site. Basal tension is also increased in the presence of TEA, and the muscle is able to produce a MR to stretch which varies in amplitude with $[Ca]_o$ and is blocked by D-600 (Stephens *et al.*, 1974). The effects of TEA are also blocked by conditions which decrease membrane permeability, such as Mg (Stephens *et al.*, 1974). Since K permeability is a prominent contributor to total membrane permeability, polarization and rectification of depolarizing pulses, (Casteels and Kuriyama, 1966) a decrease in g_K with TEA would be consistent with these findings. The possibilities were considered that TEA might alter g_{Cl} (Lux, 1971), substitute for Na or Ca or labilize ACh from nerve endings. A change in g_{Cl} is unlikely as a decrease would account for the greater r_m but would tend to hyperpolarize the membrane since E_{Cl} is less negative than RMP (Casteels and Kuriyama, 1965; Kao, 1967). The observation that electrical and mechanical activity induced by TEA is critically dependent on the $[Ca]_o$ and is blocked by a specific inhibitor of Ca influx (D-600) argues against the second possibility. Also, atropine blocks responses to electrical stimulation in normal solution but has no effect on activity in the presence of TEA (Kroeger and Stephens, 1975). Therefore, it is considered that TEA converts the quiescent, multi-unit trachealis into a functionally single-unit muscle by reducing the normally predominant resting g_K , thereby unmasking the depolarization - activation of Ca and/or Na channels.

4. Calcium Metabolism

a) Role in excitation - contraction coupling

1) Striated muscle

It is generally accepted that in order for any type of muscle fiber to contract, free myoplasmic Ca^{2+} must be raised to a critical level for activation of the actomyosin filaments. The rapid increase in $[Ca^{2+}]_i$ that leads to activation appears to be governed by 1) the release of Ca from intracellular binding sites and 2) Ca influx

(Bianchi, 1969). Sandow (1952) proposed that the AP or depolarization causes Ca influx which in some way activates the myosin ATPase system. Although this model is inadequate, available data suggests that intracellular structures are present which can take up and release Ca as rapidly as is necessary to account for the time course of activation and relaxation, and that $[Ca]_i$ may be raised by several mechanisms. Ca influx and release of Ca from the intracellular binding sites may be mediated by 1) AP's (excitation - contraction or E-C coupling), 2) depolarization (electromechanical coupling) and 3) drugs (pharmacomechanical coupling) (Somlyo and Somlyo, 1968).

Many lines of evidence are available which support the hypothesis of AP - mediated Ca influx in striated muscle. First of all, Ca is the only physiological cation which causes contraction upon microinjection at low concentrations (Heilbrunn and Wiercinski, 1947). Net influx of Ca during depolarization and AP's has been demonstrated in frog sartorius muscle (Bianchi and Shanes, 1959) and in cardiac muscle (Niedergerke, 1959). The duration of increased Ca influx was correlated with the duration of high K contracture, and substitution of NO_3 for Cl_o increased the magnitude both of the 1) Ca influx during AP's and high K depolarization and 2) of the twitches and contracture, respectively. It was difficult to envision however, how the muscle becomes fully-activated throughout its cross - section during its latent period (Hill, 1949) in view of the large diffusion distance from the cell surface to its center. Skeletal muscle contains a transverse tubular system which is continuous with the sarcolemma (Porter and Palade, 1957; Huxley, 1964) and which conducts surface depolarization to the interior (Huxley and Taylor, 1958) where the associated sarcoplasmic reticulum (SR) is capable of releasing (Weber and Herz, 1968) and binding (Ohnishi and Ebashi, 1964) Ca. Thus the diffusion distance for Ca is considerably reduced and in accordance with activation time. In addition, if Ca is the E-C coupling agent, contractile responses to stimulation should be sensitive to depletion of muscle Ca by equilibration in Ca - free medium. This has been demonstrated by stabilizing membrane permeability and blocking excitability with Na - free, choline - substituted Ringer solution; K - induced contractures show the predicted Ca sensitivity (Frank, 1965).

Further evidence for Sandow's proposal is given by the observations that 1) the $[Ca^{2+}]_i$ reflects the active state and a decrease in $[Ca^{2+}]_i$ precedes relaxation (Jöbsis and O'Connor, 1966), 2) the maximal Ca accumulation by the SR is well over the values required to account for relaxation rates (Ohnishi and Ebashi, 1964) and 3) ATPase activity of highly purified actomyosin preparation is reduced by 85% in the absence of Ca as long as troponin and tropomyosin are present (Maruyama *et al.*, 1964). It has also been shown that Ca releases the inhibition effected by the troponin - tropomyosin complex which normally prevents the interaction of actin and myosin (Wakabayashi and Ebashi, 1968). Finally, Fuchs and Briggs (1968) demonstrated that troponin could bind sufficient Ca to activate contraction. Thus the available data support the hypothesis that intracellular free $[Ca^{2+}]$ regulates the activation of muscle and that structures are available to take up and release Ca at the rates required for activation and relaxation. It is generally accepted that in mammalian twitch striated muscle fibers the initiation of contraction by a chemical stimulus (ACh) is mediated by the AP which triggers the release of Ca from the SR, raising $[Ca^{2+}]_i$ to the threshold level for activation of contraction (Sandow, 1965).

The actual mechanism of release of Ca from intracellular binding sites is poorly understood and controversial. At least two such sites have been kinetically defined and their function postulated on the basis of their association constant for binding Ca. Thus the "superficial" store is mobilized by depolarization and depleted in Ca - free solution (Shanes, 1958; Frank, 1965) while the "sequestered" store is resistant to these stimuli but mobilized by drugs (Bianchi, 1961; Edman and Schild, 1962). It is believed that the more labile store represents Ca ions in association with the membrane that enter the fiber during a twitch or contracture and are replenished by extracellular Ca ions (Frank, 1965; Lüttgau, 1963).

In twitch muscle fibers Ca release is presumed to occur from the T - tubular element and terminal sacs (SR) while the functions of the SR are served by the surface membrane in tonic skeletal fibers and ventricular fibers which lack SR (Bianchi, 1969). The $[Ca]_i$ is maintained at a low level ($< 10^{-7}$ M) by Ca pumps (ATPases) in the surface membrane, SR and mitochondria (Bianchi, 1969). The

sequestered store (s) is thought to be primarily located in the SR though it may also be located in mitochondria and may be subdivided into slowly - and nonexchanging fractions (Bianchi and Shanes, 1959). Bianchi (1969) has proposed that Ca release from the inner aspect of the membrane is effected by depolarization and/or Ca removal, which increase membrane permeability, thereby allowing a greater Ca influx and decreasing the association - constant for Ca binding. Certain agents (EDTA, caffeine) would be able to release Ca without depolarization. Stabilization is effected by hyperpolarization (repolarization) and/or Ca binding to external stabilizing sites and, as a result, myoplasmic free Ca is rapidly sequestered by the SR (in the twitch fiber) or taken up by the membrane and expelled by the membrane Ca pump. In summary, it is thought that a small Ca influx is initiated upon depolarization which releases Ca from intracellular release sites. This field however, is in a state of flux and a complete review is beyond the scope of this thesis.

2) Smooth muscle

The absence of sarcomere units implies some difference between the nature of contraction in SM and that in striated muscle, but the basic mechanism of contraction and the role of Ca^{2+} in its activation are thought to be similar in view of the findings of 1) a microsomal fraction with an ability to accumulate Ca in a manner analogous to SR (Schoenberg, 1958; Carsten, 1969) and 2) a Ca^{2+} - sensitive actomyosin system extracted from various SM's (Filo et al., 1965; Needham and Williams, 1963). Additional evidence peculiar to SM are the findings that 1) electrical stimulation causes a relatively large Ca influx (Sunnano and Miyazaki, 1968; Lüllmann and Mohns, 1969), 2) the current of the AP of most SM's appears to be carried mainly by Ca (Brading et al., 1969b; Kuriyama and Tomita, 1970; Abe, 1971; Vassort, 1975), 3) excitation and contraction can be uncoupled by removal of surface Ca (Hudgins and Weiss, 1968; Durbin and Jenkinson, 1961; Edman and Schild, 1962; Bozler, 1969) and 4) the cells of SM are small enough and the time course of contraction slow enough (Stephens et al., 1969) so that Ca^{2+} diffusion from the cell surface would be sufficient for activation of contraction. However, the effects of alterations in $[\text{Ca}]_o$ on E-C coupling are difficult to differentiate from the concomitant alterations in membrane excitability (Marshall, 1965; Bülbring and Kuriyama, 1963a;

Bülbring and Tomita, 1970). The problem is compounded by the various ways in which SM may be stimulated (Somlyo and Somlyo, 1968) and the different Ca fractions involved (Daniel, 1965).

Due to the relatively high surface area/volume ratio of SM cells (approximately 15 times that of striated muscle fibers) and the small amount of SR, it is presumed that the Ca release and uptake mechanisms reside predominantly in the surface membrane. Thus most of the cellular Ca exchanges very quickly ($t_{1/2} < 15$ minutes) and SM cells take up much more Ca per unit volume and stimulus than do striated muscle cells (Lüllmann, 1970). Various kinetically - defined Ca fractions have been discerned; the most usual however, are 1) a rapid initial component thought to rise from Ca in the extracellular space, 2) a slower component loosely bound to the cell surface and connective tissue and 3) a more tightly bound intracellular component (Schatzmann, 1961; Sperelakis, 1962; Kroeger and Stephens, 1971). This arrangement is likely to be an oversimplification as SM cells have a variety of Ca stores and multi - unit muscles have a large amount of sequestered Ca (Devine et al., 1973). The latter characteristic suggests a decreased dependence of multi - unit fibers on extracellular Ca (Bülbring and Tomita, 1970; Devine et al., 1973; Somlyo et al., 1971; McGuffee and Bagby, 1976).

Ca distribution and binding is also correlated with the ways in which SM fibers can be stimulated. The extracellular and loosely - bound membrane Ca stores are thought to be primarily utilized by AP's (E-C coupling) and depolarization (electromechanical coupling) by electrical and high - K stimulation (Somlyo and Somlyo, 1968; Daniel, 1965; Hinke, 1965). A third type of stimulation is pharmacomechanical coupling whereby certain stimulatory agonists are able to mobilize more tightly bound Ca in the absence of changes in E_m (Somlyo and Somlyo, 1968). There is considerable experimental evidence to support this idea but the most convincing is the stimulation of high K - depolarized myometrium by ACh or arterial SM by adrenaline (Edman and Schild, 1962; Hinke, 1965; Evans et al., 1958). It should be also noted that in Ca - deficient solutions the K - contracture of taenia coli (Imai and Takeda, 1967) and rabbit mesenteric vein (Somlyo and Somlyo, 1969) exhibits an initial phasic response sensitive to $[Ca]_o$.

and a subsequent tonic response independent of $[Ca]_o$. These observations suggest that high K may be also capable of mobilizing some of the bound fraction in these conditions. It is apparent, therefore, that in investigating the action of any experimental condition which produces contraction, the measurements of flux kinetics and electrical and mechanical responses must be interpreted with caution with reference to Ca pools utilized.

There is also evidence for the presence of a surface membrane Ca pump which may represent competition for the Na pump. The β - adrenergic relaxation of the pregnant rat myometrium is accompanied by a hyperpolarization thought to be due largely to an increase in P_K and possibly to a small contribution by c AMP - mediated stimulation of an electrogenic Ca pump (Marshall and Kroeger, 1973; Kroeger and Marshall, 1973; Kroeger, 1976). In the dog trachealis hypoxia plus substrate depletion causes an increase in Ca content in normal Ca solution but a decrease in content and acceleration of Ca^{45} efflux in Ca - free solution (Kroeger and Stephens, 1971). The latter results were taken to indicate impaired uptake by intracellular binding sites since these conditions were effective when Ca^{45} efflux was low and stable, and an increase in tension occurred in association with the stimulated Ca efflux. The long (> 400 minutes) half - time for the Ca^{45} efflux from the slowly - exchanging compartment in Ca - free solution suggests the presence of a large sequestered store resistant to depletion (Devine et al., 1973). These results suggest that normally, the graded depolarizations observed in trachealis produce tonic contractions which rely to a greater extent on bound intracellular Ca stores. The depolarization and increase in r_m produced by TEA may open up additional Ca channels allowing sufficient Ca influx during the AP to directly activate the contractile machinery. This hypothesis would explain the functional conversion of trachealis to a single - unit preparation producing AP's and a Ca - dependent MR to stretch (Stephens et al., 1974; Kroeger and Stephens, 1975).

b) Role in stabilization of membrane permeability

1) Nerve, striated muscle and cardiac muscle

Voltage - clamp studies on squid giant axon and myelinated nerve fibers show that ion conductances depend on the divalent cation concentration and the pH

of the bathing solution in a characteristic way. Huxley (c.f. Frankenhaeuser and Hodgkin, 1957) suggested that the action of Ca on the systems controlling g_{Na} and g_K might be explained by a change of the electric field within the membrane through absorption of Ca to negative charges fixed on the membrane surface. It was known that increasing $[Ca]_o$ raises the threshold, increases r_m (Cole, 1949) and accelerates accommodation, while reducing $[Ca]_o$ has the opposite effects and may lead to phasic electrical activity in skeletal muscle fibers (Adrian and Gelfan, 1933). In addition, removal of Ca reduces rectification in squid nerve (Steinbach *et. al.*, 1944), increases the fraction of the Na carrying system in an inactivated state in Purkinje fibers (Weidmann, 1955) and can induce the production of an anode - break response in myelinated nerve fibers (Frankenhaeuser, 1957). The data of Frankenhaeuser and Hodgkin (1957) showed that for squid giant axon reducing $[Ca]_o$ 1) increases the inward I_{Na} and the outward I_K 2) increases the rate of increase of g_{Na} and g_K under the cathode, 3) decreases threshold, 4) increases the fraction of inactive - Na carrying system in the steady state and 5) renders the axon refractory. The results of a five - fold reduction in $[Ca]_o$ were equivalent to those of a 10 to 15 mV depolarization and it was suggested that the Ca in the immediate vicinity of the membrane was important in this regard.

