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OSCILLATORY MEMBRANE POTENTIAL CHANGES IN CELLS OF MESENCHYMAL ORIGIN: THE ROLE OF AN INTRACELLULAR CALCIUM REGULATING SYSTEM

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SUMMARY

A number of mesenchymal cells (fibroblasts, macrophages and megakaryocytes) respond to a variety of stimuli with large hyperpolarizations lasting several seconds (the H.A. response). The H.A. responses can occur as repetitive trains or oscillations. These hyperpolarizations are due to an increase of the surface membrane permeability to potassium ions which is probably mediated by an increase in the cytoplasmic free calcium ion concentration. Evidence is discussed which suggests that the source of this increased calcium is, at least in part, an intracellular sequestering system, probably the endoplasmic reticulum. A model capable of producing oscillatory changes in membrane potential is proposed based on such an intracellular calcium sequestering and releasing system.

INTRODUCTION

A number of cell types of mesenchymal origin exhibit a characteristic excitability. This excitability is distinct in some regards from that studied so extensively in nerve and muscle, but it may also share important features with that of these classically excitable tissues. The mesenchymal cells in which this excitability has been demonstrated thus far include fibroblasts growing in primary culture and continuous cell lines of fibroblastic origin (Nelson, Peacock & Minna, 1972), mouse, guinea-pig and human macrophages (Gallin *et al.* 1975) and guinea-pig megakaryocytes (Miller, Sheridan & White, 1978). Intracellular recordings have revealed that these cells respond to a variety of stimuli with large membrane hyperpolarizations lasting several seconds. These hyperpolarizations, which are associated with large increases of membrane conductance, have been termed hyperpolarizing activation or H.A. responses. Such hyperpolarizations may occur spontaneously or can be elicited by electrical, mechanical or chemical stimuli. In confluent cultures of L-cells the responses can be transmitted from one cell to another. Hyperpolarizing activations may also occur spontaneously as repetitive trains in an oscillatory manner, or an evoked H.A. in a previously silent cell may be followed by a series of hyperpolarizing membrane potential oscillations lasting for several minutes (Okada *et al.* 1977*a*).

In this review we will discuss the surface membrane permeability changes res-

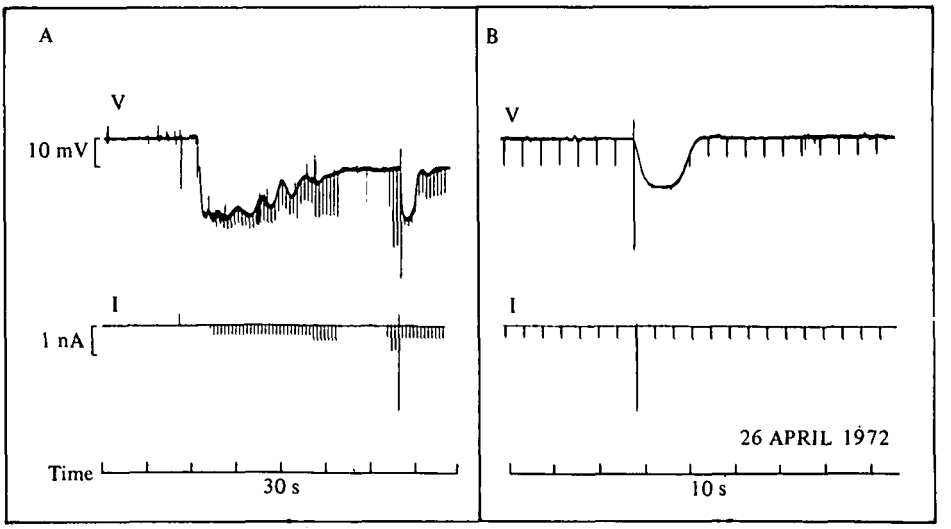

Fig. 1. Intracellularly recorded transmembrane potentials obtained from LM (TK⁻) cells selected by X-irradiation. (A) V, potential recorded by microelectrode; I, current passed through electrode; penetration of cell took place near middle of trace and typical penetration pattern of voltage change is shown. Large hyperpolarizing current pulses elicit hyperpolarizing responses. (B) Electrically elicited H.A. (Note faster time scale in B than in A.) Large change in membrane resistance shown by marked decrease in voltage response to small test current pulses. (Reproduced with permission from Nelson & Peacock, 1973.)

ponsible for the H.A. responses, summarize evidence relating to the mechanisms underlying these permeability changes and will propose a model incorporating these mechanisms, which can account for both single H.A.'s and rhythmic oscillatory membrane potential behaviour in cells exhibiting this form of excitability. Finally, based on clues provided by the forms of stimuli that elicit H.A. responses, we will discuss some possible functional implications of this form of excitability in mesenchymal cells.

