namely with identical enrichment of fractions containing synaptic membranes (*8, 17*). Finally the selective increase in cerebral enkephalinase activity after long-term administration of morphine (other enkephalin-hydrolyzing enzymes are not affected) could reflect the participation of the enzyme in the long-term control of enkephalinergic transmission (*3, 7*).

Enkephalinase inhibition in vivo should result in a facilitated enkephalinergic transmission reminiscent, for instance, of the enhanced cholinergic activity that follows administration of acetylcholinesterase inhibitors. In support of this hypothesis, it has recently been observed that thiorphan displays naloxone-sensitive antinociceptive activity in rats and mice (*13*).

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Calcium Spike Electrogenesis and Other Electrical Activity in Continuously Cultured Small Cell Carcinoma of the Lung

Abstract. *Spike electrogenesis, local depolarizing and hyperpolarizing responses, spontaneous rhythmic firing, and alternating resting potentials were measured in cells from a continuously cultured small cell carcinoma of the lung. Spike generation was blocked by $MnCl_2$. In view of this evidence for calcium-spike electrogenesis and previous evidence of secretory activity in these cells, this cell line (DMS 53) can provide a model for the study of excitation-secretion behavior in human neoplastic cells.*

Calcium-spike electrogenesis appears to be a widely occurring membrane-mediated process that participates in the initiation and regulation of excitation-secretion coupling. Endocrine and other secretory cells in both invertebrates and mammals generate Ca^{2+} spikes to signal the release of hormones and other chemical substances (*1–8*). We have studied cells of the highly malignant tumor known as small cell carcinoma of the lung (SCCL) because of their functional and growth characteristics (*9, 10*). These include the synthesis and secretion of estrogens and peptide hormones and the presence of cytoplasmic dense core vesicles, which have been associated with the synthesis and storage of peptide hormones. Although spike electrogenesis has been reported in cells isolated from a primary tumor SCCL (*11*), neither the ionic mechanism nor other electrical potentials were described. We have now determined the types of electrogenic activity that are present in long-term continuous cultures and report that extracellular calcium ions carry a major portion of depolarizing transmembrane current.

Table 1. Switching between two levels of membrane resting potential. Data were obtained during a prolonged impalement of DMS 53 cell 5/16/80D. The 12 observed epochs of decreased resting potential occurred as four discrete periods interspersed with periods of normal resting potential. *N* is the number of 150-msec observation epochs.

Resting potential (mV)	Class of resting potential measurement	
	Normal (N = 15)	Spontaneously decreased (N = 12)
Average	− 32.8	− 21.3
Median	− 32.0	− 19.2
S.D.	± 3.5	± 4.6

Cells of a continuous SCCL line (DMS 53) were trypsinized for subculture with 0.25 percent trypsin in calcium- and magnesium-free Hanks balanced salt solution (*9*). The cells (2.5×10^6) were plated in 35-mm Falcon plastic petri dishes in 5 ml of Waymouth's MB 752/1 medium containing 20 percent fetal calf serum and 25 m*M* Hepes buffer. Cultures were examined 4 days after plating; at this time the monolayer was approximately half confluent. The culture dish was mounted on a temperature-controlled (35 ± 1°C) stage of a Zeiss inverted phase-contrast microscope. Microelectrodes, filled with 3*M* KCl and having resistances of 45 to 55 megohms, were coupled to a bridge circuit to allow intracellular injection of constant-current pulses. Recordings of transmembrane potential and injected current were obtained from a cathode-ray oscilloscope with a Polaroid camera or were directly led into an Able-40 (New England Digital) computer for analysis. Software for real-time data acquisition and analysis were developed specifically for this project.

Resting potentials recorded in these cells displayed two significant characteristics: (i) relatively low values and (ii) a switching back and forth between two values (Table 1). There is a tendency to dismiss low-voltage membrane poten-

