Voltage-dependent Ion Channels in Small-Cell Lung Cancer Cells

Joseph J. Pancrazio, Michael P. Viglione, Imad A. Tabbara, and Yong I. Kim

Departments of Biomedical Engineering [J. J. P., Y. I. K.], Neurology [M. P. V., Y. I. K.], and Internal Medicine [I. A. T.], University of Virginia Health Sciences Center, Charlottesville, Virginia 22908

ABSTRACT

Small-cell carcinoma of the lung is a highly lethal form of cancer associated with a wide variety of paraneoplastic syndromes. Using the patch-clamp technique, we have directly demonstrated the presence of voltage-gated K⁺, Na⁺, and Ca²⁺ channels in three cell lines of human small-cell carcinoma, NCI-H128, NCI-H69, and NCI-H146. Whole-cell currents were measured from the tumor cells held at −80 mV and depolarized to −60 to +120 mV. Outward K⁺ current (Iₖ), which was found in every cell tested, reached 1.58 ± 0.12 nA (mean ± SE, n = 24 cells) for H128 cells and 2.14 ± 0.18 nA (n = 41) for H69 cells in response to a test potential of +80 mV. Unlike H69 and H128 tumor cells, Iₖ from H146 cells occasionally exhibited partial inactivation during the 60-ms pulse length and reached 0.94 ± 0.15 nA (n = 18) in response to a +80 mV test potential. Iₖ from each of the cell lines was significantly reduced by 4-aminopyridine and tetraethylammonium. The rapidly inactivating inward Na⁺ current (I₅), recorded in H146 cells and about 30% of the H69 and H128 cells tested, demonstrated a peak amplitude of 58 ± 6 pA (n = 11) at 0 mV and a reversal potential of 47 ± 2 mV (n = 11). Externally applied tetrodotoxin quickly suppressed I₅. For the H128 and H69 tumor cells, inward Ca²⁺ current (I₉), observed in about 25% of the cells exposed to 10 mM Ca²⁺, peaked at 2.5 ± 0.4 ms (n = 5) with an amplitude of 46 ± 14 pA (n = 5) at +20 mV and partially inactivated over the 40-ms depolarization. In H128 cells exposed to isotonic Ba²⁺ (110 mM), inward currents with time courses similar to those of I₉ were recorded. Nearly all H146 tumor cells demonstrated a significant inward Ca²⁺ current which peaked with an amplitude of 93 ± 16 pA (n = 26) at +30 to +40 mV in the presence of 10 mM Ca²⁺. Application of test potentials 2 s in duration revealed that H146 I₉ inactivated in a voltage-dependent manner with a time constant on the order of seconds. Adjustment of the holding potential from −80 mV to −40 mV had no observable effect on the amplitude of the evoked current.

These voltage-dependent ion channels may have integral roles in several small-cell carcinoma bioelectric phenomena, including secretion, resting membrane potential, and action potential generation. Furthermore, a current hypothesis concerning the autoimmune etiology of Lambert-Eaton syndrome suggests that small-cell carcinoma tumor cells possess Ca²⁺ channels which may serve as the antigenic stimulus for the production of these autoantibodies. Our finding is consistent with this hypothesis.

INTRODUCTION

Nearly 25% of all pulmonary cancer cases are classified as small cell carcinoma of the lung (1). Although of indefinite origin (2), SCCL³ is considered a neuroendocrine-like tumor of the amine precursor uptake and decarboxylation series. SCCL is the most commonly encountered form of cancer in paraneoplastic neurological disorders (3). Of particular interest is the autoimmune disorder Lambert-Eaton syndrome characterized by a deficient presynaptic release of acetylcholine at the neuromuscular junction (4, 5). Nearly 70% of LES cases are associated with SCCL (6). LES autoantibodies have been shown to inhibit the function of voltage-dependent Ca²⁺ channels (7–9). This finding raises the question of whether the putative Ca²⁺ channels in SCC cells serve as the antigenic stimulus for the production of autoantibodies which destructively cross-react with presynaptic voltage-sensitive Ca²⁺ channels and thereby impair neuromuscular transmission.