I. CHARACTERISTICS OF THE HYPERPOLARIZING ACTIVATION (H.A.) RESPONSES

The hyperpolarizing increase in membrane conductance which constitutes the H.A. response is illustrated in Fig. 1. Results obtained from L-cells are generally representative, and we will describe them in most detail. L-cells are a fibroblastic cell line initially adapted to culture in 1948 (Sanford, Earle & Likely, 1948). They normally have a doubling time of some 20 h, but can be caused to stop dividing and to increase in size by X-irradiation (Whitmore *et al.* 1958; Nelson & Peacock, 1973). The large cells so obtained are quite suitable for electrophysiological studies involving intracellular microelectrode recording and stimulating techniques (Nelson & Peacock, 1972, 1973). Such recordings have shown that the cells have a relatively low resting membrane potential (-15 to -20 mV) and a large membrane input resistance (20–30 MΩ) and time constant (10–15 ms). The resting membrane potential is relatively insensitive to changes in extracellular K^+ concentration and this, in conjunction with flux measurements (Lamb & MacKinnon, 1971*a*, *b*), indicates that th

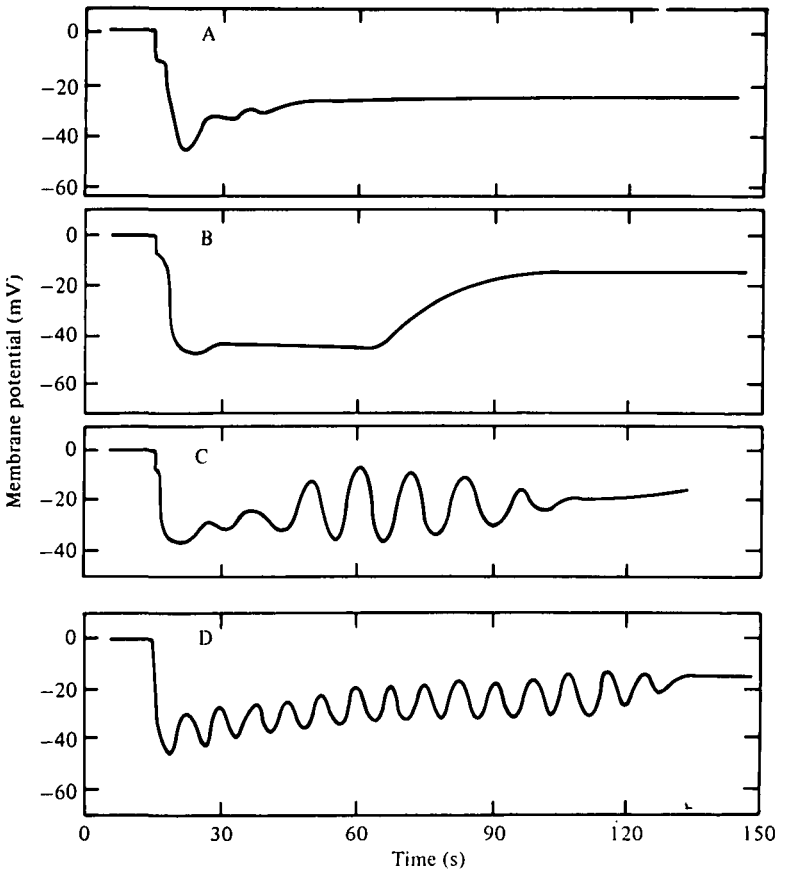

Fig. 2. Various patterns of recorded transmembrane potential on penetration of L cells. Note the transient small negativities immediately after penetration (near the beginning of A, B and C). More prolonged, larger negativities were then seen followed by a variably timed return to a steady lesser negativity (A and B). Variable oscillatory potentials were seen in some cells, as shown in (C) and (D). (Reproduced with permission from Nelson *et al.* 1972.)

permeabilities of the L-cell membrane to Na^+ and K^+ are roughly equal at rest. Stimulation of the L-cells by a variety of means can elicit a 20–30 mV hyperpolarization of the membrane lasting for several seconds; during this hyperpolarization the surface membrane conductance is increased 2- to 10-fold. The H.A. may also occur spontaneously and repetitively (Fig. 2) (Nelson *et al.* 1972) and, as described in detail by Okada and co-workers (Okada *et al.* 1977*a*, *b*, 1978), represents an oscillatory mechanism in L-cells.