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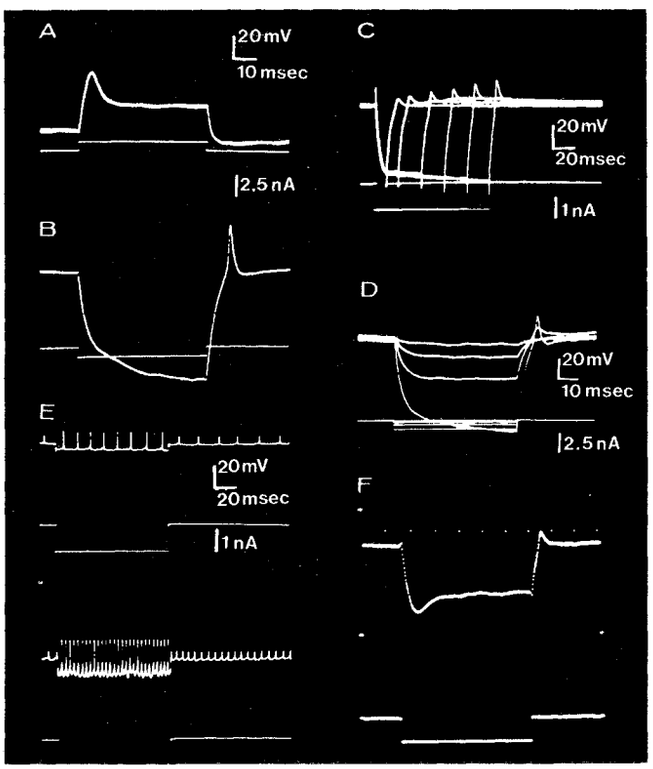
tials on the basis of ionic leak resulting from membrane injury from the impalement, but the persistence of the maintained potential differences and the high input impedances (>250 megohms) suggest that these cells do maintain only a low-voltage transmembrane potential. Similar low-voltage potentials have been reported for various cells in culture, particularly secretory cells (*1–4*, *12*). In some experiments, the resting potential abruptly changed from about −32 mV to −20 mV and then just as abruptly returned to the original level (*13*). Such switching behavior has been reported in cultured macrophages (*12*).

Depolarizing pulses of constant current evoked local potentials (Fig. 1A) followed by steady-state plateaus (*14*). Overshooting action potentials were elicited by anodal break excitation (Fig. 1B). The amplitude and rate of rise of the spikes were directly related to the duration (Fig. 1C) and intensity (Fig. 1D) of the stimulus pulse. Latency and overshoot of the active response varied independently.

The spike response was followed by a brief phase of hyperpolarization (Fig. 1, B–D). Postspike hyperpolarization has been studied in a number of mammalian neurons where it has been related to an electrogenic ion pump, to prolonged potassium conductance, or to a combination of these factors (*15*, *16*). A phase of hyperpolarization, seen as an undershoot, occurs when injected depolarizing current is switched off (Fig. 1A). The mechanism and significance of this response after hyperpolarization in the SCCL are unknown. Another class of responses sometimes observed during injection of hyperpolarizing current occurs as an early change in hyperpolarizing potential in response to the hyperpolarizing current pulse (Figs. 1F and 2A).

Spontaneous firing was encountered in a cell that, despite a low (−15 mV) resting potential, produced rhythmic (50 Hz), small (10 mV) potentials (Fig. 1E, upper trace) that increased both in amplitude (40 mV) and frequency (320 Hz) when a hyperpolarizing current was injected (Fig. 1E, lower trace). The frequency of firing was greater after such pulsing. Although repetitive firing could be a "rebound response" to hyperpolarization, this cell was firing before currents were injected. These frequencies are much faster than those reported in mammalian endocrine cells (*4*) or in cultured macrophages (*12*). Unlike cells that function as pacemakers, in which hyper-