SCC cells possess dense core granules (10) and secrete a vast array of ectopic hormones in a calcium-dependent manner (11). SCC cells are known to demonstrate properties indicative of membrane excitability (2, 12, 13). Specifically, these tumor cells exhibit calcium spike electrogensis (12) and membrane potential "switching" (2). Although some of these bioelectric phenomena have been examined with intracellular recording, there is a paucity of information regarding the types and biophysical characteristics of the SCC ion channels governing secretion, resting membrane potential, and action potential generation.

The objective of our study was to identify the major voltage-dependent ionic currents present in SCC cells. We utilized the whole-cell patch-clamp technique (14) to demonstrate the presence of voltage-gated Ca²⁺, Na⁺, and K⁺ currents in three established SCC cell lines. A preliminary account of this work has appeared previously (15).

MATERIALS AND METHODS

Cell Culture. The well-characterized human SCC cell lines NCI-H69 and NCI-H146 were obtained from American Type Culture Collection (Rockville, MD). The human SCC cell line NCI-H146 was generously provided by A. Gazdar and H. Oie of the National Cancer Institute (Bethesda, MD). NCI-H69 and NCI-H128 were maintained in RPMI 1640 with 10% heat-inactivated fetal calf serum (Gibco, Grand Island, NY). NCI-H146 was also maintained in RPMI 1640 but with 5% heat-inactivated fetal calf serum. Flasks containing the cells were incubated at 37°C in 5% CO₂ and air. Cultures were subcultured once per week by mechanical dissociation. In order to immobilize the tumor cells for each experiment, the cells were affixed to glass coverslips coated with poly-l-lysine.

Solutions. The compositions of the external and internal (inside the patch pipette) solutions used in the patch-clamp experiments are listed in Table 1. Solutions A, B, and C were used within the patch pipette, whereas solutions D, E, F, G, and H were used externally. All solutions were adjusted to pH 7.2 with NaOH, with the exception of solution G which was adjusted to pH 7.3 with tetraethylammonium hydroxide. Every solution was filtered through a 0.2-μm Millipore filter before use.

Whole-Cell Patch-Clamp Recording. Fig. 1 illustrates the procedure for establishing the WCR configuration. WCR allows voltage-clamp measurements from small diameter cells with excellent signal-to-noise ratio (16). Recordings were made at room temperature (22°C) with standard patch-clamp techniques (14) 1 min after establishing the WRC configuration by using a List EPC-7 patch-clamp amplifier (List Electronic, Darmstadt, Federal Republic of Germany). Patch electrodes, fashioned from Kimax capillary tubes (outer diameter, 1.5–1.8 mm), were coated with Sylgard-184 resin to reduce capacitance. Patch electrode tips were heat polished by using the List L/M-CPZ-101 (List Electronic) pipet forging system.

Ensemble Fluctuation Analysis. Ensemble fluctuation analysis (17, 18) of K⁺ channels was also performed to approximate single channel conductance and channel density. A linear least-squares fit of the
In order to record the inward Na⁺ current, solutions A and E were used. To isolate the outward K⁺ current, the internal and external solutions were B and F. To assess the inward Ca²⁺ current, solutions A and D were used. When Ba²⁺ replaced Ca²⁺ as a charge carrier to measure Ba²⁺ inward current, solutions C, G, and H were used.

Table 1  Internal (A, B, C) and external (D, E, F, G, and H) solutions used to measure whole-cell currents in SCC cells

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td></td>
<td></td>
<td>130</td>
<td>140</td>
<td>140</td>
<td>130</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KCl</td>
<td></td>
<td>129</td>
<td></td>
<td></td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CaCl₂</td>
<td>1</td>
<td></td>
<td></td>
<td>2.8</td>
<td>2.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MgCl₂</td>
<td>1</td>
<td>10</td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATP</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>120</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CoCl₂</td>
<td></td>
<td></td>
<td>130</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CoCl₃</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BaCl₂</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>110</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>EGTA (NaOH)⁴⁺</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EGTA (KOH)⁴⁻</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EGTA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TEA-Cl</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TTX</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HEPES (NaOH)⁴⁺</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HEPES (KOH)⁴⁻</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HEPES</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10</td>
</tr>
</tbody>
</table>

* EGTA, ethylendiglycol bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid; HEPES, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid.
* A stock solution containing EGTA (0.1 m) and NaOH (0.22 m) was initially prepared. The contribution of NaOH to test solution A was 24.2 mM.
* A stock solution containing EGTA (0.1 m) and KOH (0.22 m) was initially prepared. The contribution of NaOH to test solution B was 24.2 mM.
* A stock solution containing HEPES (1 m) and NaOH (0.27 m) was initially prepared. The contribution of NaOH to test solutions A, D, E, and F was 2.7 mM.
* A stock solution containing HEPES (1 m) and NaOH (0.25 m) was initially prepared. The contribution of NaOH to test solution B was 2.5 mM.