In later studies, Hille (1968) and Hille *et. al.* (1975) observed a shift in the peak g_{Na} in myelinated nerve of 20 - 23 mV per 10 - fold change in $[Ca]_o$ but reported little dependence of steady - state g_K on alterations in $[Ca]_o$. Mozhayeva and Naumov (1970) however, observed a shift in steady state g_K of +15 mV per 10 - fold increase in $[Ca]_o$. Hille *et. al.* (1975) have proposed a model whereby divalent cations (Ca^{2+} and Mg^{2+}) and protons (H^+) are assumed to affect the surface potential either by chemically binding to the fixed charges (determined by dissociation constants), or by developing a diffuse double layer and screening off the fixed charges (determined by ion valencies), thus increasing the electric field within the membrane. All effects of varying the cation concentrations are assumed to be restricted to the outer surface of the membrane, the internal double layer remaining unaffected. Ion conductances therefore, are supposed to be

controlled by the resulting electric field in the membrane.

In skeletal muscle Ca (and Mg) affect the threshold of 1) delayed rectification and 2) contraction in a similar way. The threshold and the steady - state g_K are shifted from 8 mV (Constantin, 1968) to 23.5 mV (Dörrscheidt - Käfer, 1976) per 10 - fold change in $[Ca]_o$, depending on the nature of the agent used to inhibit g_{Na} . Thus, the nature of the Ca - dependence of the contraction threshold is the same as that of both g_{Na} and g_K , and the threshold shifts may reflect identical changes in surface potential (Dörrscheidt - Käfer, 1976). A causal relationship between contraction threshold and the threshold of delayed rectification has been ruled out however (Adrian et. al., 1969). Dörrscheidt - Käfer (1976) has proposed a model similar to that of Hille for nerve but with the fixed negative charges also present on the outer surface of the tubular membrane. The ion conductances and the contraction threshold would be controlled by the electric field in the membrane resulting from the binding and screening effect of external Ca.

An example of the regulatory effect of Ca on membrane conductance and potential is given by the decreased tension which occurs when striated muscle fibers are depolarized in high K solution after having been soaked in Ca - free medium. It has been postulated this effect is due to the progressive decline in RMP in the absence of external Ca (Lüttgau and Oetliker, 1968). This depolarization, if sustained for a short time, breaks the link between E_m and the contractile machinery; the muscle remains in a relaxed state, although the membrane is sufficiently depolarized to have produced considerable tension had this degree of depolarization been induced initially. The degree of tension which can be developed when the partially - depolarized muscle (by Ca - deficiency) is completely depolarized depends on the degree of the depolarization. This effect is represented by a restoration - potential curve (Lüttgau and Oetliker, 1968). This and a tension - potential curve, like contraction and conductance thresholds, are shifted by alterations in $[Ca]_o$. Therefore, in striated muscle fibers, the most prominent effects of Ca_o seem to be exerted through its control of E_m .

The selective ion permeability of cardiac fibers is also regulated by Ca as demonstrated by Nayler (1965), who observed decrements of E_m of toad ventricle in association with Ca - deficiency and Ca chelating agents (Chelex -

100 and ethylenediaminetetraacetic acid - EDTA), and subsequent restoration of RMP by addition of the divalent cations zinc (Zn^{2+}), strontium (Sr^{2+}) and Ca^{2+} . Ca also stabilizes the membrane potential against increases in $[K]_o$ (Aidley, 1970). Weidmann (1955) showed that a raised $[Ca]_o$ increases the threshold for AP generation but produces no change in the rate of spontaneous depolarization in pacemaker regions. An increased rate was observed however, with lowered $[Ca]_o$, the effect attributable to the reduced threshold. With reference to the Na - activation mechanisms, the effects of high and low $[Ca]_o$ are similar to those in nerve (Weidmann, 1955).

Recent studies involving the iontophoretic injection of Ca into individual heart fibers strongly suggest that intracellular Ca plays an important role in the control of surface and junctional membrane conductance. Intracellular injection produces electrical uncoupling between canine Purkinje fibers and a concomitant increase in input resistance (De Mello, 1975). This effect is 1) maintained by injury to the cell membrane and 2) spontaneously reversed by cessation of Ca injection, the rate of reversal being reduced in the presence of caffeine and low temperature. These results indicate that the recovery of electrical coupling after cessation of injection is related to the re-establishment of the low $[Ca^{2+}]_i$, which is normally maintained by active uptake of Ca by the SR (Weber and Herz, 1968) and active extrusion by the surface membrane. Similar studies suggest that the level of intracellular free Ca^{2+} can affect the g_K in cardiac muscle. Iontophoretic injection of Ca into sheep Purkinje fibers led to 1) shortening of the AP duration which was more pronounced with greater amounts of injected Ca and shorter delays between Ca injection and AP, and 2) membrane hyperpolarization (Isenberg, 1975). These results are consistent with an increase in g_K due to the presence of elevated $[Ca^{2+}]_i$. The possibility of g_K being increased by the concomitant depolarization by Ca injection was ruled out by the observation that the shortening of the AP was the same during a voltage clamp. The foregoing evidence suggests, therefore, that the membrane potential of heart fibers may be intimately regulated by the control which both intracellular and external Ca apparently exert over ion conductances, particularly g_K . It thus appears that an

elevation in $[Ca]_o$ decreases g_{Na} but increases g_K , accounting for the observed membrane hyperpolarization.

2) Smooth muscle

The functional state of the smooth muscle cell membrane is also maintained by the presence of Ca_o . An increase in $[Ca]_o$ hyperpolarizes the membrane, this (Bülbring and Kuriyama, 1963a; Marshall, 1965) effect being accompanied by a reduction of r_m (Bülbring and Tomita, 1968a,b, 1969c; Brading et. al., 1969b). A decrease in $[Ca]_o$ depolarizes the membrane and increases r_m except at concentrations $< 0.2 - 0.6$ mM at which a decrease in r_m has been observed (Brading and Tomita, 1968b). Replacement of Na_o with tris or sucrose after the taenia coli is depolarized in Ca - deficient solution causes a repolarization and restoration of normal r_m (Brading and Tomita, 1968b; Brading et. al., 1969b). The changes in r_m at $[Ca]_o$'s > 0.6 mM are consistent with an effect of Ca on g_K , but since the depolarization in Ca - free solution is smaller if Na is removed, g_{Na} may also be increased (Brading and Tomita, 1968b; Brading et. al., 1969b). There is apparently little effect on g_{Cl} as the same effects are obtained when Cl_o is replaced with a less permeant anion (Kuriyama, 1964). It is conceivable that Ca also regulates the integrity of the junctional membrane although Tomita (1969) found no change in the longitudinal internal resistance ($r_i + r_j$) of the taenia coli after at least 15 minutes exposure to altered Ca solutions.

Excess Ca_o also 1) increases the threshold for AP production, partly as a result of the hyperpolarization and partly due to a shift of the firing level toward a less negative potential, and 3) decreases the latency between the onset of the stimulus and the peak of the spike (Brading et. al., 1969b). In spite of the increase in threshold and decrease in r_m , the spike amplitude and dV/dt of the taenia coli are greater in elevated $[Ca]_o$. This may be due in spite of the inhibition of any depolarization - activated increase of g_{Na} and/or to the ability of the (assumed) activated Ca channels to admit sufficient Ca to overcome the opposing effects on threshold and r_m . On the other hand, the presence of spike activity in nominally Ca - free solution (Bülbring and Tomita, 1970) might be explained by the maintenance of spike activity by Ca bound to the cell membrane. This site

may be replenished by an active membrane Ca pump, at the same time maintaining P_{Ca} at a high enough level to support AP's. In favour of the idea that Ca is supplied in Ca - deficient solution by a membrane store rather than by an extracellular supply are the observations that 1) abolition of the spike by removing Ca_o is much slower than the loss of Ca by diffusion from the extracellular space (Brading and Jones, 1969), 2) the effects of Ca - depletion are highly temperature dependent (Brading et. al., 1969a) and 3) the effects of Ca - depletion are modified by the presence or absence of other ions (Bülbring and Tomita, 1970). Thus membrane potential and resistance are maintained in Ca - deficient solution by Mg (Kuriyama and Tomita, 1970) and abolition of AP activity is faster in the presence of Mg than in its absence (Bülbring and Tomita, 1970). This latter effect may be explained by a competition between Mg and Ca for membrane sites (Goodford, 1967) and the fact that Mg can not replace Ca in carrying the inward current of the spike (Kuriyama and Tomita, 1967).

Additional evidence for a membrane Ca store is the finding in barnacle muscle fibers, which produce a Ca - spike, that 1) the spike amplitude is related to the amount of Ca adsorbed at the membrane rather than to $[Ca]_o$, and 2) that divalent cations bind competitively with the same membrane sites as Ca (Hagiwara and Takahashi, 1967). With reference to SM it has been suggested that the reduction of ionic strength by replacing Na Cl with sucrose may increase Ca adsorption at the membrane and potentiate the stabilizing action of Ca (Tomita, 1970). Evidence in this regard is the observation that exposure to pure sucrose solution, although a drastic shock to osmotic regulation, increases the binding of Ca with the result that subsequent removal of Ca from the tissue is very slow (Sparrow et. al., 1967).

It appears that the most important interaction at the membrane is that between Ca and g_K . From measurement of radioactive ^{45}Ca efflux Goodford (1965) tentatively deduced that the amount of membrane Ca might be modified by competition with K, as these both serve as extracellular counter-cations to the fixed negative charges. In view of the evidence for the stabilizing ability of Ca it is more likely that Ca controls g_K . It has been postulated (Tomita, 1970; Tomita and Watanabe, 1973) that an increase of Ca at the inner layer of the membrane increases g_K , as it

does in heart muscle (De Mello, 1975; Isenberg, 1975), and that active removal of Ca from the cell reduces g_K . It is observed that in taenia coli, in high $[Ca]_o$ or low $[Na]_o$, the amplitude of the ETP decreases due to high $[Ca]_i$ (Tomita and Watanabe, 1973). Thus it is apparent that g_K may be controlled by Ca on both sides of the membrane. This mechanism in turn should provide for a very sensitive control of E_m and r_m .

Another aspect of the interaction of Na, K and Ca is the possibility that the value of g_{Na}/g_K has a strong influence on the activation of Ca channels. The depolarization of the rat hepatic portal vein produced by high K solution increased g_K which was reflected in a lower r_m and which suppressed electrical and mechanical activity (Hermsmeyer, 1976). These effects were reversed by Ba^{2+} which reduced g_K , and it was concluded that the spike pattern depends at least in part on the combination of effects of g_K and E_m . Similar findings have been reported with the application of procaine (Kurihara, 1975) and TEA (Mekata, 1971; Kroeger and Stephens, 1975) which also suppress g_K and produce, or potentiate, phasic electrical and mechanical activity as discussed above (section 3). Furthermore, Vassort (1975) has recently adduced a very rapid and sensitive interaction between the inward Ca current and g_K in the myometrium. The author has identified a fast outward K current in addition to the delayed rectifying I_K . This rapid outward current is characterized by 1) rapid inactivation, 2) partial inactivation at the RMP, 3) reversal of inactivation by conditioning hyperpolarization and 4) inhibition by TEA. This current normally coincides with the inward I_{Ca} and is responsible for the fast repolarization and low amplitude of the AP. Vassort has thus proposed that the influx of Ca transiently increases the local $[Ca]_i$ at the inner side of the membrane, inducing a momentary increase in g_K (i.e. a shift in the K activation curve). Following this, the I_K declines due to inactivation and the decrease of the local $[Ca]_i$. The results of the study suggested that the g_K activation curve is sensitive to E_m and to the g_{Ca} activation curve. This transient outward current probably influences the firing behaviour of SM cells and allows its control by the nervous system and circulating hormones: any small hyperpolarization would thus inhibit the electrical activity of the cell.

In conclusion, it is becoming increasingly apparent that the excitability of SM's, or lack of it, has a basis in a sensitive interaction between g_K and the $[Ca]$ on either side of the cell membrane. The single - unit muscles are spontaneously active and this activity appears to rely mainly on neurohumors and circulating agents for its modulation (Bülbring and Tomita, 1969a,c) and mainly on external Ca for the carrier of dynamic current and activator of contraction. Multi - unit preparations are more dependent on nervous control and likely utilize internal bound stores of Ca to a greater extent. The potential for any activation appears to be masked in multi - unit preparations by a relatively high resting g_K , resulting in a lower g_{Na}/g_K .

METHODS

A. Dissection

Trachealis muscle was obtained from the cervical tracheae of mongrel dogs anaesthetized with 35 mg/kg pentobarbital (nembutal, Abbott) injected i.v. The dogs were then killed by intracardiac injection of saturated KCl solution.

The trachealis muscle (musculus transversus tracheae) composes the most dorsal layer of the paries membranaceus which spans the incomplete dorsal ends of the cartilaginous rings.

Dissection was carried out in the plane of separation between the trachealis dorsally and the tunica fibrosa of the elastica mucosae trachea ventrally (Stephens, Kroeger and Mehta, 1969). Separation was easy and the muscle layer could be dissected out with minimal handling. Care was taken to remove a thin, tough layer of connective tissue on the ventral side which would impede microelectrode penetration of the muscle cells. A rectangular strip of muscle approximately 10 mm long in the lateral transverse plane and 3 to 4 mm wide in the craniocaudal plane of the trachea was cut out and placed in a bath containing Krebs-Ringer normal solution (NS). The composition of this and other solutions is given in Table 1. It was equilibrated with a 95% O₂ - 5% CO₂ mixture so as to yield a PO₂ of 600 mm Hg, a PCO₂ of 45 mm Hg and a pH of 7.40, at a temperature of 37° ± 1° C. Dissection time averaged 20 minutes and the muscle was kept moist during this time.