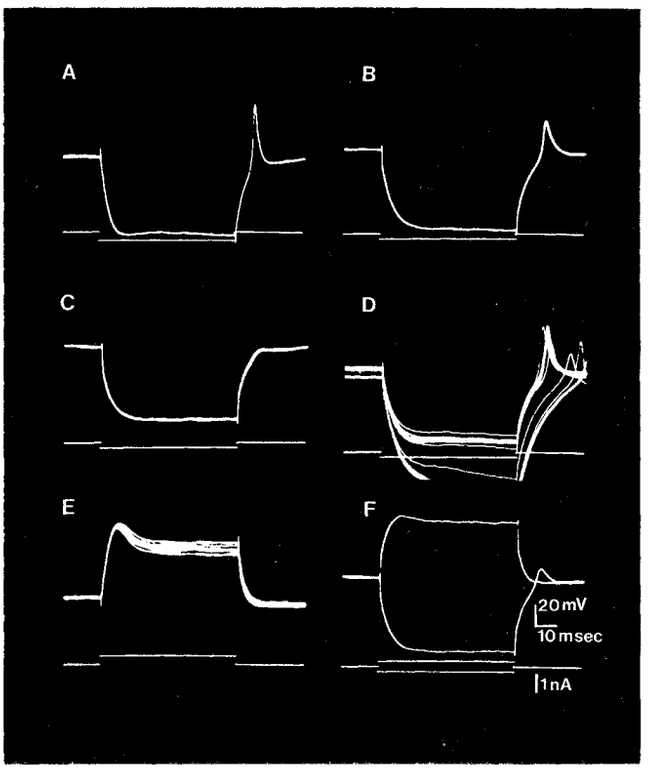


Fig. 1 (left). Responses of DMS 53 cells to injected current pulses. In each record, intracellular potentials are displayed on the upper trace, and injected current is shown on the lower trace. Resting potential in (A) to (D) was −41 mV. (A) Local response to a depolarizing current pulse. Rates of rise and fall for the local membrane response were +13.5 and −13.9 V/sec, respectively. (B) A spike triggered by anodal break excitation after injection of a hyperpolarizing current pulse. Rates of rise and fall for the anodal break spike were +67.1 and −24.7 V/sec, respectively. (C) Positive relation between the amplitude of the active membrane response to anodal break and increasing stimulus duration. The rate of rise during the active response increased with its magnitude. (D) Positive relation between amplitude of anodal break response and magnitude of hyperpolarizing current pulse. As before, the rate of rise is positively related to the magnitude of the stimulus. The maximum dV/dt during the active responses of these cells ranged from 0 to +67 V/sec. (E) Spontaneous rhythmic firing in a DMS 53 cell. In the upper record, an injected hyperpolarizing current produced an accelerated discharge rate and an increased amplitude of potentials. In the lower record, the normal discharge rate was greater than before, and again acceleration and potentiation were seen in response to injection of a hyperpolarizing current. (F) Nonovershooting anodal break response in a cell with low resting potential. The dotted line indicates 0 mV; in the left margin, the upper mark is at + 30 mV and the lower mark is at − 100 mV. Current injection was − 0.52 nA. These two traces were displayed data from an experiment recorded by computer on a flexible disk. Successive points represent measurements taken every 200 μsec. Fig. 2 (right). Effects of 4 m*M* $MnCl_2$ on active membrane responses to injected current, displayed as in Fig. 1. (A) Anodal break spike before manganese was added to the bath. (B) Five seconds after adding the $MnCl_2$, the anodal break response was delayed and diminished. (C) Ten seconds after adding $MnCl_2$, the spike was abolished. (D) Successive sweeps show recovery of spike after its abolition by $MnCl_2$. (E) The local response to depolarizing current included an invariant early potential followed by a variable repolarization. (F) In the presence of $MnCl_2$, the repolarization component of the local response was abolished.

polarization slows the inherent rate of discharge, this cell increased its rate of spontaneous firing when it was hyperpolarized. Such behavior seems paradoxical but may be related to excessive inactivation of inward current at a low resting potential, which is then relieved by a hyperpolarizing pulse.

Spike electrogenesis was blocked by the addition of 4 m*M* $MnCl_2$ to the bath (Fig. 2, A–C), suggesting that these action potentials are Ca^{2+} spikes. Calcium spike electrogenesis appears to be a frequent correlate in cells that exhibit secretory behavior, cells as diverse as *Aplysia* bag cells (*5*) and rat pituitary cells (*1*). A gradual inactivation of the repolarizing current after a local depolarizing response (Fig. 2E) appeared when a depolarizing pulse of constant current and constant duration was repeated at a rate of 1 Hz. The depolarizing local potential elicited by the first pulse repolarized rapidly, and then the slope of the repolarization phase diminished stepwise with each of about six pulses, after which the repolarization phase became extinguished. The repolarization phase could be reactivated either by conditioning pulses of hyperpolarizing current or by a rest period. The process of inactivation of the repolarization phase was greatly accelerated in the presence of $MnCl_2$ (Fig. 2F). This suggests that a Ca^{2+}-activated K^+ gate may be involved in this process (*17*) or that the local response consists of a fast and a slow potential, the latter being dependent on readily available calcium. These two time-related phenomena—the switching of membrane potential and the inactivation of repolarization of the local response—may be examples of a dynamic electrophysiological state that exists in certain cells (*12*).