RESULTS

To date we have observed three different voltage-gated ion currents in the SCC cells, those carried by K⁺, Na⁺, and Ca²⁺. All three cell lines could be subjected to whole-cell current measurement with relative ease. For most of the cells tested, seal resistance reached the gigaohm level. The size of SCC cells varied widely in each cell line; the H69 and H128 whole-cell inward currents, larger (higher capacitance) cells were preferred when assessing Na⁺ and Ca²⁺ currents. Statistical significance of a drug treatment was evaluated by Student’s t test. Values of P < 0.01 were considered to be significant.
quantitative analysis of any bioelectrical parameters.

K⁺ Current. The slowly activating outward current, recorded in the presence of up to 5 μM TTX, was readily apparent in every cell tested. In H69 and H128 cells this current did not show signs of inactivation over the 60-ms depolarization (Fig. 2A) and persisted even 20 min after initiating WCR. Although all of the H146 cells tested exhibited a prominent outward current, inactivation was occasionally observed during the 60-ms test pulse. Furthermore, as depicted in Fig. 3, the form and time course of the outward current varied among different H146 cells.

Several lines of evidence suggest that the outward current observed from all three cell lines was carried mainly by K⁺ ions and that the putative contribution of Cl⁻ to this current was not significant. Firstly, the outward current (Iₒ) was not apparent after internal application of TEA and CsCl. Secondly, Iₒ exhibited sensitivity to 4-AP added to the external bath, which resulted in a 60% reduction in the current amplitude from all three cell lines. For a step potential to +80 mV, 5 mM 4-AP significantly diminished (P < 0.01) H128 Iₒ from 178 ± 28 pA/pF (mean ± SE, n = 9 cells) to 66 ± 4 pA/pF (n = 8). The effect of 4-AP was also observed on a single cell during WCR. Fig. 2B depicts Iₒ evoked by a test potential of +80 mV before and 18 min after addition of 5 mM 4-AP to the bath solution, where Iₒ is shown to be reduced by approximately 60%. Thirdly, the combination of externally applied TEA (30 mM) and 4-AP (2 mM) diminished Iₒ by more than 90%.

As illustrated in Fig. 2C and Fig. 3, Iₒ increased in response to larger step potentials. Occasionally, saturation was present at potentials larger than +90 mV. The conductance of Iₒ, Gₒ, was estimated:

\[ Gₒ = Iₒ/(V - Eₒ) \]

where Iₒ is the peak current, V is the magnitude of the test potential, and Eₒ is the equilibrium potential. Eₒ, calculated by using the Nernst equation, with [K⁺]ᵲ = 155.7 mM and [K⁺]ᵰ = 2.8 mM, was -101 mV, Gₒ(V) and V were fitted to the Boltzmann function:

\[ Gₒ(V)/Gₒ,max = [1 + exp((V - Vₜ)/kₜ)]^{-1} \]

where Gₒ,max is maximal conductance, Vₜ is the potential at which Gₒ is half-maximal, and kₜ is the slope factor. Neither Vₜ nor kₜ was significantly different among the three SCC cell lines. Overall, Vₜ was 27.6 ± 1.4 mV (n = 40) and kₜ was -19.3 ± 0.6 mV (n = 40).

The H69 tumor cell line seemed to possess a larger Iₒ than the H128 and H146 cell lines. Peak Iₒ, in response to a +80 mV test potential, reached 244 ± 16 pA/pF (n = 41) for the H69 tumor cells, but only 160 ± 14 pA/pF (n = 24) for the H128 tumor cells and only 103 ± 14 pA/pF (n = 18) for the H146 tumor cells. Mean cell membrane capacitance was 8.7 ± 0.4 pF (n = 41), 10.6 ± 0.7 pF (n = 24), and 9.3 ± 0.7 pF (n = 18) for H69, H128, and H146 tumor cells, respectively.