B. Apparatus

Stimulation and recording was carried out in a plexiglass bath whose design was modified from that described by Abe and Tomita (1968) (figure 1). The bath consists of an outer, water-filled jacket for temperature control and an inner muscle chamber. Incoming solutions enter both ends of the inner chamber via rubber tubing coiled in the outer chamber for temperature equilibration. The inner chamber contains 2 Ag - Ag Cl stimulating electrodes, one at one end and one in the center. The central electrode thereby divides the bath into stimulating and recording chambers which hold about 3 and 5 ml of solution respectively. Both electrodes are chloride-plated in order to minimize polarization. The central electrode is insulated with Araldite epoxy resin on the side facing the stimulating

Table 1

Composition (mM) of Muscle Perfusates

	NS	NS- TEA	Low Cl TEA	Low Cl High Ca T EA
Na Cl	118.5	118.5	29.6	29.6
Na HCO ₃	23.8	23.8	23.8	23.8
KCl	4.7	4.7	-	-
KH ₂ PO ₄	1.2	1.2	1.2	1.2
Dextrose	5.5	5.5	5.5	5.5
Mg Cl ₂	1.17	1.17	-	-
Ca Cl ₂	2.0	2.0	-	-
K ₂ SO ₄	-	-	2.3	2.3
Mg SO ₄	-	-	1.17	1.17
Ca SO ₄	-	-	2.0	6.0
TEA·Cl	-	20.0	20.0	20.0
Na Isethionate	-	-	88.8	88.8
Na	142.3	142.3	142.2	142.2
Cl	126.37	146.37	49.6	49.6
K	5.9	5.9	5.8	5.8
Mg	1.17	1.17	1.17	1.17
Ca	2.0	2.0	2.0	6.0
PO ₄	1.2	1.2	1.2	1.2
SO ₄	-	-	5.47	5.47
HCO ₃	23.8	23.8	23.8	23.8
Dextrose	5.5	5.5	5.5	5.5
TEA	-	20.0	20.0	20.0
Isethionate	-	-	88.8	88.8

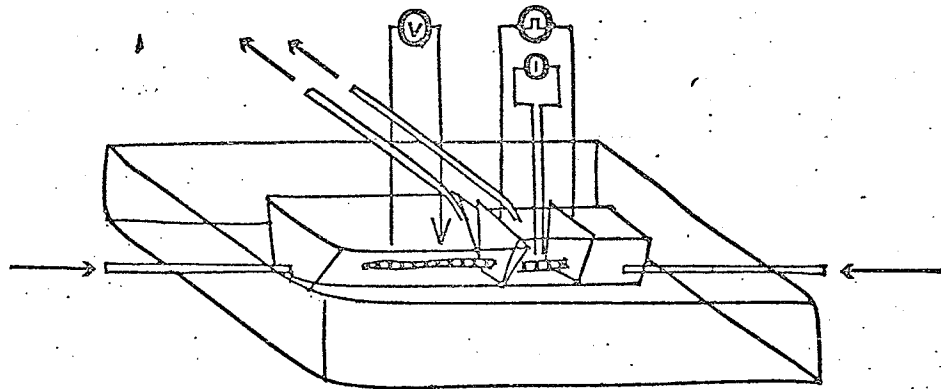


Figure 6. Schematic diagram of apparatus for stimulating and recording (described in text).

chamber and with celluloid on the other side. There is a space between the metal and the celluloid for current flow and a small oval hole through which the muscle strip is passed horizontally. The insulation and small aperture combine to reduce shunting of the current by the solution, thus focusing the current on the muscle and reducing the stimulus intensity necessary to stimulate the preparation. An independent pair of Ag - Ag Cl wires are placed in the solution in the stimulating chamber to monitor stimulus intensity. Solutions were propelled by gravity from overhead reservoirs. Flow rates were monitored with glass drip tubes and maintained at about 3 ml/min. Appropriate placement of suction ports maintained the bathing medium at the desired level in the bath.

After passing the muscle strip through the central stimulating electrode, it was attached by a loop of braided 3 - 0 surgical silk to a stationary glass hook in the stimulating chamber. A length of silk tied to the other end was passed around a vertical pulley and attached to a Grass FT.03 C force transducer for measurement of isometric tension. The output of the force gauge was balanced by a bridge circuit and recorded on tape with a Philips Analog - 7 tape recorder.

After mounting, the preparation was slowly stretched until a resting tension of 0.5 to 0.8 g was maintained. This procedure approximates the optimal length (Stephens, Kroeger and Mehta, 1969), yielding final muscle dimensions of about $20 \times 3.0 \times 0.75$ mm. One-half to 1 hour was allowed for equilibration in the normal Krebs-Ringer solution at 37°C. After this period, the muscle was perfused with 20 mM TEA solution which invariably caused a dramatic rise in baseline tension after a delay of 3 to 5 minutes. When the tension had stabilized, the microelectrode experiments were begun.

Microelectrodes were pulled from glass capillary tubing (0.8 mm O.D.) using a David Kopf two-stage vertical puller. Coil temperature and pull were adjusted so as to produce tips of no greater than 0.5 micron O.D. with shanks of fairly sharp taper to lend greater physical strength. The electrodes were then placed in a beaker of freshly - filtered 2 M KCl which had just been heated to boiling temperature. Finally, the beaker was placed in a vacuum chamber for 1/2 hour to boil and fill the electrodes under reduced pressure. Microelectrodes were occasionally

checked microscopically for adequate configuration (tip invisible under $\times 600$ magnification) and for complete filling without bubbles. For subsequent use each microelectrode was mounted on a 1 cm Ag - Ag Cl wire soldered to a 6 cm length of thin, flat, Z-folded foil. This in turn was mounted on a WPI electrode holder clamped to an X-Y-Z micromanipulator. The output was connected to a WPI 750 high impedance, direct-coupled amplifier whose output was recorded on tape. The circuit was completed by a salt bridge (2 M KCl stabilized in agar) indifferent electrode positioned in the recording chamber and connected to the amplifier ground input. Dynamic calibration of the system was effected by a Bioelectric Instruments Inc. CA5 calibrator in series with the salt bridge and amplifier. After positioning the "floating" electrode above the muscle it was lowered into the solution. Precise positioning was facilitated by a vernier scale on the micromanipulator and by a metric scale parallel to the muscle strip. The microelectrode was then checked electrically for high impedance (inversely proportional to tip diameter) and used only if > 10 megohms. The possibility of a drifting baseline due to polarization at the metal - KCl junction was minimized by chloriding the electrode wire before use. The "floating" assembly prevents contraction - induced extrusion of the microelectrode, thus allowing simultaneous recording of electrical and mechanical activity (Marshall and O'Brien, 1967).

Stimulation was effected by a Grass S-44 constant voltage (output $R = 250$ ohms) stimulator whose output was isolated from ground by a WPI photon-coupled isolation unit. The two electrodes monitoring stimulus intensity were connected to the differential amplifier of a modified Tektronix dual beam oscilloscope, whose output was also recorded on tape. A sync. signal for oscilloscope triggering was recorded on another channel while the fifth input was short - circuited for flutter compensation. The outputs of the first four channels were connected to a flutter compensation unit (bandpass 0 to 500 Hz) and then displayed in parallel on a Tektronix dual-beam oscilloscope and a Gould Brush 2400 pen recorder. Membrane potential and stimulus intensity were calibrated with the dynamic calibration unit. All recordings could be played back and displayed on either the Brush pen recorder or on 35 mm, high speed negative film with a Nikon-Kohden camera mounted on the

oscilloscope. All experimentation was carried out within a shielded room.

C. Protocol

1. Effect of Cl reduction on r_m

The amplitude of the AP can be increased simply by an Ohm's Law function due to passage of a constant current across an increased r_m (Csapo and Kuriyama, 1963). It has been shown that changes in cell cable properties are most effectively studied by measurement of the spatial spread of the electrotonic potential (ETP) elicited by extracellular stimulation (Tomita, 1966 a; Abe and Tomita, 1968).

After a microelectrode proved to be suitable for use by an impedance check, it was positioned along the central longitudinal axis of the muscle at a distance of 2 mm from the partition electrode. The electrode was lowered cautiously until small amplitude electrical activity signaled contact with the muscle surface. Cell impalement was effected by manual table tapping, since a high velocity, small amplitude thrust minimizes membrane trauma. The criteria accepted for successful impalement were 1) an instantaneous increase in negative voltage, 2) voltage achieved held steady for at least 10 to 15 seconds, 3) upon withdrawal of the electrode the initial zero baseline was regained and 4) electrode impedance was unchanged.

When a successful penetration was thought to have been achieved, hyperpolarizing square wave pulses of four amplitudes ranging from 15 to 100 mV/cm (see figure 8) were applied at a frequency of 0.3 hertz. This frequency allowed sufficient time for any active anode-break response to occur and for the resting membrane potential (RMP) to be regained, while a pulse duration of 0.6 to 1.0 second permitted the ETP's to reach steady-state amplitude. The rectification seen in response to depolarizing stimuli in normal and TEA-containing solutions have been reported previously (Kroeger and Stephens, 1975). If a record was obtained with stable membrane potential, negligible stimulus artifact (due to incomplete penetration by microelectrode) and return to baseline upon withdrawal with no change in electrode impedance, the electrode was advanced to distances of 4 and 6 mm and the steps repeated. When satisfactory records were obtained in the TEA solution (NS-TEA), the muscle was equilibrated for 1/2 hour in Cl - deficient TEA solution (see Table 1) and measurements at 2, 4 and 6 mm repeated.

A concentration of 20 mM TEA was chosen for these experiments as it lies midway along the dose-response curve for TEA and trachealis. To make Cl⁻ deficient solution, seventy-five percent of the Na Cl was substituted equimolar with Na isethionate, a less permeant anion, producing a net Cl⁻ concentration of 49.6 mM, 33% of that in the normal solution.

2. Effect of Ca and D-600 on AP's

The main purpose of this study was to elucidate the role of Ca in carrying the current of the AP in the presence of TEA. The presence of Ca is a prerequisite for electrical and mechanical activity in most types of muscle, including TSM, as well as for stabilization of resting and active membrane conductance to various physiological ions. Consequently we wished to 1) analyze the contribution of Ca to dynamic voltage transients devoid of an electrotonic component but 2) still obtain measurements of changes in r_m throughout the experiment. Trains of anode-break excited AP's at a distance of 4 to 6 mm from the stimulating electrode fitted this experimental design quite adequately. Recording at this distance would also tend to eliminate all but fully-conducted AP's.

Upon reinsertion of the microelectrode into the muscle cell at 6 mm in Cl⁻ deficient TEA solution, pulses of 20 to 30 mV/cm (see figure 15) at the same frequency as stated above were applied to the muscle strip. When the RMP and active responses were stable, the bathing medium was switched to Cl⁻-deficient TEA solution containing 6.0 mM Ca. The spikes usually required about 5 minutes to exhibit the full effect of the intervention. When a plateau in spike amplitude and/or rate of rise (dV/dt) was observed, D-600, a specific antagonist of dynamic Ca channels (Kolhardt et al., 1972) was applied to the bath to yield an initial bath concentration of about 10^{-5} M.

D. Analysis

1. Evaluation of changes in r_m

In order to relate changes in ETP's to changes in r_m during the action of Cl⁻-replacement, cable theory was applied (Hodgkin and Rushton, 1946; Fatt and Katz, 1951, such that

$$\frac{V'}{V} = \sqrt{\frac{r'_m r'_i}{r_m r_i}} \frac{\exp(-x/\lambda')}{\exp(-x/\lambda)}$$

However, several investigators (Abe and Tomita, 1968; Ohashi, 1970) recorded at very small distances ($\lambda < 0.5$ mm) from the stimulating electrode, therefore simplifying the above equation to

$$\left(\frac{V'}{V}\right)^2 = \frac{r'_m}{r_m}$$

This simplification can lead to large errors due to simultaneous changes in r_m and λ . Increasing x to values greater than λ (necessary in this study since high surface tension at the partition made positioning the microelectrode very difficult) will increase this error. Consequently, changes in r_m were estimated as described by Kroeger and Marshall (1973): assuming that r_i (internal plasma resistance) is not affected by Cl-substitution, then

$$\frac{r'_m}{r_m} = \left(\frac{\lambda'}{\lambda}\right)^2 \quad \text{and} \quad \lambda' = \lambda \sqrt{\frac{r'_m}{r_m}}$$

Let $A = \frac{r'_m}{r_m}$, then $\lambda' = \lambda \sqrt{A}$ and

$$\left(\frac{V'}{V}\right)^2 = A \left(\frac{\exp(-x/\lambda \sqrt{A})}{\exp(-x/\lambda)} \right)^2 \quad (24)$$

Equation 24 was solved graphically for A by plotting the numerical value of the right side of the equation as a function of A . The value of A , from the graph, that satisfied equation 24 is the estimate of $\frac{r'_m}{r_m}$.

RESULTS

A. Effect of Cl-replacement on the Electrical Activity of TEA-treated TSM.

1. Spontaneous spikes

The electrical activity in the presence of 33 mM TEA - containing normal solution has previously been shown (Kroeger and Stephens, 1975) to consist of small, slowly-rising AP's discharging at low frequency in a one-to-one relation with individual twitches. Furthermore, these spikes are conducted slowly and decrementally. These findings are consistent with a potentiation of r_m due to a reduction of g_K by TEA, and it was felt that decreasing the normal Cl leakage current would serve to increase r_m even further. This action should in turn result in greater ETP and AP amplitudes. Preliminary experiments provided positive results as evidenced by the train of spontaneous spikes shown in figure 7. Partial replacement of external Cl with isethionate produced a transient depolarization (repolarizing within 15 minutes) and approximately a 60% increase in spike height as well as stabilization of frequency. This, then, provided us with a good model with which to test the hypothesis that Ca is an essential physiological cation for carrying the current of the AP and for activating contractile proteins in the presence of TEA. The problem still remained, however, to determine whether or not the augmented electrical activity in response to the reduction of I_{Cl} was due solely to an increment in r_m or to additional Ca current activated upon depolarization. It was decided, therefore, to determine the relative contribution of the change in r_m by measuring changes in the amplitude, and consequently, the spread of the ETP.