Although spike electrogenesis was long held to be peculiar to nerves and muscle, new investigations reveal the presence of electrogenesis in cells that are clearly not nerve or muscle. Our findings demonstrate the presence of a mechanism for spike generation, implicate calcium ions as the major charge carrier, and provide evidence for other forms of electrical activity in these lung tumor cells.

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13. The mean ± standard deviation (S.D.) of the input resistance (R_i) at the original membrane potential was 242 ± 155 megohms, whereas that of the switched membrane potential was 209 ± 167 (Table 1). In view of these data, we propose that switching is not the result of injury or conductance changes but may be due to a metabolic mechanism.
14. The steady-state current-voltage curves were linear in response to anodal pulses but exhibited delayed rectification in response to cathodal pulses. Test current pulses ranged from −3 nA to +3nA.
15. The postspike hyperpolarization was not voltage-dependent but rather was cell-specific, occurring in about one of every four cells examined.
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Divalent Cation Ionophores Stimulate Resorption and Inhibit DNA Synthesis in Cultured Fetal Rat Bone

Abstract. *Two divalent cation ionophores, A23187 and Ionomycin, which are selective for calcium, stimulated the resorption of fetal rat long bones in organ culture at 0.1 to 1 micromolar but not at higher concentrations. Both agents inhibited DNA synthesis at concentrations that stimulated resorption. These results might explain the differences in ionophore effects on bone previously reported, and they imply that cell replication is not required for osteoclast formation in fetal rat long bone cultures.*

Dziak and Stern (*1*) have shown that, like parathyroid hormone (PTH), the divalent cation ionophore A23187 stimulates resorption in cultured fetal rat long bones, enhances intracellular calcium entry, and increases adenosine 3′,5′-monophosphate (cyclic AMP) in cells isolated from fetal rat calvaria. Dietrich and Paddock (*2*) found that both A23187 and PTH inhibited the incorporation of tritiated proline into collagen in cultured newborn rat calvaria. However, in cultured newborn mouse calvaria, Ivey *et al.* (*3*) found that A23187 acts only as an inhibitor of bone resorption and has no effects on the accumulation of cyclic AMP in the medium. Differences between the action of PTH and A23187 have also been described in the rat. Hahn *et al.* (*4*) showed that calcitonin did not inhibit resorption stimulated by A23187 in fetal rat long bones, while Dietrich and Paddock (*2*) found that despite the similarities of their effects on collagen synthesis in fetal rat calvaria, A23187, unlike PTH, inhibited noncollagen protein synthesis and incorporation of tritiated thymidine into DNA. We have used 19-day fetal rat long bone cultures to compare the effects of PTH, A23187, and Ionomycin (*5*). Ionomycin, which is a divalent cation polyether ionophore previously untested on bone, is more selective for calcium than is A23187.

Bone resorption was assessed by measuring the release of previously incorporated ^{45}Ca (*6*). DNA and protein synthesis were assessed by measuring the incorporation of tritiated thymidine and tritiated amino acids, respectively. Long bone shafts labeled in utero with ^{45}Ca were cultured in 0.5 ml of modified BGJ medium (Gibco) supplemented with 5 percent fetal calf serum (Gibco) that was treated with dextran-coated charcoal to reduce endogenous bone resorbing activity (*7*). During the last 2 hours of culture, 1.5 μCi of tritiated thymidine or tritiated amino acid mixture (New England Nuclear) was added to each dish. At the end of the experiments bones were extracted in 5 percent trichloroacetic acid (TCA). The radioactivity from the ^{45}Ca was counted in the medium and TCA extract; the ^{3}H was counted in the TCA extracts and the bones after they were digested with NCS (Amersham). The ^{3}H counts were divided by the total ^{45}Ca counts of each bone to normalize for differences in fetal bone size. In most experiments, bones from the fetuses of a single litter were used to minimize variation caused by any nonuniformity of ^{45}Ca labeling. In some experiments the activity of the lysosomal enzyme β-glucuronidase was measured in the medium (*8*). For histologic studies the bones were placed in Millonig's fixative for 2 to 4 days and then dehydrated and embedded in meth-

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