Kinetic analysis of the H69 and H128 Iₒ was performed at different depolarizing potentials. H146 Iₒ was not included in this analysis due to the variable nature of this current. The time-dependent potassium conductance, Gₒ(t), was fitted during the activation phase of the H69 and H128 current with a Hodgkin-Huxley parameter (19) by using a nonlinear least-squares method:

\[ Gₒ(t) = Gₒ,n(t)^p \]

where Gₒ,n is the maximal K⁺ conductance for a particular voltage, n(t) is the activation parameter, and p is the activation exponent. Fits with p = 1, 2, and 4 were attempted for each current record. At all potentials, Gₒ(t) was best fitted with p = 1. The activation time constant, rₚ, was sensitive to voltage (Fig. 4); rₚ decreased from 15.9 ± 1.0 ms (n = 17) at 0 mV to 5.2 ± 0.5 ms (n = 18) at +80 mV.

Ensemble fluctuation analysis was used to examine the single-channel events underlying H128 Iₒ. For a step potential to +30 mV, single-channel current and channel density were estimated to be 1.4 ± 0.3 pA (n = 5) and 63 ± 14 channels/pF (n = 5), respectively. Mean cell membrane capacitance was 10.4 ± 1.5 pF (n = 5).

Na⁺ Current. The rapidly activating inward current, illustrated in Fig. 5A, was recorded after suppressing any Ca²⁺ current with Ca²⁺-free buffer and externally applied Cd²⁺ (1 mM). In addition, Iₒ was blocked with internally perfused CsCl (120 mM) and TEA (20 mM). This inward current was observed in H146 cells and in about 30% of H69 and H128 cells (n = 61) tested. The following observations indicate that the predominant charge carrier for this current is Na⁺. Firstly, within the range of test potentials used (−60 to +90 mV), activation of this current (Iₒ,n) occurred within 0.4–2.0 ms followed by complete inactivation within 1–30 ms at the different depolarizations. Secondly, with more positive test potentials, Iₒ,n reversed its polarity as the voltage passed the equilibrium potential for
The absolute voltage values are given for each trace; \( C^\prime = 15.0 \) pF. B, pharmacological sensitivity of \( I_Na \) to TTX. \( I_Na \) was completely suppressed 6 min after application of TTX. Peak \( I_Na \) abolished /Na. C, \( I-V \) relationship for peak \( I_Na \). Voltage pulses ranged from \(-60 \) mV to +90 mV; \( C^\prime = 22.2 \) pF. TTX quickly abolished /Na. \( I_Na \) evoked by a test potential to 0 mV before holding potential of -80 mV. A, \( I_Na \) was elicited in response to five test potentials. External and internal pipette solutions used and stimulating voltage protocols were the same as in Fig. 2. Inset, time course of \( I_Na \) from a single cell occurring at low (-21 and -11 mV) and high (+52 and +101 mV) depolarizing voltages; calibration bars, 10 ms, 0.6 nA. The time constant of activation, \( \tau_a \), was best fitted by a single exponential function to the activation phase of \( I_Na \). Mean ± SE for data obtained from 17-18 cells (NCI-H128) are shown.

\[ \text{Na}^+ \text{, } E_{Na} \text{. The experimental } E_{Na} \text{ agreed reasonably well with the theoretical } E_{Na} \text{ predicted by the Nernst equation, with } [\text{Na}^+]_i = 26.9 \text{ mM and } [\text{Na}^+]_o = 142.7 \text{ mM, was } 42.2 \text{ mV. Experimentally, } E_{Na} \text{ was estimated to be } 47 \pm 2 \text{ mV ( } n = 11 \text{).} \]

Thirdly, addition of TTX to the external bath abolished /Na. \( C^\prime = 22.2 \) pF. TTX quickly abolished /Na. \( I_Na \) was completely suppressed 6 min after application of TTX. Peak \( I_{Na} \) versus the applied voltage is shown in Fig. 5C. The \( I_{Na} \) peak, which was largest in response to a depolarization to -10 to 0 mV, reached 3.2 ± 0.4 pA/pF (\( n = 11 \)). Mean cell membrane capacitance was 19.0 ± 1.7 pF (\( n = 11 \)).