2. Electrotonic potentials

Since the height of the steady-state ETP elicited by extracellular stimulation is directly proportional to r_m , measurement of changes in ETP amplitude provides the most accurate estimate of changes in r_m . The ETP produced by hyperpolarizing (anodal) current was recorded at three distances from the site of stimulation in the normal and Cl-deficient TEA solutions. The protocol was chosen because the spatial decay of the ETP has been demonstrated in this (Kroeger and ^(Stephens, 1975) and in several other smooth muscle preparations to be an exponential function (given by a straight line on a semilog plot). The longitudinal potential field was considered to be the current axis, assuming that the current intensity flowing through the tissue was proportional

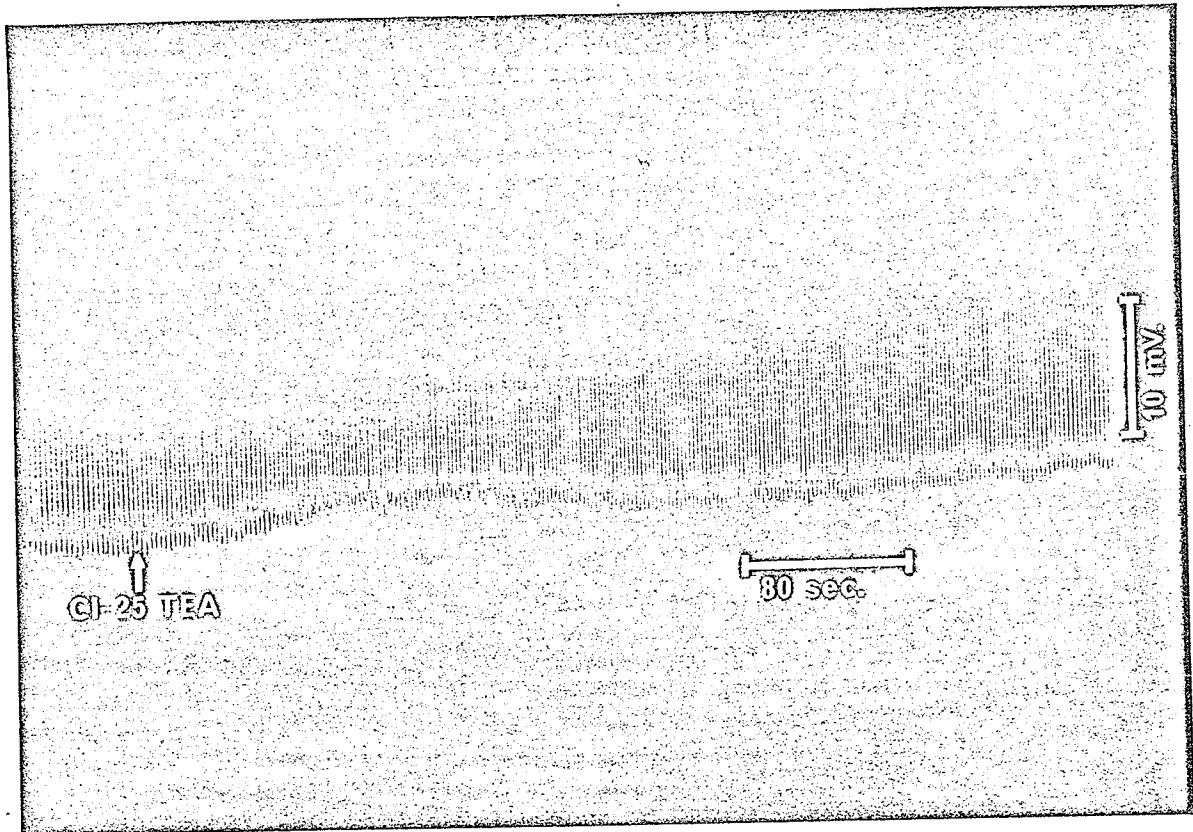


Figure 7. Effect of reduction of $[Cl]_o$ from 146 to 50 mM (isethionate substitution) on a train of spontaneous AP's in the presence of 33 mM TEA.

to the applied potential field (Abe and Tomita, 1968). Recordings from a typical experiment are shown in figures 8 and 9 and means from seven experiments are presented in Table 6. The effect of the Cl⁻ substitution was to increase the amplitude of the ETP by 43% at 2 mm and by 51% at 4 mm both highly significant at $P < 0.01$ by factorial analysis of variance. For each experiment the ETP amplitude was plotted for each distance and solution as a function of the stimulus intensity. Figure 10 shows such a typical current - voltage (I - E) relationship while mean plots from 7 experiments are plotted in figure 11. Note the linear relationship between the ETP amplitude and increasing stimulus intensity, signifying the absence of rectification due to alterations in membrane permeability as observed in the depolarizing direction (Kroeger and Stephens, 1975; Abe and Tomita, 1968). The data obtained in the control solution were replotted on a semilog scale (Figure 12) which graphically represents the spatial decay of the ETP. The mean data points were joined by straight lines on the assumption that the decay was exponential. The length constant (λ) calculated from the decay in control solution was 3.05 mm which shows fair agreement with the value of 2.8 mm obtained by Kroeger and Stephens (1975). Acceptable data was not obtained in low Cl⁻ - TEA solution with which to plot the spatial decay and derive λ .

3. Evaluation of the Change in r_m

Other workers (Abe and Tomita, 1968; Ohashi, 1970) have been able to record transmembrane potentials with microelectrodes placed immediately next to the stimulating partition, and consequently have used a simplified form of the cable equation (see Methods) to calculate the actual change in r_m . The high surface tension present at the stimulating partition in this study made impalements at less than 1.0 mm with the floating microelectrode virtually impossible. Therefore, a graphical solution (Kroeger and Marshall, 1973) to the unmodified cable equation was employed. Substituting a value of 3.05 for λ and hypothetical values for A, a plot of $\left(\frac{V'}{V}\right)^2$ versus $\frac{r'_m}{r_m}$ was calculated for distances of 2 and

4 mm as shown in figure 13. From this graph values for $\frac{r'_m}{r_m}$ of 1.59 and 1.45

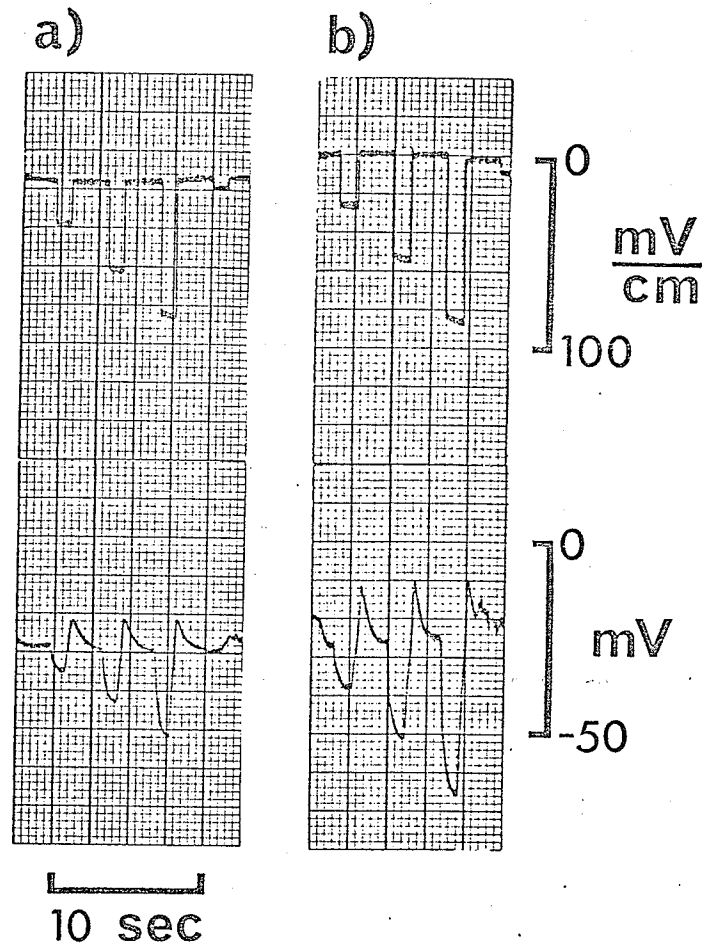


Figure 8. Typical recording showing the effect of Cl⁻ deficiency on the amplitude of the ETP. Panel a - hyperpolarizing square - wave stimuli (upper trace) and resultant ETP's (lower trace) in 146 mM Cl⁻ TEA. Panel b - same as panel a except recorded in 50 mM Cl⁻ TEA (isethionate substitution). Note active transients produced by anode - break stimulation.

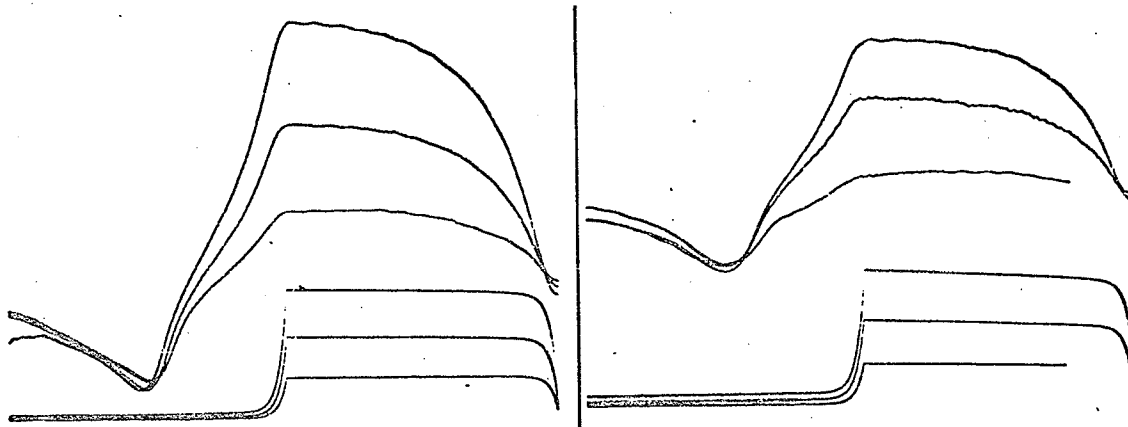


Figure 9. Effect of Cl^- deficiency on the amplitude of the ETP; typical recordings with expanded time base, superimposed. Panel a - square - wave stimuli and ETP's in 146 mM Cl^- - TEA. Panel b - same as above in 50 mM Cl^- - TEA.

Table 6

Effect of Cl⁻ deficiency on Steady - State

ETP amplitude and r_m

	ETP Amplitude (mV) [†]		
	2 mm	4 mm	6 mm
NS - TEA (control)	37.9 ± 3.13 ⁽⁷⁾	19.6 ± 1.95 ⁽⁷⁾	13.8 ± 0.90 ⁽³⁾
Low Cl ⁻ - TEA	54.6 ± 6.66	29.3 ± 4.24	19.7 ± 0.94
V'/V ^{††}	1.43**	1.51**	1.44
r'_m/r_m ^{†††}	1.59	1.45	1.29

Means ± S.E.'s with number of experiments in brackets.

[†] At a stimulus intensity of 100 mV/cm.

^{††} Ratio of ETP amplitude in test (V') and control solutions.

^{†††} Calculated from graphical solution (see Methods and Figure 8).

* $P < 0.05$

** $P < 0.01$

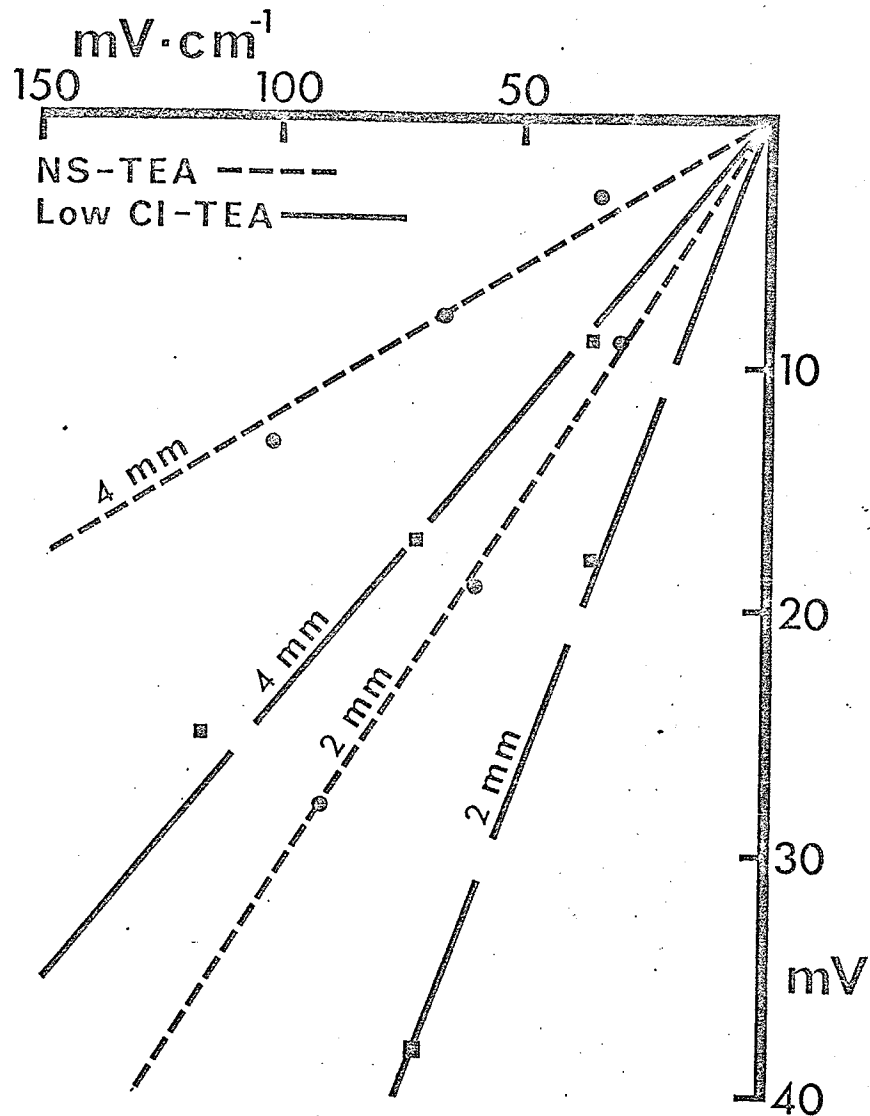


Figure 10. Effect of Cl^- deficiency ($\text{Cl}^-_o = 50 \text{ mM}$) on current - voltage relations at 2 and 4 mm from the stimulating partition, typical experiment. Ordinate, steady - state electrotonic potential (mV); abscissa, applied stimulus (mV/cm).