The H.A. response is conveniently elicited by a brief ($\sim$ 100 msec) hyperpolarizing current pulse of 10–50 nA. Even though these are large current pulses, it appears that the effective stimulus is a voltage gradient across the membrane since in macrophages which have much higher input resistances, the current required to evoke an H.A. is an order of magnitude smaller. Large depolarizing pulses may also elicit the response, but at considerably higher threshold, if at all. In macrophages, depolarizing pulses occasionally evoked H.A. in cells when hyperpolarizing pulses did not (Gallin *et al.* 1975). When one H.A. has been elicited, the threshold for eliciting a second

response is typically increased for several seconds. However, repetitive H.A.s can occur spontaneously or in response to single stimuli as will be discussed below. When two adjacent L-cells are impaled, an H.A. elicited in one cell frequently is followed, after a latency of a few seconds, by an H.A. in the adjacent cell. L-cells are sometimes coupled electrically, but propagation of the H.A. occurs between cells which are not directly coupled electrically (Nelson & Peacock, 1973). Gentle mechanical stimulation provided by touching the cell surface some distance away from the impaling micropipette is a very effective means for eliciting H.A. responses. On the other hand, L-cells that are spontaneously oscillating are sometimes made to stop by mechanical stimulation (Okada *et al.* 1977*a*). We have found that iontophoretic application of acetylcholine was effective in some L-cells in producing H.A. responses and that this could be blocked by atropine but not curare (Nelson & Peacock, 1972). In experiments using L-cells from another source (Okada *et al.* 1977*a*) such cholinergic chemosensitivity was not found, but these cells were responsive to other chemical stimuli (Tsuchiya, Okada & Inouye, 1978). Macrophages produce large hyperpolarizations, sometimes followed by oscillations, in response to serum components and artificial peptides known to function as chemotactic stimuli (Gallin & Gallin, 1977).

II. THE MECHANISM UNDERLYING THE H.A. RESPONSE

When steady polarizing currents are passed across the L-cell or macrophage membrane to alter the membrane potential and the H.A. is elicited at different steady membrane potential levels, a reversal potential for the H.A. can be demonstrated. In normal (5·4 mM) K^+ solutions this is about -80 mV in L-cells (Nelson *et al.* 1972; Okada, Tsuchiya & Inouye, 1977*b*) and -54 mV in macrophages (Gallin *et al.* 1975). The H.A. reversal potential is markedly affected by changes in the external K^+ concentration (Fig. 3) but is very little affected by changes in external Na^+ or Cl^- concentrations (Okada *et al.* 1977*b*). Okada *et al.* (1977*b*) have also shown that oscillating H.A.s are insensitive to tetradotoxin, which blocks electrically excitable Na^+ channels in many tissues (Narahashi *et al.* 1960), but are abolished by tetraethyl ammonium (TEA), which blocks certain K^+ channels (Hagiwara & Saito, 1959). These experiments leave little doubt that the H.A. response consists of a large increase in the surface-membrane permeability to K^+ ions.

A rise in the free intracellular Ca^{2+} concentration has been shown in a number of cell types to produce an increase in the surface membrane K^+ permeability (Romero & Whittam, 1971; and reviewed by Meech, 1978). This appears to be the case in L-cells also; injection of calcium ions intracellularly produces a membrane hyperpolarization accompanied by an increase in surface membrane conductance (Nelson & Peacock, 1973; Henkart & Nelson, 1979; Okada *et al.* 1978, abstr.). Brief pulses of Ca^{2+} injections produced graded responses with a time course closely similar to the H.A. response (Fig. 4). The question of the mechanism of the H.A. response then becomes, what is the source of the calcium which produces the surface membrane permeability change?

An obvious possibility is that ingress of calcium ions from the external medium across the surface membrane accounts for the rise in intracellular calcium which generates the H.A. response. Gallin *et al.* (1975) showed that 1·5 mM EGTA (which