The time to peak, \( t_p \), and inactivation time constant, \( \tau_i \), were calculated for \( Na^+ \) currents elicited by test potentials from -30 to +30 mV. To quantify \( \tau_i \), the inactivation phase of \( I_{Na} \) was fitted to a single exponential decay function. Both \( t_p \) and \( \tau_i \) demonstrated considerable voltage sensitivity (Fig. 6). As the test potential grew larger, \( t_p \) fell from 2.06 ± 0.07 ms (\( n = 10 \)) at -30 mV to 0.44 ± 0.01 ms (\( n = 11 \)) at +30 mV. Likewise, \( \tau_i \) decreased from 10.3 ± 1.9 ms (\( n = 4 \)) at -30 mV to 0.37 ± 0.04 ms (\( n = 5 \)) at +30 mV.

**Ca**^2+ Current in H128 and H69 Tumor Cells. A second inward current was identified with 10 mM Ca**2+** (Fig. 7A) or 110 mM Ba**2+** (Fig. 7B) in the external medium after both \( I_Ca \) and \( I_{Na} \) were blocked. This current, which activated significantly slower than \( I_{Na} \) and expressed a steady-state component, was observed in about 25% of the 42 cells tested. Two observations suggest that this second inward current was largely carried by Ca**2+** ions. Firstly, isotonic barium, highly permeant through voltage-sensitive Ca**2+** channels, produced a profound inward current (Fig. 7B), similar in form and time course as the current (\( I_{Ca} \)) measured with 10 mM Ca**2+** buffer. Secondly, in the presence of external cobalt (1 mM) and no Ca**2+** in the external bath, this inward current was not observed (\( n = 48 \)).

Both steady-state and peak \( I_{Ca} \) were maximal in response to a depolarization to +10 to +20 mV and reversed at approximately +40 to +50 mV. The inward \( I_{Ca} \) peaked and partially inactivated over the 40-ms pulse duration. Fig. 7C illustrates the voltage dependence of both peak and steady-state \( I_{Ca} \). Peak \( I_{Ca} \) and steady-state \( I_{Ca} \) were measured to be 1.7 ± 0.2 pA/pF (\( n = 5 \)) and 0.9 ± 0.1 pA/pF (\( n = 5 \)), respectively. Mean cell membrane capacitance was 31.6 ± 10.5 pF (\( n = 5 \)). In addition, the time to peak (\( t_p \)) was determined for \( I_{Ca} \) at step potentials ranging from -30 mV to +30 mV; \( t_p \) decreased from 14.1 ± 1.1 ms (\( n = 5 \)) at -30 mV to 4.5 ± 0.2 ms (\( n = 4 \)) at +30 mV.

**Ca**^2+ Current in H146 Tumor Cells. After \( I_{Na} \) and \( I_{Ca} \) were suppressed, nearly all H146 cells (23 of 26 cells tested) readily demonstrated a profound inward current (Fig. 8A). This inward current exhibited pharmacological sensitivity to externally applied Co**2+** (1 mM) and Ba**2+** (Fig. 8B), similar to the H69 and H128 \( I_{Ca} \), suggesting that this current is also carried by Ca**2+** ions.

Unlike the H69 and H128 \( I_{Ca} \), the H146 inward current often reaches a steady-state amplitude with little inactivation over the 40-ms depolarization. Fig. 8C shows the voltage dependence of the H146 \( I_{Ca} \); peak \( I_{Ca} \) evoked by test pulses of +30 to +40 mV; \( C^\prime = 22.2 \) pF. TTX quickly abolished \( I_{Na} \) at potentials ranging from -30 mV to +30 mV; \( t_p \) decreased from 14.1 ± 1.1 ms (\( n = 5 \)) at -30 mV to 4.5 ± 0.2 ms (\( n = 4 \)) at +30 mV.

**Ca**^2+ Current in H128 and H69 Tumor Cells. A second inward current was identified with 10 mM Ca**2+** (Fig. 7A) or 110 mM Ba**2+** (Fig. 7B) in the external medium after both \( I_Ca \) and \( I_{Na} \) were blocked. This current, which activated significantly slower than \( I_{Na} \) and expressed a steady-state component, was observed in about 25% of the 42 cells tested. Two observations suggest that this second inward current was largely carried by Ca**2+** ions. Firstly, isotonic barium, highly permeant through voltage-sensitive Ca**2+** channels, produced a profound inward current (Fig. 7B), similar in form and time course as the current (\( I_{Ca} \)) measured with 10 mM Ca**2+** buffer. Secondly, in the presence of external cobalt (1 mM) and no Ca**2+** in the external bath, this inward current was not observed (\( n = 48 \)).