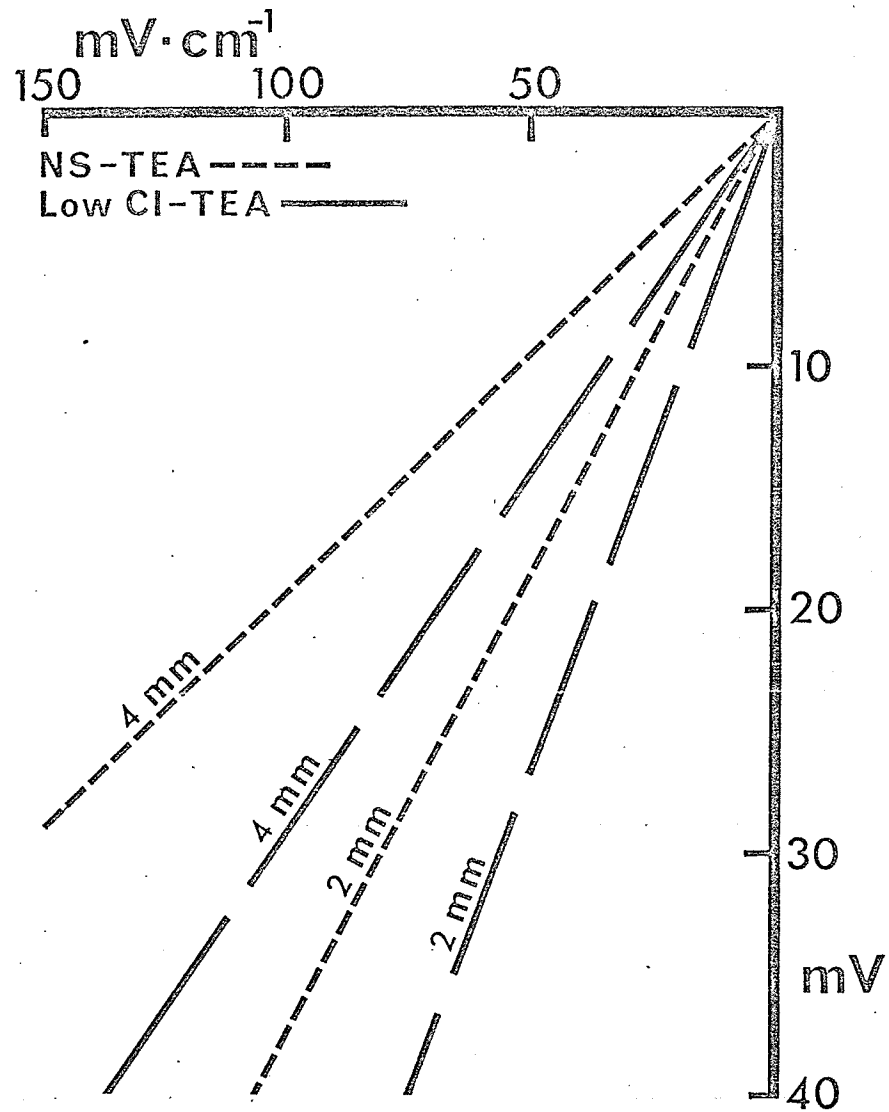


Figure 11. Effect of Cl^- deficiency ($\text{Cl}_o = 50 \text{ mM}$) on the current - voltage relations at 2 and 4 mm from the stimulating partition, mean slopes of 7 experiments.

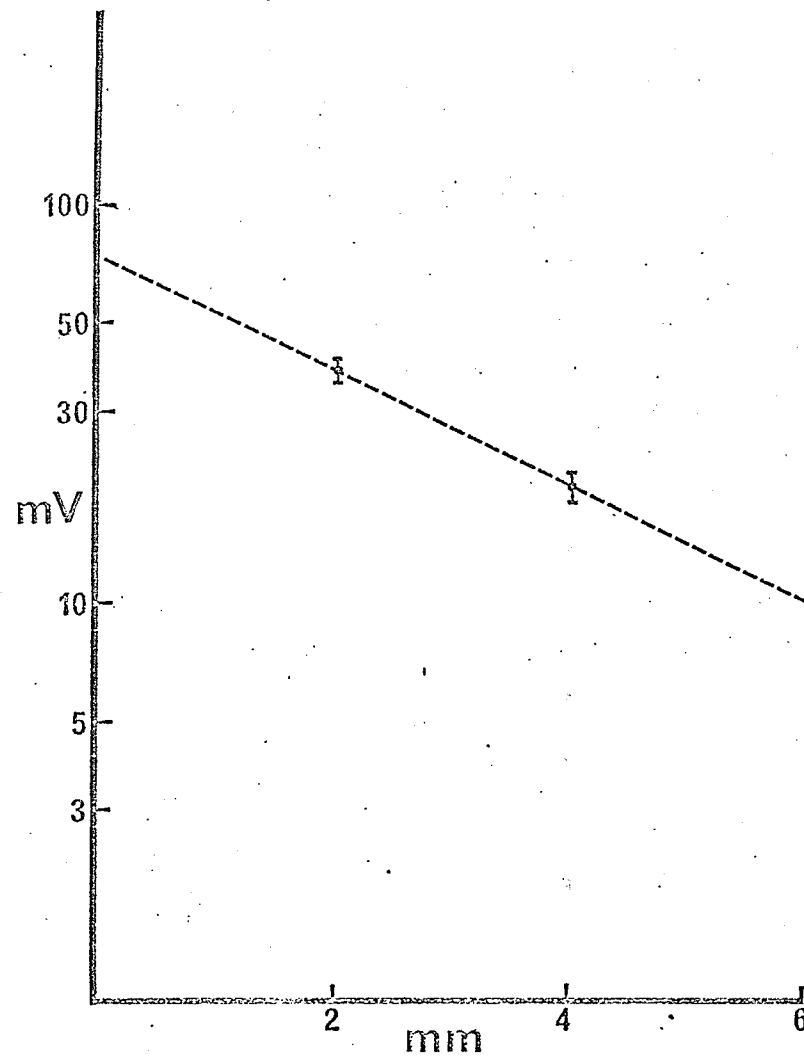


Figure 12. Spatial decay of the steady-state ETP in normal solution containing TEA; means $\pm$ S.E.'s of 7 experiments.

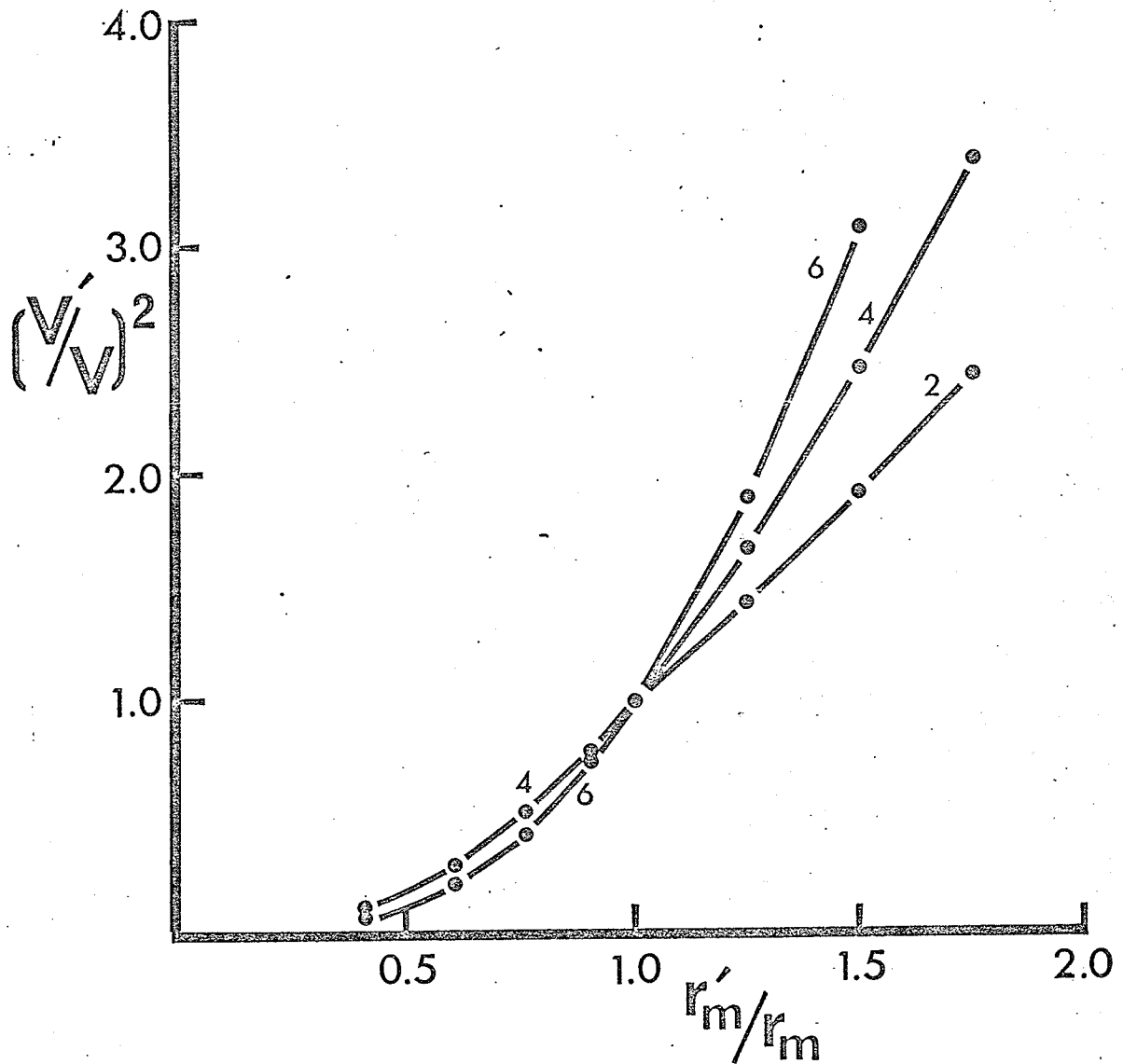


Figure 13. Graphical solution of the effect of Cl - deficiency on r . Hypothetical values for r'_m/r_m , experimental distances of 2, 4 and 6 mm and a space constant of 3.05 mm were substituted into the equation $(V'/V)^2 = r'_m/r_m \left(\frac{\exp \frac{-x}{\lambda r'_m/r_m}}{\exp -x/\lambda} \right)^2$ to obtain graph points. Experimentally - derived values for $(V'/V)^2$ were then plotted to arrive at the corresponding r'_m/r_m values.

respectively were obtained. Since specific membrane resistance is a rather uniform physical parameter and the values for $\frac{r'_m}{r_m}$ were not significantly

different, the two values were averaged to yield a ratio for $\frac{r'_m}{r_m}$ of 1.53 as shown

in figure 14.

4. Action potentials

AP's were evoked by anode-break excitation (see figure 9) and means from 16 experiments regarding the effect of Cl-deficiency on the AP parameters are presented in table 7. The mean results of these experiments were not significantly different ($P < 0.05$) from those of the 7 experiments for which resistance measurements were obtained. Maximum dV/dt values were calculated by measurements of maximum slope from pen recordings at high paper speed (expanded time base). The ionic intervention resulted in the AP height being augmented by 73% (significantly different from control at the $P < 0.01$ level by the Student's "t" test) and maximum dV/dt by 160% (difference significant at the $P < 0.05$ level). These results confirm the preliminary observations regarding the effect of Cl-deficiency on spontaneous AP's, but also show that a greater r_m cannot account for the dramatic potentiation of the dV/dt of the AP. The relationship between the changes in r_m and AP parameters in Cl-deficient solution is presented in figure 14.

B. The Role of Ca in the Phasic Electrical and Mechanical Activity in the Presence of TEA

1. Effect of high Ca

The myogenic response (MR) of the trachealis in response to stretch could not be demonstrated in vitro except in the presence of TEA (Stephens, Kroeger, and Kromer, 1974), but may be an important mechanism in disease states involving forced respiratory manoeuvres (Olson ^{et. al., 1967}). TEA also produces AP's and twitches (Kroeger and Stephens, 1975) and the magnitude of the MR bears a direct relationship with the concentration of external Ca. Therefore, it is believed that the MR may be mediated by AP's whose magnitude may be regulated by Ca in two ways: 1) by carrying at least part of the inward current of the AP and 2) by controlling membrane

Table 7

Effect of Cl⁻ deficiency on AP Height and
Maximum dV/dt

	AP Height (mV)	AP dV/dt _{max} (mV/sec)
NS - TEA (control)	13.8 ± 1.07 ⁽¹⁶⁾	95.4 ± 18.38 ⁽¹⁶⁾
Low Cl ⁻ - TEA	23.2 ± 1.79	229.6 ± 53.54
% of control	173**	257*

Means ± S.E.'s with number of experiments given in brackets.