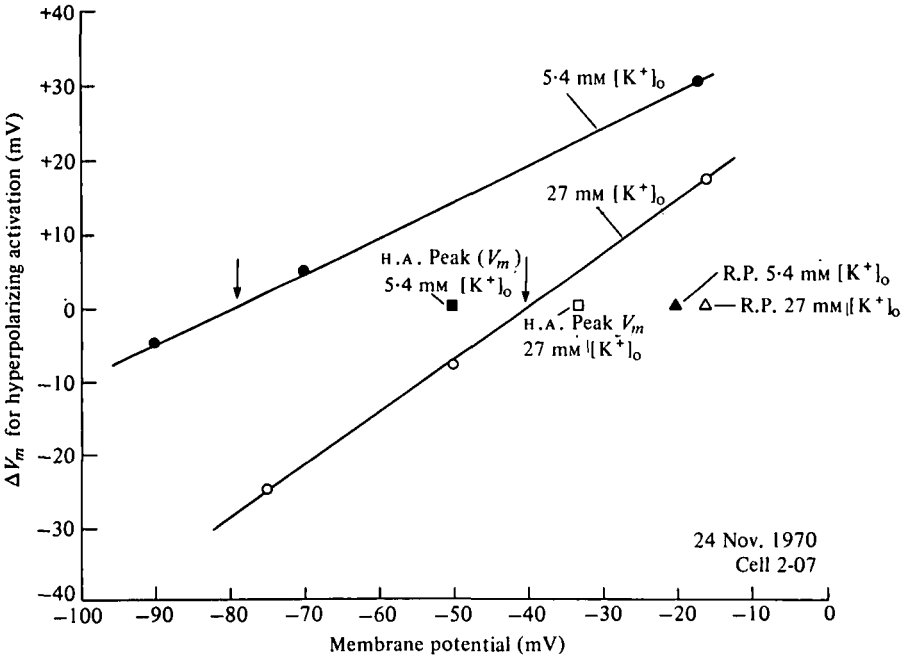

Fig. 3. The membrane potential change occuring during an H.A. response as a function of steady membrane potential. ΔV_m = the steady membrane potential minus the membrane potential reached at the peak of the H.A. This steady membrane potential was adjusted by passing steady currents through the intracellular electrode. The open circles correspond to data obtained when the extracellular potassium concentration was 27 mM and chloride was 140 mM. The filled circles correspond to data obtained from the same cell within less than 10 min after the external medium was changed so that it contained 5·4 mM-K^+, 118 mM-Cl^- and 44 mM mannitol. The arrows indicate the reversal potentials for the H.A. under the two conditions of extracellular potassium concentration. Resting potentials at the indicated external potassium concentrations are indicated by the triangles. (Modified from Nelson *et al.* 1972; reproduced with permission.)

chelates Ca^{2+}) in the extracellular medium abolished both spontaneous and evoked H.A.s in macrophages. A23187 (an ionophore facilitating divalent ion penetration through membranes (Reed & Lardy, 1972)) by contrast, produced prolonged membrane hyperpolarizations. In this latter circumstance, if EGTA were added to the medium in the presence of the ionophore repolarization of the cell promptly occurred. Okada *et al.* (1978) also reported that external Ca removal abolished oscillating H.A.s in L-cells. Our results from experiments involving altered external Ca^{2+} ion concentrations, however, have been complex. We observed in L-cells that were transferred to and allowed to remain for some time in Ca-free, EGTA-containing medium before recording was begun that H.A.s appeared to be abolished. Occasionally, however, an H.A. could be elicited or even occurred spontaneously after several hours in Ca-free medium. Upon more careful examination of the results of these and other experiments we have been led to consider the release of intracellularly sequestered calcium as a source for the calcium involved in hyperpolarizing responses in L-cells (Henkart & Nelson, 1979). We summarize this evidence here.

(A) There is no electrophysiological evidence for obligatory Ca^{2+} ingress preceding the H.A. If an increase in Ca^{2+} permeability preceded the H.A. and Ca^{2+} ions entered

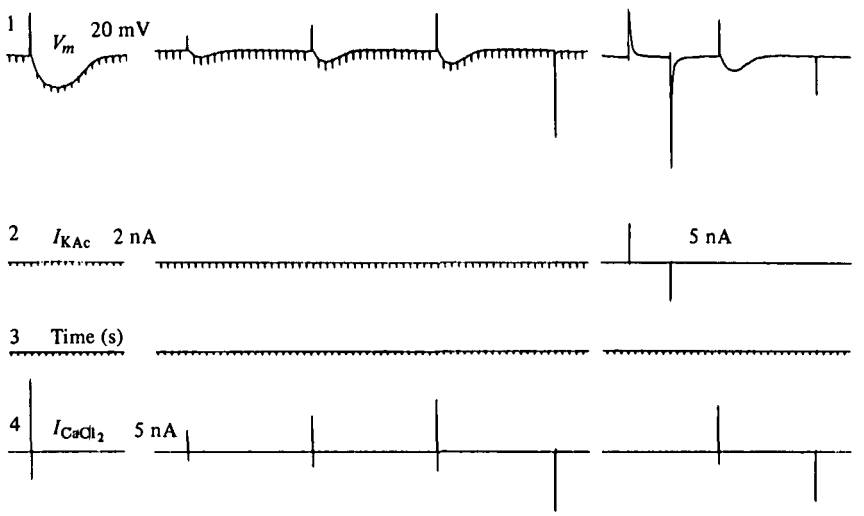

Fig. 4. Effect of calcium injection into L-cells. Two electrodes, one filled with 2 m-K acetate and one filled with 92 mM-$CaCl_2$, were inserted into a single L-cell. The four traces are simultaneous records. Line 1 (V_m) represents membrane potential recorded by the KAc pipette. Line 2 (I_{KAc}) is current passed through the KAc-filled pipette. Small constant current pulses are passed throughout most of the record from the KAc pipette to measure membrane resistance. Line 3 is a time marker, and Line 4 (I_{Ca}) is current passed through the $CaCl_2$ pipette. Outward current pulses (injecting Ca^{2+}) through the $CaCl_2$ pipette produce hyperpolarizations seen in trace 1. The amplitude of the hyperpolarizations is proportional to the size of the current pulses. Inward current pulses through the CaCl electrode did not produce any active membrane response. Comparable pulses of either polarity through the KAc pipette (end of trace 2) failed to elicit active hyperpolarizing responses. (Reproduced with permission from Henkart & Nelson, 1979.)