Both steady-state and peak \( I_{Ca} \) were maximal in response to a depolarization to +10 to +20 mV and reversed at approximately +40 to +50 mV. The inward \( I_{Ca} \) peaked and partially inactivated over the 40-ms pulse duration. Fig. 7C illustrates the voltage dependence of both peak and steady-state \( I_{Ca} \). Peak \( I_{Ca} \) and steady-state \( I_{Ca} \) were measured to be 1.7 ± 0.2 pA/pF (\( n = 5 \)) and 0.9 ± 0.1 pA/pF (\( n = 5 \)), respectively. Mean cell membrane capacitance was 31.6 ± 10.5 pF (\( n = 5 \)). In addition, the time to peak (\( t_p \)) was determined for \( I_{Ca} \) at step potentials ranging from -30 mV to +30 mV; \( t_p \) decreased from 14.1 ± 1.1 ms (\( n = 5 \)) at -30 mV to 4.5 ± 0.2 ms (\( n = 4 \)) at +30 mV.

**Ca**^2+ Current in H146 Tumor Cells. After \( I_{Na} \) and \( I_{Ca} \) were suppressed, nearly all H146 cells (23 of 26 cells tested) readily demonstrated a profound inward current (Fig. 8A). This inward current exhibited pharmacological sensitivity to externally applied Co**2+** (1 mM) and Ba**2+** (Fig. 8B), similar to the H69 and H128 \( I_{Ca} \), suggesting that this current is also carried by Ca**2+** ions.

Unlike the H69 and H128 \( I_{Ca} \), the H146 inward current often reaches a steady-state amplitude with little inactivation over the 40-ms depolarization. Fig. 8C shows the voltage dependence of the H146 \( I_{Ca} \); peak \( I_{Ca} \) evoked by test pulses of +30 to +40 mV; \( C^\prime = 22.2 \) pF. TTX quickly abolished \( I_{Na} \) at potentials ranging from -30 mV to +30 mV; \( t_p \) decreased from 14.1 ± 1.1 ms (\( n = 5 \)) at -30 mV to 4.5 ± 0.2 ms (\( n = 4 \)) at +30 mV.**
**K⁺, Na⁺, AND Ca²⁺ CHANNELS IN LUNG CANCER CELLS**

Fig. 7. Voltage-activated calcium currents (I₉Ca) measured from SCC cells (NCI-H128). Depolarizing voltage pulses of 40 ms in duration were applied every 2 s from a holding potential of −80 mV. A, I₉Ca was elicited by five test pulses whose absolute voltage is given in each record, Cm = 28.3 pF. External and internal pipette solutions used were A and D (see Table 1). B, current through voltage-dependent Ca²⁺ channels with Ba²⁺ (110 mM) as the charge carrier. External and internal pipette solutions used were C and G (see Table 1). Ba²⁺ currents are shown for five test potentials as indicated; Cm = 31.6 pF. C, I-V relationships for peak and steady-state I₉Ca. Voltage pulses ranged from −60 to +90 mV; Cm = 64.5 pF.

Fig. 8. Voltage-activated calcium currents (I₉Ca) measured from H146 cells. Depolarizing voltage pulses of 40 ms in duration were applied every 2 s from a holding potential of −80 mV. A, I₉Ca was elicited by five test pulses whose absolute voltage is given in each record, Cm = 7.97 pF. External and internal pipette solutions used were A and D (see Table 1). B, current through voltage-dependent Ca²⁺ channels with Ba²⁺ (10 mM) as the charge carrier. External and internal pipette solutions used were C and H (see Table 1). Ba²⁺ currents are shown for five test potentials as indicated; Cm = 10.1 pF. C, I-V relationships for steady-state I₉Ca from the cell shown in A; voltage pulses ranged from −60 to +90 mV.

DISCUSSION

The main objective of these experiments was to identify the major voltage-gated currents present in SCC cells. We have observed three currents, I₉K, INa, and I₉Ca at voltages ranging from −60 to +120 mV. These currents were distinguished by their different time courses, voltage dependence, and pharmacological sensitivity to agents such as TEA, 4-AP, TTX, Ba²⁺, and Co²⁺.