* P < 0.05

** P < 0.01

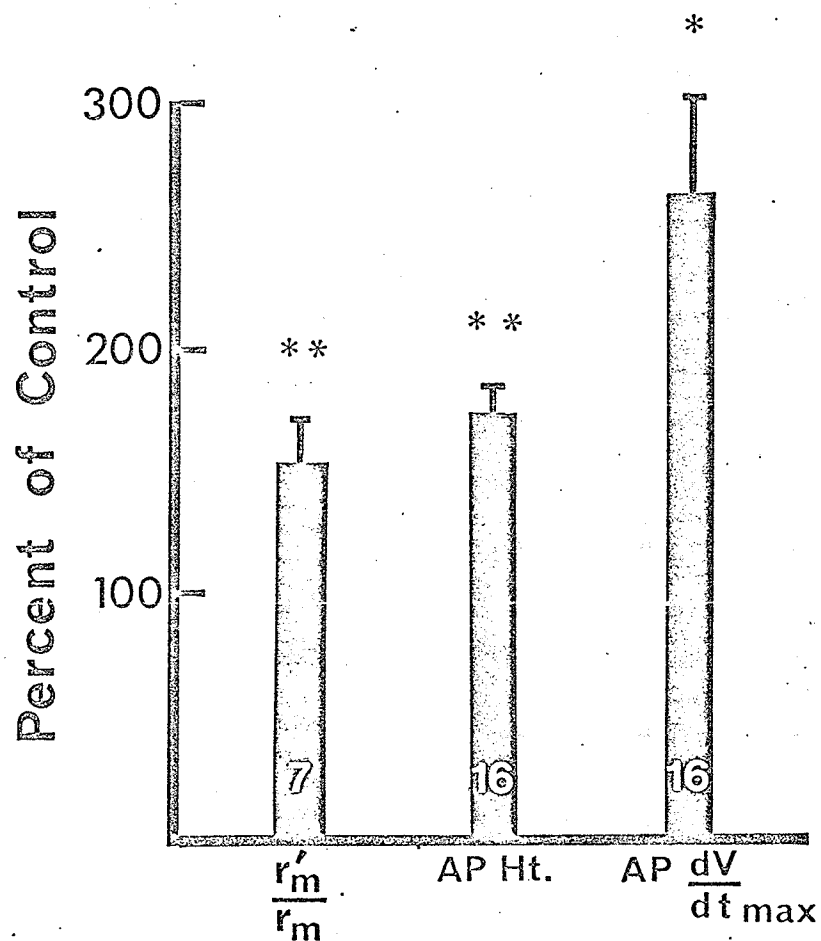


Figure 14. Effect of Cl⁻ deficiency (Cl_i = 50 mM) on r_m , AP height and maximum AP dV/dt , percent of control values in NS - TEA (Cl_i = 146 mM). Mean + S.E. with number of experiments given by numbers inside bars. *P < 0.05, **P < 0.01.

conductance properties. To test these hypotheses it is necessary to show an effect of high Ca_o and of a specific inhibitor of dynamic inward I_{Ca} on both active voltage transients and r_m simultaneously. Consequently, the effect of high Ca_o (6.0mM) on anode - break stimulated AP's and tension was investigated. This method of stimulation produces hyperpolarizing ETP's which provide a continuous measurement proportional to r_m .

Figure 15 shows a typical recording of AP's and mechanical responses in the presence of Cl-deficient TEA solution containing 2.0 mM Ca which was switched to one containing 6.0 mM Ca when the activity was stable. It is evident that the AP was substantially increased in high Ca solution as were the corresponding twitches. Figures 16, 17 and 18 present the overall results of exposure to high Ca (included are the effects of D-600 for comparison). Figure 16 shows the effect of high Ca on AP height which was increased by 47% from a mean of 15.6 to 22.4 mV which was significantly different at the $P < 0.01$ level by paired "t" test. Figure 17 shows the increase in the maximum dV/dt of the AP which amounted to 222%, from 69.5 to 185.7 mV/sec (significantly different at the $P < 0.05$ level). Thus, both high Ca and Cl - deficiency produced a much greater potentiation of the rate of rise of the AP than of the height, the former proportional to the dynamic ionic current of the AP. A concomitant reduction in the ETP amplitude by 49% ($P < 0.05$) was observed in the presence of high Ca as seen in figure 15. The actual change in r_m was calculated as before and amounted to a 41% decrease. The ETP's in figure 15 were dramatically reduced by 2/3, a fall in r_m of 58%. There are two possible reasons for this decline which will be discussed below.

These changes in r_m in response to alterations in the availability of external Ca point toward a role for Ca associated with the cell membrane in regulating ion permeability as it does in other preparations (Frankenhaeuser and Hodgkin, 1957; Hagiwara, 1966; Kuriyama and Tomita, 1970). However, an even more interesting phenomenon can be discerned in the records in figure 15. The regularity of the ETP and AP amplitudes observed in normal Ca dramatically changed to an alternating

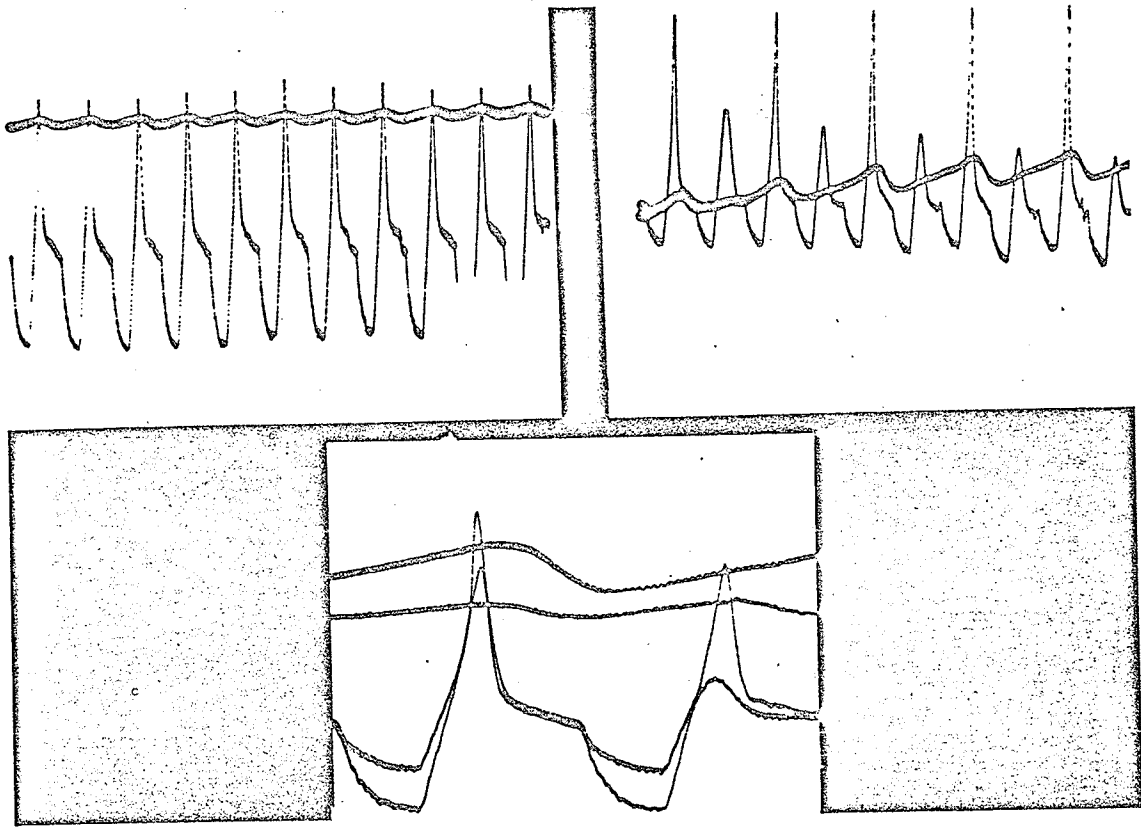


Figure 15. Effect of high Ca on paced AP's and mechanical responses (increasing tension represented by a downward deflection); stimuli delivered by hyperpolarizing square-wave pulses which elicited downward - going ETP's and active anode - break responses, $\times = 4$. Panel a - normal Ca^{2+} (2.0 mM). Panel b - 6.0 mM Ca . Panel c - representative responses from panels a and b superimposed with time base expanded and Mechanical responses doubled to show phasic nature more clearly.

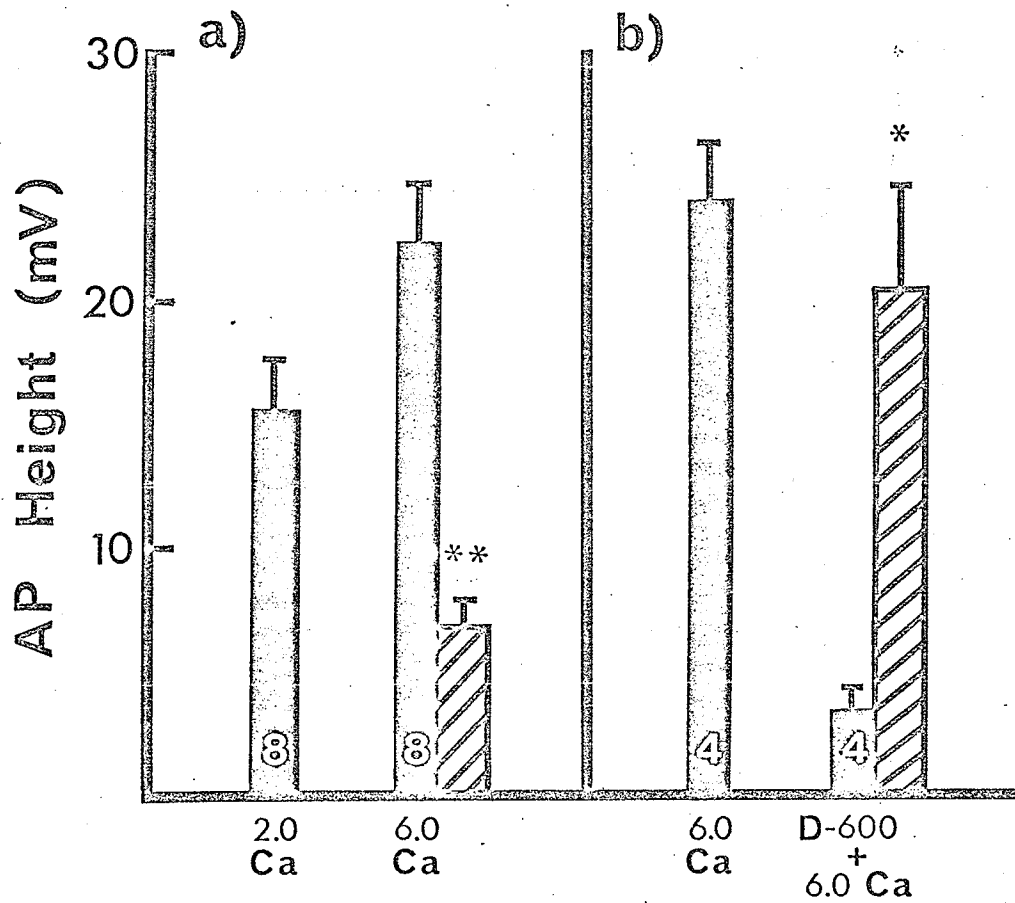


Figure 16. Effect of a) high Ca (6.0 mM) and b) D-600 (10⁻⁵ M) in the presence of high Ca on AP height (mV). Mean ± S.E. with number of experiments given inside bars. Shaded bars represent actual mean values while hatched bars represent magnitude of change. *P < 0.05, **P < 0.01.

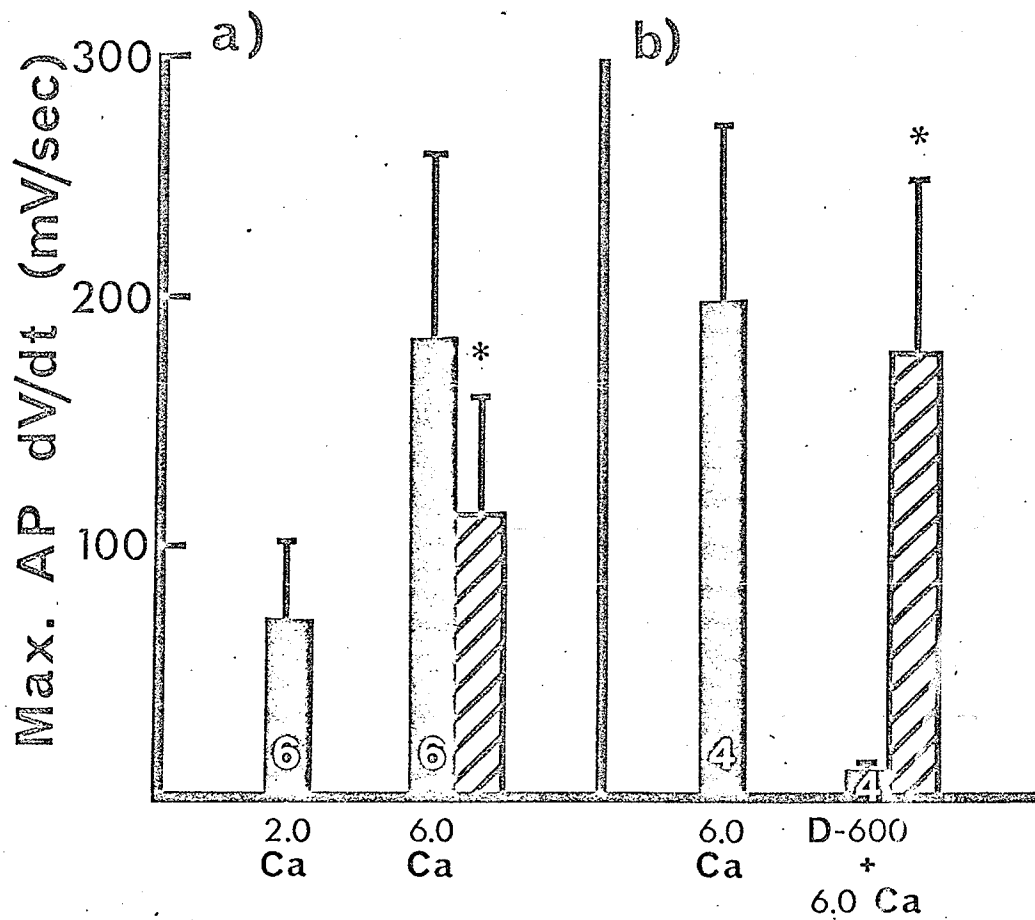


Figure 17. Effect of a) high Ca (6.0 mM) and b) D-600 (10^{-5} M) in the presence of high Ca on maximum AP dV/dt (mV/sec). Mean $\pm$ S.E. with number of experiments given inside bars. Shaded bars represent mean values while hatched bars represent magnitude of change. *P < 0.05.

rhythm. At the same time the twitches, which reflect intracellular free Ca levels at the myofilaments, also respond with alternation to the height and dV/dt of the initiating spikes. Thus it appears that both the activation of dynamic Ca channels and the permeability to other ions are sensitive to beat to beat fluctuations in intracellular Ca levels. Possible mechanisms are discussed below.