Table 1. *Effect of removing external calcium on* H.A. *responses (taken from Henkart & Nelson,* 1979)

Solutions	No. of cells	No. producing H.A.	Time at which last H.A. seen
2 Ca, 1 Mg	40	33 (83 %)	—
0 Ca, 3 Mg 0·3 Na, EGTA	71	38 (54 %)	115
0 Ca, 3 Mg 1·5 mM EGTA	16	10 (63 %)	90

the cell from the external medium, some depolarization of the membrane should be evident. Although this is occasionally seen in macrophages, megakaryocytes and L-cells, it is not generally seen in L-cells and is certainly not required preceding H.A. generation. An inhibitor of voltage-sensitive calcium channels, D-600, has no effect on the H.A.

(B) Cobalt ions block the H.A. response, but this requires rather high concentrations and prolonged incubations (e.g. 5 mM-Co^{2+} requires approximately 4 h to block). At a period of less than 1 h even in 15 mM cobalt, large H.A. responses were seen in some cells.

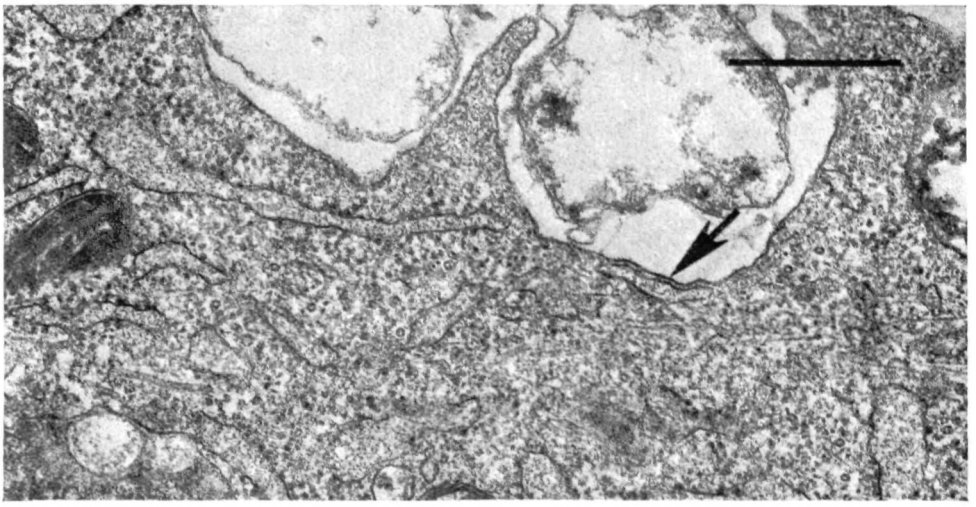

Fig. 5. Electron micrograph of an area of the surface of an L cell. The arrow indicates a subsurface cistern of the endoplasmic reticulum. The surface and outer ER membranes are parallel over a few tenths of a micrometre in this section. Serial sections show that the portion of ER in apposition to the surface membrane is continous with the long ribosome-bearing ER cistern to the left in the micrograph. The cell in this area appears to be engulfing a bit of membranous debris. ×45 000. The bar indicates 0·5 μm.

Table 2. *The effect of varying the external concentration of calcium on the amplitude of* H.A. *responses*

(Data represents means ± standard errors of the mean. Abstracted from Henkart & Nelson, 1979.)

Solution	No. of cells	V_m	$\Delta V_{H.A}$	R_{in}
2 mM-Ca^{2+} 1 mM-Mg^{2+}	7	15·6 ± 1·5	20·7 ± 4·2	34·7 ± 5·6
0 mM-Ca^{2+} 3 mM-Mg^{2+} 0·3 mM-Na EGTA	15	9·9 ± 0·5	15·0 ± 3·4	*21·5 ± 1·8
2 mM-Ca^{2+} 1 mM-Mg^{2+}	7	20·6 ± 1·6	25·1 ± 3·2	21·2 ± 4·1
10 mM-Ca^{2+}	8	22·3 ± 1·2	24·8 ± 3·9	*60·3 ± 15·7

* Shows values that are different from corresponding controls at the $p < 0.05$.

(C) Zero-Ca^{2+}, EGTA-containing solutions reduce the proportion of cells showing H.A. responses (Table 1), but in those cells showing responses, the amplitude of the responses is not significantly reduced (Table 2). We have observed large H.A. responses in L-cells as much as $1\frac{1}{3}$–2 h after switching cultures to balanced salt solutions containing no calcium and with 0·5–1·5 mM EGTA.

(D) The amplitude of the H.A. response is not increased in high (10 mM) Ca^{2+} solution (Table 2).

We feel that these results are incompatible with extracellular calcium as the sole or even predominant source for the calcium which generates the H.A. responses in L-cells. Parallel morphological studies revealed a possible basis for coupling between stimuli delivered to the surface membrane and a potential intracellular store of releasable calcium (Fig. 5). In L-cells the endoplasmic reticulum (ER) forms morphologically specialized appositions with the surface membrane which are similar to appositions between the sarcoplasmic reticulum and the transverse tubular system or surface membrane at diads or triads in striated muscle. In muscle these specialized appositions are thought to be the sites of coupling between the surface membrane and the intracellular calcium-releasing system involved in muscle contraction, the sarcoplasmic reticulum. Similar appositions between the ER and surface membrane have been described in neurones (Rosenbluth, 1962; Henkart, Landis & Reese, 1976) where the ER has also been shown to be a Ca-sequestering system (Henkart, Reese & Brinley, 1978).

Despite our conclusion that intracellular release of calcium is involved in the generation of the H.A. response it is clear that removal of extracellular Ca^{2+} has a strong impact on the H.A. mechanism as indicated by the decrease in the proportion of cells in which H.A.s can be evoked. We postulate that the functioning of the coupling mechanisms between the surface membrane and the internal calcium sequestering (and releasing) system may be dependent on or affected by extracellular Ca^{2+} and is eventually inactivated in the absence of extracellular Ca^{2+}. We propose the concept of 'permissive' extracellular calcium to describe this phenomenon. It should be noted that the possibility of such a situation implies that caution should be exercised in

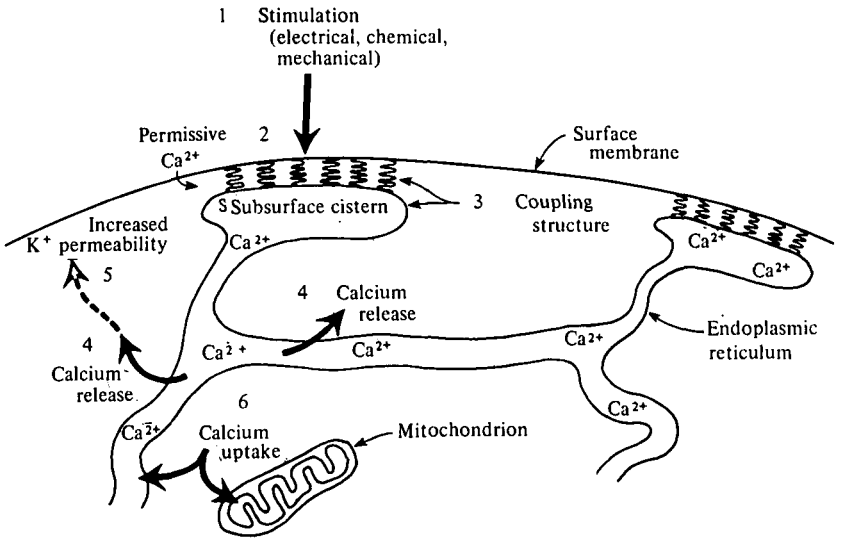

Fig. 6. Proposed model for the cellular events involved in the H.A. response and oscillatory membrane potential change in L-cells and macrophages. See text.

interpreting the results of experiments involving low Ca^{2+} solutions. Effects seen in such experiments may be due to the removal of Ca^{2+} as a charge-carrying species crossing the surface membrane, but we feel that possible effects of low Ca^{2+} solutions on the functioning of intracellular systems should be considered as well.

The combined functional and morphological observations described above have led us to suggest the model shown in Fig. 6. The proposed sequence of events involved in an elicited H.A. response are: (1) stimulation delivered to the surface membrane is coupled by some (2) 'permissive Ca^{2+}'-dependent step to (3) release of Ca^{2+} from the subsurface cisterns and/or (4) other regions of the endoplasmic reticulum. This released calcium affects (5) the surface membrane in such a manner as to increase its potassium permeability. The cycle is terminated (6) by Ca^{2+} removal from the cytoplasm by uptake into ER or mitochondria or extrusion through the surface membrane. This is analogous to the sequence of events in muscle where myofibril contraction is the major effect of Ca^{2+} released from the SR. We discuss below other possible physiological consequences.

Evidence supporting one aspect of the above model has been provided by studies of Ca^{2+} uptake in fibroblast microsomes (Hoffman-Berling, 1964; Moore & Pastan, 1977*a*, *b*). A non-mitrochondrial subcellular fraction, probably distinct from surface membrane vesicles is capable of accumulating calcium; the source of the microsomal fraction is probably ER membranes, which suggests that the ER system would be able to maintain an elevated Ca^{2+} concentration within its lumen.