2. Effect of D-600

Additional evidence for a critical requirement of the AP for external Ca in TEA - containing solution came with the application of D-600 (an antagonist of dynamic, depolarization - activated Ca influx) which abolished the MR to stretch (Stephens, Kroeger and Kromer, 1974). This agent has no independent effect on ouabain contracture, E_m , cAMP levels, tissue Ca content or actomyosin ATPase (Marshall and Kroeger, 1973; Bose, personal communication). Since $[Ca^{+2}]_i$ must rise to a critical threshold level for contraction to take place, demonstration of contraction produced by AP - mediated Ca influx requires first, an elevated background of Ca influx as evidenced by increments in AP parameters and tension. Subsequent cessation of the electrical activity together with relaxation after exposure to a specific antagonist would provide the necessary additional evidence.

In this study D-600 was introduced during a stable train of AP's in the presence of Cl - deficient TEA containing 6.0 mM Ca. The initial bath concentration of D-600 in the flow-through system was $10^{-5} M$, and figure 18 shows a typical result of this treatment. Inhibition of the spikes, twitches and basal tension was rapid (within 90 seconds) and almost complete. Although 10 to 20 minutes is required for this agent to exert its full effect, only the first minute or two was recorded since the major inhibition was produced within this time and the electrode penetration was difficult to maintain much longer. Figures 15 and 16 show that AP height was reduced by 84%, from 24.2 to 3.3 mV (significantly different at the $P < 0.05$ level by paired "t" test) and maximum dV/dt by 94%, from 214.3 to 10.5 mV/sec (difference significant at $P < 0.05$). These results, therefore, lend additional support for 1) the utilization of the external (or superficial) Ca pool for the production of phasic electrical and mechanical activity in the presence of TEA and

2) for the activation of a greater inward, dynamic Ca current upon depolarization.

An increase in r_m also occurred during D-600 treatment in 3 experiments as evidenced by the 66% increase in the ETP amplitude over that in high Ca solution. This change correlated with a 59% increase in r_m . The record in figure 18 shows a substantial augmentation of the ETP, providing further evidence for a regulatory role for membrane Ca over membrane permeability and a requirement for input of Ca from external sources. Figure 19 presents the overall percent changes in r_m and AP parameters effected by D-600 treatment.

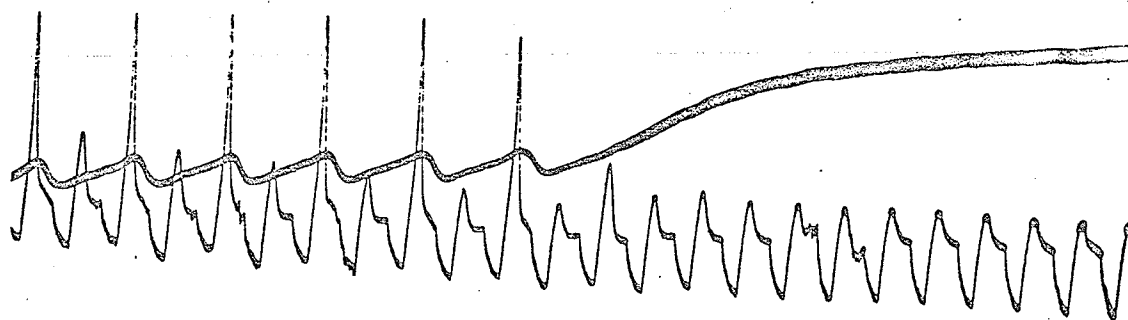


Figure 18. Effect of D-600 (10^{-5} M) on paced AP's and mechanical responses (downward deflection with increasing tension) in the presence of 50 mM Cl-TEA containing 6.0 mM Ca; AP's elicited at break of hyperpolarizing pulses which produce downward - going ETP's, $x = 4$.

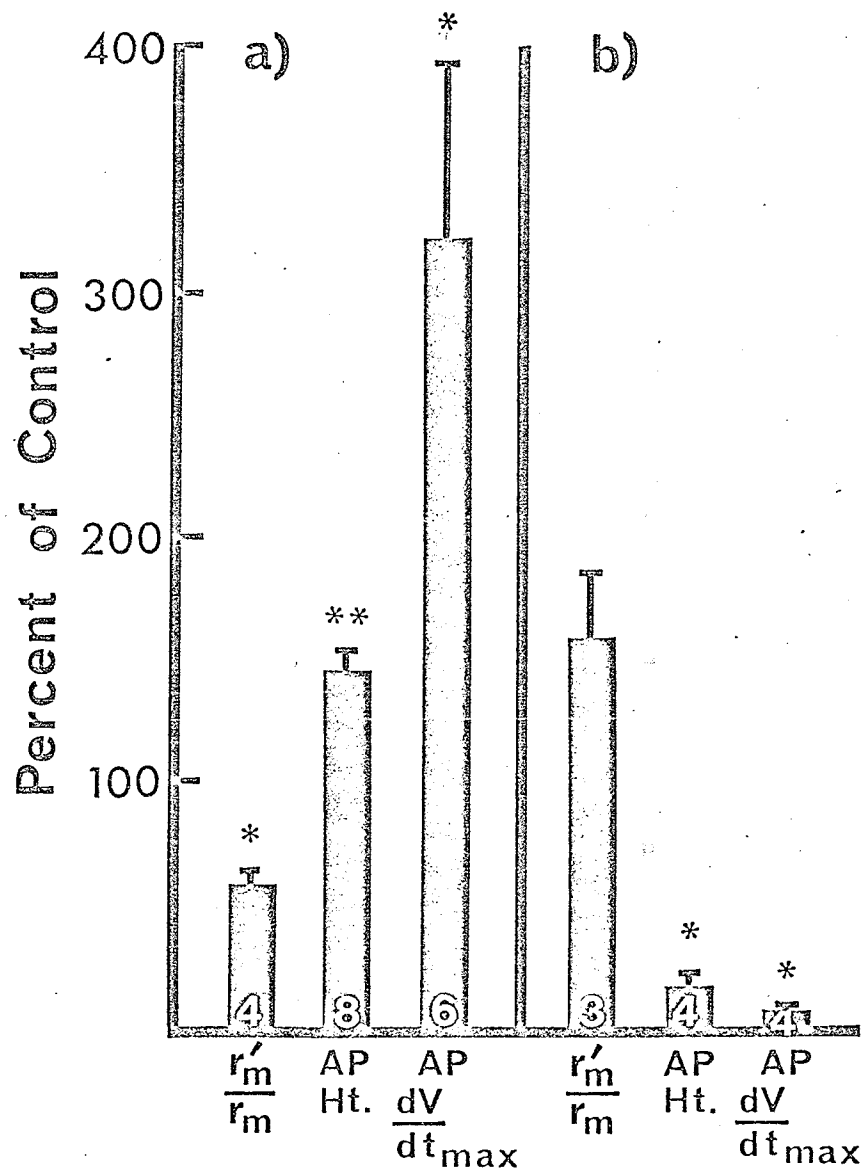


Figure 19. Effect of a) high Ca (6.0 mM) and b) D-600 (10⁻⁵ M) in the presence of high Ca on r'_m , AP height and maximum AP dV/dt . Percent of control values measured in 2.0 mM Ca (panel a) and in 6.0 mM Ca (panel b). Mean $\pm$ S.E. with number of experiments. *P < 0.05 **P < 0.01.

DISCUSSION

A. Effect of TEA and Cl⁻ deficiency on Electrophysiological Parameters

The present results showed that Cl⁻ deficiency increased 1) the amplitude of the ETP at a distance of 2 mm by 43% and at 4 mm by 51% corresponding to a mean increase in r_m of 53%, 2) the height of the AP by 73% and 3) the maximum dV/dt of the AP by 160%.

1. Membrane resistance

Since TEA produces spontaneous phasic electrical and mechanical activity presumably via a reduction in g_K (Kroeger and Stephens, 1975), the present experiments were designed to test the hypothesis that a further independent increase in r_m would potentiate this activity. The method of recording intracellularly the hyperpolarizing ETP's produced by external stimulation was therefore employed as it provides a convenient measure of r_m devoid of active responses (Abe and Tomita, 1968; Kroeger and Marshall, 1973).

Previous work undertaken to characterize the smooth muscle of the canine trachealis in terms of classical biophysical, ultrastructural and electrophysical parameters showed that TEA of the numerous agents tested, was the only one which produced spontaneous, phasic electrical and mechanical activity. In addition, a decrease in E_m and an increase in cell r_m were observed (Kroeger and Stephens, 1975). These observations were consistent with an inhibition of resting g_K (Stanfield, 1970; Ito *et al.*, 1970) which is a prominent contributor to 1) total membrane permeability (Hodgkin and Horowicz, 1959b; Casteels, 1969a, 1970), 2) E_m (Adrian, 1956; Casteels and Kuriyama, 1965), 3) rectification of depolarizing ETP's (Hodgkin and Huxley, 1952b,c; Inomata and Kao, 1976) and 4) excitability (Hermsmeyer, 1976; Tomita, 1966b). Therefore it was considered that although at rest, g_{Na}/g_K is relatively high in SM (Casteels, 1970; Inomata and Kao, 1976), g_K of trachealis might normally be great enough to prevent any substantial effect of depolarization - activated g_{Na} on E_m . The effect of these depolarization - activated channels should therefore be unmasked by TEA (Mekata, 1971; Kroeger and Stephens, 1975). Since TEA increases r_m of trachealis, a further increase in r_m should produce proportionally greater voltage transients on the principle of Ohm's Law (Kuriyama, 1963a; Abe, 1971), assuming a constant current across an increased r_m .

The significant increase in r_m observed in the presence of Cl - deficient solution (isethionate substitution) in the present study indicates that g_{Cl} is relatively high in trachealis as it is in other SM's (Barr, 1959; Durbin and Monson, 1961). In addition, Cl is not passively distributed in SM but is thought to be actively taken up by the cell (Kao, 1967; Casteels, 1965); thus E_{Cl} is less negative than E_m (Kao and Nishayama, 1964). The influence of g_{Cl} on r_m , E_m and electrical activity can be investigated by measuring the effects on these parameters of a reduction in g_{Cl} effected by partial substitution of external Cl with less permeant anions (Bülbring and Tomita, 1969; Abe, 1971). The resultant reduction in g_m is reflected as a greater r_m (Ohashi, 1970) and thus greater voltage transients (according to Ohm's Law) while E_{Cl} (and therefore E_m) becomes transiently more positive due to decreased $[Cl]_o$. Since the anion substitute is less permeant than Cl, the net movement of Cl is outward which gradually shifts E_{Cl} toward (but not as far as) its normal resting level. Anion substitutes such as SO_3^{2-} or $C_6H_5SO_3^-$ (less permeant) increase the amplitude of the ETP and the membrane time constant as a function of the increase in r_m but SO_3^{2-} is divalent and is thought to be slightly toxic, while neither anion is impermeable (Kuriyama, 1963a; Abe, 1971). Isethionate however, lacks these defects and appears to be the most suitable substitute for Cl (Kroeger and Marshall, 1973).

The theoretically - expected increase in r_m effected by Cl-deficiency may be calculated from approximate values for $g_k : g_{Na} : g_{Cl}$. For taenia coli this ratio has been shown to be approximately 1 : 0.1 : 0.4 (Casteels, 1970). The equivalent resistances are thus $\frac{1}{r_m} = \frac{1}{r_k} + \frac{1}{r_{Na}} + \frac{1}{r_{Cl}} = 1.5$ and $r_m = 0.66$.

With total elimination of I_{Cl} , $\frac{1}{r_m} = 1.1$ and $r_m = 0.91$, such that the maximum

possible increase would be $\frac{r'_m}{r_m} = \frac{0.91}{0.66} = 1.38$. With substitution of $\frac{2}{3}$ of $[Cl]_o$

as in the present study, $\frac{r'_m}{r_m}$ would be approximately 1.25. Consequently, the value of 1.53 obtained in this study suggests either 1) a very high g_{Cl} in trachealis or 2) experimental error. In the latter case it is an error in the right direction since this value is compared to concomitant increases in AP parameters (see below) in order to determine the voltate - dependence of dynamic ion channels.

Although Cl⁻ substitution during this study produced significant increases in the amplitude of the ETP, acceptable records were obtained only at two distances in each solution, thus making it difficult to calculate reasonable estimates of the space constant (λ) in the test and control solutions. However, the spatial decay of the steady-state ETP has been shown to be an exponential function in the taenia coli (single-unit), vas deferens of the guinea pig (intermediate, Abe and Tomita, 1968), the pregnant and non-pregnant rat uterus (Abe, 1971) and the dog trachealis (Kroeger and Stephens, 1975) both in normal and in TEA-containing solution. Therefore, mean ETP amplitudes at 2 and 4 mm from 7 experiments were plotted on a semilogarithmic graph and joined by a straight line for the control solution (see figure 12). The λ measured in this way is 3.05 mm which is similar to that obtained previously (Kroeger and Stephens, 1975).

With reference to an assessment of the method employed for analysis of changes in r_m , it should be noted that the technique relies on the following assumptions: 1) that the muscle possesses cable-like properties, 2) that junctional resistance remains unaltered during current flow and the action of agents and ionic interventions, 3) that the current passing through the tissue is directly proportional to the applied voltage and 4) that this current is unaffected by reasonable changes in r_m (Bolton, 1972). Evidence regarding these assumptions is the following: 1) the spatial decay of the ETP is smoothly exponential for a distance of many cell lengths (Abe and Tomita, 1968); 2) present techniques are inadequate to measure r_j directly and accurately, but it appears that, at least in the taenia coli, r_j is resistant to the osmotic perturbations of hypertonic

sucrose solution (Tomita, 1966a; Sparrow et al., 1967) and to alterations in $[Ca]_o$ (Tomita, 1969); 3) any error invoked by assuming direct proportionality between stimulating current and applied voltage is very small and can be neglected (Bolton, 1972); 4) ^{the} above also applies for errors produced by variations in current flow with changes in r_m (Bolton, 1972), although any such error is eliminated when the amplitude of the ETP is plotted as a function of distance semilogarithmically, thus eliminating the current factor.