III. OSCILLATORY PHENOMENA

As stated earlier, L-cells and macrophages may exhibit regular oscillations in membrane potential and conductance; the cycle of these oscillations resembles in most regards the H.A. response described above. (An interesting difference is the greater

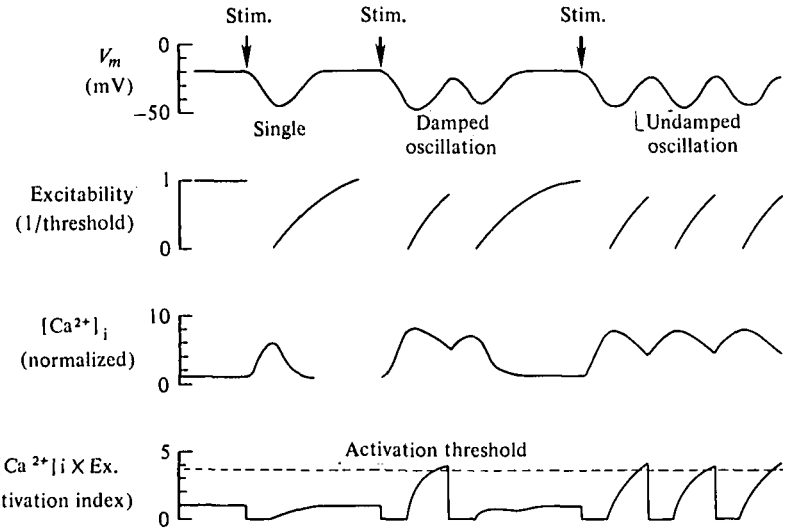

Fig. 7. Graphical diagram of hypothetical processes postulated to account for oscillatory behaviour in L cells. Excitability plots show the recovery of the capacity for an L cell to respond to a stimulus with a second H.A. cycle following the occurrence of an H.A. This would correspond to the reciprocal of the electrical current required to elicit the second response. $[Ca^{2+}]$ shows the proposed fold increase in intracellular calcium ion concentration that would occur to produce the increase in membrane potential and conductance that constitutes an H.A. response. The lowest graph shows the product of the two middle graphs and represents an index of the probability of occurrence of an H.A. response (the activation index). Thus, the product of excitability times the calcium level must exceed some level (the activation threshold) in order for an H.A. to occur. Different values for the rise and time course of cytoplasmic free calcium concentration and the recovery time course could give either single responses (first stimulus), damped oscillations (second stimulus) or undamped oscillations (third stimulus).

sensitivity of oscillations to metabolic inhibitors (Okada *et al.* 1977*a*).) What might be the basis for such oscillatory behaviour?

The most obvious possibility would assign the oscillatory mechanism to the surface membrane. One such hypothesis would be that a relatively high resting conductance to calcium at the low resting potential of mesenchymal cells would lead to the activation of the hyperpolarizing K^+ conductance; the hyperpolarization would inactivate the Ca^{2+} conductance and calcium extrusion would lead to a decrease in K^+ conductance, with consequent return to the resting membrane potential and reactivation of the Ca^{2+} conductance.

Given the evidence for the involvement of internal Ca^{2+} stores, however, we suggest an alternative or additional possibility for the generation of oscillatory behaviour, based on the parameters of calcium release and uptake by the intracellular system. We postulate that the probability of occurrence of an H.A. response (that is, the large-scale release of calcium by the ER) is a function of intracellular calcium concentration. A certain level of free cytosol calcium predisposes toward release of Ca^{2+} from the ER. (This is reminiscent of Ca release by Ca from muscle SR (Ford & Podolsky, 1970; Endo, Tanaka & Ogawa, 1970). Further, as noted, an increased threshold for eliciting the H.A. occurs following the occurrence of an H.A. (Nelson *et al.* 1972). These properties of the system, coupled with some finite time course of calcium removal after an H.A., would be expected to generate oscillatory behaviour given appropriate kinetic

values for the different processes. A schematic depiction of the system we postulate is shown in Fig. 7. It is interesting that in skinned muscle fibres, oscillatory contractions have been obtained by appropriate combinations of caffeine (affecting Ca^{2+} uptake by the SR) and cytoplasmic calcium and EGTA concentrations (Endo *et al.* 1970). In this situation the SR uptake and release kinetics are clearly the basis for the oscillatory phenomena. The observations that at least under some conditions calcium ions in the bathing medium can produce release of calcium from the SR, lends plausibility to our postulate (above) that certain cytosol Ca^{2+} concentrations may lead to further release of Ca^{2+} from the ER.

We have emphasized possible analogies between muscle and mesenchymal cells with reference to an intracellular compartment which sequesters calcium ions and which can be caused to release this calcium by stimuli delivered to the surface membrane. While the evidence, we feel, justifies such an anology, it should also be pointed out that there are a number of uncertainties connected with the analogy. As discussed earlier, the nature of the 'permissive' calcium is not well defined. Even though there are no apparent barriers to diffusion between the L-cell surfaces and the culture fluid (Henkart & Nelson, 1979), it may be that local pools of calcium near or adsorbed to the inner or outer surface of the membrane exist and their movement through the membrane or from the inner membrane surface into the cytoplasm can release intracellular calcium. A situation of this sort may occur in muscle, since Barrett & Barrett (1978) have shown that sufficiently high concentrations of EGTA can block excitation contraction coupling. It is also possible that the relative dominance of calcium entering from outside the cell and calcium released from intracellular sites may participate to various extents in different cell types in producing hyperpolarizing responses.

Our treatment of oscillatory phenomena implies that a regenerative calcium-releasing-calcium sequence contributes to oscillatory behaviour. With calcium injections, however, we have found an essentially graded relationship between the amount of calcium injected and the degree of hyperpolarization produced, rather than a threshold type of relationship. Clearly further work is required to clarify these basic issues of the cellular mechanisms involved in the hyperpolarizing response.

IV. PHYSIOLOGICAL SIGNIFICANCE OF THE HYPERPOLARIZING RESPONSES

In general terms, the electrophysiological evidence demonstrates a system which translates stimuli impinging on the surface membrane of mesenchymal cells into substantial changes in free cytoplasmic calcium concentrations. These changes could either be transient or, in the case of sustained oscillations, of longer duration. There are a number of possible functions that may, in turn, be controlled by this cytoplasmic calcium.

The importance of intracellular calcium for microtubule assembly (e.g. Schliwa, 1976) and contractile activity of actin and myosin (reviewed by Hitchcock, 1977) have been documented in a variety of cells. This suggests that control of cell shape and locomotory activity might be a function of an H.A.-generating mechanism. Consonant

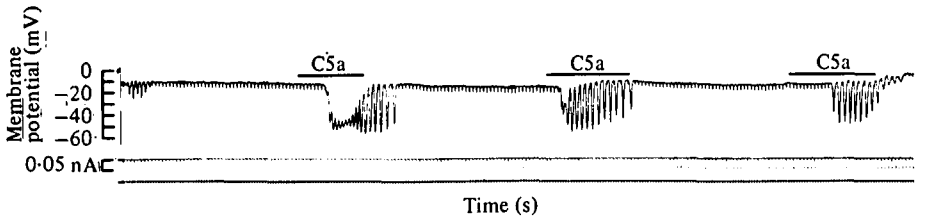

Fig. 8. Recording from a human macrophage exposed to a chemotactic factor, C5a, applied from a blunt microelectrode. Partially purified C5a, a cleavage product of the fifth component of complement from endotoxin activated human serum, was prepared as described by Gallin & Gallin (1977). Small current pulses of less than 0·5 nA used to measure membrane resistance are superimposed on the tracing. Repetitive oscillations follow the initial response to the application of C5a. The cell resting membrane potential = −11 mV. (Reproduced with permission from Gallin, Seligman & Gallin, 1979.)

with this suggestion are the observations of Gallin & Gallin (1977), who have shown that in human macrophages, endotoxin-activated serum produced more pronounced H.A. responses than did control serum and that this property of the activated sera was due to chemotactically active components in the sera (Fig. 8). They conclude that 'ion fluxes associated with membrane potential changes are early events in macrophage activation by chemotactic factors'. Thus the changes in calcium ion concentration which are manifested in the H.A. response may also play a critical role in the regulation of cell motility involved in chemotactic responses. Tsuchiya *et al.* (1978), in a preliminary communication, report that serum proteins can elicit H.A. responses and propose that the H.A. is a 'receptor potential which is closely related to cell membrane movement'. In support of this they also note that cytochalasin B, which inhibited the cell motility reversibly, inhibited hyperpolarizations in their strain of L-cells.

Megakaryocytes, as precursors to blood platelets, have been proposed to exhibit certain functional parallels with muscle cells. Chemical and mechanical stimuli to platelets may release calcium within the platelet which in turn produces contraction of thrombosthenin and platelet aggregation (Miller, Sheridan & White, 1978). The H.A. responses in megakaryocytes may reflect such Ca^{2+} release within the megakaryocyte, but detailed study of this system has not been published.

In addition to the large increase in cytosol calcium concentration, a corresponding transient decrease in Ca^{2+} concentration within the ER would be expected if release of calcium from the ER is involved in the H.A. response. Since processing of some newly synthesized proteins occurs through the ER, it seems plausible that alteration in calcium concentrations, particularly during repetitive stimuli or prolonged oscillations in this structure, might have some regulatory effect on protein translocation into the ER and, hence, on synthesis and secretion.

Finally, we would suggest that the cell biological processes revealed in the H.A. response in mesenchymal cells may have some counterpart in neurones. Increased potassium permeability is involved in a variety of post-activation hyperpolarizations and oscillatory or pacemaker potentials. Ca^{2+} ingress across the surface membrane is undoubtedly involved in many of these processes; subsurface cisterns are prominent in many neurones, however, and the ER has been shown to sequester Ca^{2+} in squid axon (Henkart *et al.* 1978) and in synaptic terminals (Blaustein *et al.* 1978; McGraw,

Somlyo & Blaustein, 1978). The possibility that this intracellular Ca^{2+} sequestering system is a dynamic one in neurones and is accessible to surface stimuli should be entertained. Intracellular events clearly can contribute dramatically to the regulation of surface membrane behaviour.

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