The Abe - Tomita apparatus also has several limitations whose effects must be kept in mind when designing experiments and analyzing data. These limitations and necessary refinements include 1) maintenance of a constant tissue length (after equilibration) in order to prevent the superimposition of any myogenic responses to changes in length on those due to agents and test conditions, 2) substantial loss of polarizing current to the bath unless focused on the muscle strip by suitable insulation of the central stimulating electrode, 3) the necessity of employing a floating microelectrode system in order to record E_m and tension simultaneously and 4) the presence of surface tension at the partition electrode which in our hands precluded recording of E_m at distances less than 1 mm from the partition with the floating arrangement. Since a substantial error may be incurred in the event that x is assumed to be $\ll \lambda$ and the cable equation simplified by neglecting the term $-x/\lambda$ (Bolton, 1972), the cable equation was applied in its original form (Hodgkin and Rushton, 1946) to calculations of the increase in r_m on the basis of the observed increase in the ETP (Kroeger and Marshall, 1973).

2. Action potential parameters

Since TEA produces an elevation of r_m in parallel with the production of AP's (Kroeger and Stephens, 1975) it was suggested that TEA, by reducing the resting g_K , unmasks the ability of any dynamic depolarization - activated Na and/or Ca channels to produce AP's (Mekata, 1971). In addition, the ability to produce a MR to stretch in the presence of TEA was considered to be correlated with the ability of a mechanical stress to depolarize the membrane and produce (or increase the rate of discharge of) AP's (Burnstock and Prosser, 1960a).

In the present investigation, a further increase in r_m was effected independently

of that produced in TEA, by reducing I_{Cl} with Cl - deficient solution as discussed above. The object of these studies was to ascertain whether AP parameters would be increased by the greater depolarization on the basis of Ohm's Law (assuming a constant current across an increased r_m) or whether additional dynamic ion channels would be activated and produce even greater increases in these parameters. The amplitude of the AP was increased roughly in proportion to the increase in r_m while the maximum rate of rise, which is determined by the dynamic inward ionic current (Kao, 1967), was increased by about a factor of 2 $\left(\frac{160\%}{73\%}\right)$ over the

increase in the amplitude. This data therefore provides evidence for additional current being provided by depolarization - activated ion channels in Cl - deficient solution. It has been suggested (Anderson, 1969) that membrane leakage - current (predominantly I_{Cl}) may reduce the ability of the active transient current to depolarize the muscle. Therefore, it appears that such a significant voltage - dependence of the dynamic ion channels may afford the muscle a considerable sensitivity to changes in its ionic environment and, more important, to the influences of nerve - mediated and circulating neurohumors (Bülbring and Tomita, 1969a).

The importance of g_K to SM activity generally is indicated by several findings similar to those regarding the actions of TEA on r_m and spontaneous membrane activity. Tomita (1966b) has suggested that the presence of a low g_K might be the main factor contributing to a low E_m and spontaneous activity. Low K efflux rates in single - unit SM's such as dog carotid artery (Barr, 1961b) and rat uterus (Daniel et. al., 1962) suggested the presence of a low g_K in these spontaneously active preparations. In the hepatic portal vein, high K depolarizes the membrane which increases g_K (thus decreasing r_m) and converts bursts of spikes and accompanying contractions to single spiking and aborted contractions; Ba^{2+} reduces g_K and increases the number of spikes per burst as well as contraction amplitude (Hermes-meyer, 1976). In the taenia coli, adrenaline hyperpolarizes the membrane, reduces the amplitude of the ETP and suppresses spike activity, these actions being consistent with an increase in g_K (Bülbring and Tomita, 1969a). In addition, procaine increases r_m of guinea - pig urinary bladder, depolarizes the membrane,

reduces the maximum rate of fall of the AP as well as the duration, and enhances spike activity (Kurihara, 1975). Similar effects are obtained with TEA (Mekata, 1971; Kroeger and Stephens, 1975) which is known to inhibit the steady state g_K (Tasaki and Hagiwara, 1957) and the resting g_K (Stanfield, 1970). It is evident therefore, that phasic electrical activity may be dependent on g_K , both in an absolute sense (Ohm's Law basis) and for the control of dynamic ion conductance.

B. Role of External Ca in TEA - induced electrical and mechanical activity

1. Current of the action potential and E-C coupling

The present experiments showed that in the presence of Cl - deficient TEA solution, 1) increasing $[Ca]_o$ from 2.0 to 6.0 mM increased AP height by 47% and dV/dt_{max} by 222% but decreased r_m by 41% and 2) D - 600 reduced AP height by 84% and dV/dt_{max} by 94% in the presence of 6.0 mM Ca but increased r_m by 59%.

The main purpose of the present investigation was to obtain quantitative evidence regarding the role for external Ca 1) as a carrier of the dynamic inward current of the AP produced by TEA and 2) as "activator" of contraction at the myofilaments or as "trigger" of contraction by releasing sequestered Ca. That the MR to stretch is critically dependent on $[Ca]_o$ and is abolished by a specific antagonist of dynamic Ca influx (D-600) provides indirect evidence that the dynamic ionic current of these AP's is carried mainly by Ca. Thus it appears that TEA converts the normally quiescent, multi - unit trachealis into a functionally single - unit SM with Ca - dependent electrical activity. This is of interest in view of the findings 1) that other single - unit preparations (visceral and uterine SM) appear to utilize external Ca for production of AP's (Brading et. al., 1969b; Abe, 1971; Vassort, 1975) and 2) that multi - unit SM's have a large intracellular store of sequestered Ca resistant to depletion in Ca - free media (Devine et. al., 1973; Kroeger and Stephens, 1971). Consequently, it was of interest to us to define 1) the ionic basis of the AP of trachealis in the presence of TEA and 2) the relationship between depolarization and activation of dynamic ion conductance.

The protocol involved measurement of the effect of 1) elevated $[Ca]_o$ and 2) D-600 on the height and dV/dt_{max} of AP's produced in the presence of

Cl - deficient TEA. Coincidentally, the AP height was increased in 6.0 mM Ca by an amount similar to that produced by increasing r_m , but the increase in $dV/dt_{\max}$ was 4.5 - fold greater, indicating a direct correlation between the current of the AP and the $[Ca]_o$. This augmentation in the $dV/dt_{\max}$ is especially dramatic in view of the 160% increase in this parameter already effected by Cl - deficiency, and the decrease in r_m by 41% in high Ca_o . Stronger evidence for a predominant Ca current was provided by the application of D-600 which inhibited this high influx to a mere 6% (as indicated by $dV/dt_{\max}$) of its value in 6.0 mM Ca. The small component of the AP (2 to 3 mV) remaining after D-600 treatment may represent 1) a small Na component, 2) a D-600 - resistant Ca component (Bülbring and Tomita, 1970; Kohlhardt et al., 1972) or 3) an insufficient time allowed for the agent to exert its full effect, as 10 to 20 minutes is normally required (Kroeger, unpublished observations); this however, was difficult to achieve since electrode penetration was not maintained for more than several minutes. The major inhibition however, was always produced within one minute, providing strong evidence for a major role for external Ca in the AP of trachealis in the presence of TEA.

The possibility of extracellular Ca acting as activator Ca in the present study was tested by calculating Ca influx using estimates of membrane capacitance (C_m), cell surface area, extracellular space and internal free Ca^{2+} concentration (Marshall and Kroeger, 1973; Hodgkin and Katz, 1949). Assuming that C_m of the trachealis is similar to that of the guinea pig taenia coli, 2 - 3 $\mu F/cm^2$ (Abe and Tomita, 1968; Tomita, 1966), then the amount of charge crossing the membrane per mV AP is

$$\begin{aligned} Q &= C_m \cdot V \\ &= 3 \times 10^{-6} \text{ F/cm}^2 \cdot 10^{-3} \text{ V} \\ &= 3 \times 10^{-9} \text{ coulomb/cm}^2 \end{aligned}$$

Since 96,500 coulomb/eq. = 10^{-5} eq./coulomb, this amount of charge influx is equivalent to

$$\begin{aligned} Q &= 3 \times 10^{-9} \text{ coulomb/cm}^2 \cdot 10^{-5} \text{ eq./coul.} \\ &= 3 \times 10^{-14} \text{ eq./cm}^2 \end{aligned}$$

For a surface area of $6 \times 10^3 \text{ cm}^2/\text{g}$ (Goodford, 1970), the increase in cellular concentration of Ca is

$$\begin{aligned} Q &= 3 \times 10^{-14} \text{ eq./cm}^2 \cdot 6 \times 10^3 \text{ cm}^2/\text{g} \\ &= 1.8 \times 10^{-10} \text{ eq./g} \\ &= 1.8 \times 10^{-7} \text{ eq./kg} \end{aligned}$$

The ECS in TSM is roughly 50% (Stephens, unpublished observations) of the tissue mass, which therefore doubles the value for ion influx, but Ca^{2+} ions are divalent so that the approximate Ca influx is $1.8 \times 10^{-7} \text{ mol/kg/mV}$. Assuming that the range of intracellular free Ca^{2+} ions required for threshold to maximal activation of the myofilaments is 2×10^{-7} to $1.3 \times 10^{-6} \text{ mol/g actomyosin}$ (Bianchi, 1969) and that the trachealis contains 5 mg actomyosin/g wet muscle (Needham and Schoenberg, 1967), then the $[\text{Ca}^{2+}]_i$ required for activation is 1.0×10^{-9} to $6.5 \times 10^{-9} \text{ mol Ca/g muscle}$ $= 1.0 \times 10^{-6}$ to $6.5 \times 10^{-6} \text{ mol Ca/kg}$. Therefore, AP's of 6 mV should be sufficient to provide enough Ca influx for direct activation while 36 mV would be required for maximal contraction. Consequently, the AP's recorded in NS - TEA ($\bar{X} = 13.8 \text{ mV}$) and in Cl - deficient TEA ($\bar{X} = 23.2 \text{ mV}$) would be more than adequate for providing activator Ca.

2. Membrane stabilization

In the presence of 6.0 mM Ca 1) the ETP was reduced by an amount which correlated to a mean reduction of r_m by 41% and 2) r_m was increased almost to its original value seen in 2.0 mM Ca by the action of D-600 in the continued presence of 6.0 mM Ca. In one experiment (see figure 15) r_m was decreased by 58% in high Ca solution, suggesting a strong influence of Ca on r_m . The concomitant hyperpolarization is consistent with an increase in g_K as proposed for heart muscle (De Mello, 1975; Isenberg, 1975) and SM (Tomita and Watanabe, 1973; Bülbring and Tomita, 1970).

The decline in r_m observed during the action of elevated $[\text{Ca}]_o$ may be explained in two ways. One possibility is that the resistance at intercellular junctions (r_j) is increased directly by the presence of elevated levels of external Ca or through increases in internal Ca during surface electrical activity as evidenced by increased tone. These conditions induce electrical uncoupling as demonstrated

in Purkinje fibers (De Mello, 1975), and in salivary gland cells (Loewenstein et al., 1967), thus reducing current spread and the spatial decay of the ETP. Assuming, however, an average cell length of $100\ \mu$ and no overlap of cells (Nagai and Prosser, 1963), AP's were recorded at least 600 cell lengths from the partition (stimulating) electrode and exhibited greater amplitude and rate of rise in high Ca_o . These results do not indicate whether r_j and intercellular electrical coupling are altered by elevated Ca levels but it is evident that AP conduction is not impaired.

The other possible explanation for the reduction of r_m in high Ca_o is that membrane g_K is increased by an increase in the local $[Ca]$ at the membrane. The location of this action may be indicated by the recordings shown in figures 15 and 19. The amplitude of the ETP is decreased considerably in the presence of 6.0 mM Ca, suggesting a primary action by Ca at the outer aspect of the membrane. The stable amplitudes of the ETP as well as the AP and twitch in high Ca_o change however, to an alternating rhythm. Immediately after a full, all - or - none spike, the tension rises steeply and the following ETP is the greater of the two alternating amplitudes. This ETP (produced by a hyperpolarizing stimulus) gives rise to an abortive AP which causes only a slight slowing of the relaxation phase. The next ETP is smaller but gives rise to a full AP and a large twitch. It appears that the onset of the stimulus immediately following a full AP occurs before that AP is completed. The subsequent production of a small AP suggests that the g_{Ca} is not sufficiently reactivated to produce a full spike even though r_m is greater. When the smaller AP is completed, the next ETP, although smaller, is followed by a full spike. Application of D-600 increases r_m and abolishes the alternating pattern. Although several models may be proposed describing the interaction between intracellular Ca gradients and membrane ion conductances (i.e. resting g_K and dynamic g_{Ca}), no evidence in this regard is available on which to base speculation. These data however, suggest an intimate moment - to - moment control of membrane excitability and in turn suggests the possibility of a regulatory influence by Ca of spontaneity in SM.

It should be noted that measurements of r_m are steady - state measurements. The substantial increase in the dV/dt_{max} of the AP in the presence of Ca (discussed above in section B, part 1) might be due in part to a delay in the activation of g_K . However, this event would lead to an increase in AP duration which was not observed. Therefore, it is suggested that the increased dV/dt_{max} is the result of a greater inward current instead of a change in the temporal relationships of the Ca and K currents.

In conclusion, TEA appears to convert the normally multi-unit canine trachealis into a functionally single - unit SM exhibiting spontaneous phasic electrical and mechanical activity. The evidence presented in this and previous studies suggests that this activity is produced via a suppression of the resting g_K by TEA, allowing 1) the manifestation of dynamic, depolarization - activated Ca influx and 2) a greater sensitivity to local intra - and extracellular Ca levels and stimulating neurohumors.

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