Although all tumor cells exhibited a prominent I₉K, not all H69 and H128 cells demonstrated measurable inward currents. There are three possible explanations for this apparent absence: (a) SCC tumor cells may only express inward current channels during a fraction of their life cycle; (b) channels may be expressed by only the “most neuroendocrine” members of the cell population; and (c) some tumor cells may express inward current channels in such a low density that WCR is not sufficient to measure the current. In fact, we often selected larger, higher capacitance, cells to observe any inward current more readily.

Voltage-dependent Potassium Current. I₉K in H69 and H128 tumor cells, like other delayed rectifiers, demonstrated: (a) sensitivity to both 4-AP and TEA; (b) a voltage-dependent time constant of activation; and (c) little inactivation over the duration of the test potential. The time course of activation for SCC tumor cell I₉K was best fitted by a single exponential, although multi-exponential fits are more prevalent in the “classic” delayed rectifier currents (20–22). Similar findings have been reported in rat type II alveolar epithelial cells (23) and human T-lymphocytes (24). The ensemble variance estimate of single-channel current was comparable to those reported for the K⁺ channels at the frog node of Ranvier (25) and the “slow kinetics"
K+ channels of the mouse neuroblastoma cells (26).

Similar to H69 and H128 Ica, the H146 outward current was sensitive to 4-AP and TEA. However, SCC cells appear to express K+ currents which may be heterogeneous between different cell lines as well as among cells in a single cell line. Although H69 and H128 Ica was consistent from cell to cell, the time course of H146 Ica was quite variable. Possibly, H146 Ica may be carried by multiple K+ channel types expressed in proportions that vary between cells.

There is one notable difference between SCC cell Ica and the classic delayed rectifier currents found in the squid axon (20), and striated muscle fiber (21, 22). SCC cell Ica seemed to activate at more positive potentials. The voltage at which Gca was half-maximal, V½, was about +28 mV for SCC cells; however, V½ of the classic delayed rectifier currents are substantially lower, ranging from ~20 mV (21) to ~35 mV (22).

Voltage-dependent Sodium Current. When present, Ica in SCC cells exhibited activation and inactivation within milliseconds and complete suppression after addition of TTX to the bath. In addition, the time to peak, tpe, and inactivation time constant, τinac, for SCC Ica were comparable to those of chick dorsal root ganglion cells (27), bovine chromaffin cells (28), and bovine lactotrophs (29).

Voltage-dependent Calcium Current. SCC cells, like many secretory cells, may generate Ca2+ spikes which are presumed to initiate the release of hormones and peptides (12). When present, H69 and H128 Ica showed voltage-dependent activation kinetics and partial inactivation over the duration of the 40-ms test potential. In contrast, H146 Ica demonstrated no inactivation over the 40-ms depolarization.

The types of Ca2+ channels underlying Ica remain to be determined. In our study, H69 and H128 Ica resembled the form and the time course of the GH3 clonal pituitary cell Ca2+ current (30), leading to the speculation that SCC cells may possess T- and L-type Ca2+ channels (31). Two findings suggest that the H146 Ica is largely carried by L-type Ca2+ channels: (a) H146 Ica inactivates with a voltage-dependent time constant ranging from 20 to 2 s comparable to the L-type current found in chick dorsal root ganglion cells (32); and (b) in several cells, H146 Ica was not inactivated by adjusting the holding potential from ~80 to ~40 mV (32, 33). Past pharmacological studies have consistently implied the presence of L-type Ca2+ channels in SCC tumor cells. Roberts et al. (7) showed that the dihydropyridine nifedipine, an L-type Ca2+ channel antagonist, reduced K+ stimulated Ca2+ uptake in SCC tumor cells. Furthermore, a recent study by De Alzpurua et al. (34) indicated that SCC depolarization-dependent Ca2+ is inhibited by both ω-conotoxin and adrenaline. Their results indicate that in addition to “L-like” channels, “N-like” channels may also be present in SCC tumor cells.

In conclusion, this is the first direct demonstration of voltage-dependent ion currents in SCC cells. These findings establish the presence of at least three voltage-gated ion channel types, K+, Na+, and Ca2+. These ion channels may have integral roles in several SCC cellular phenomena such as secretion, resting membrane potential, and action potential generation. Most notably, our finding of the voltage-sensitive 7Ca is consistent with the hypothesis that the triggering factor in the production of the LES autoantibodies may be the SCC voltage-gated Ca2+ channels.

REFERENCES
Voltage-dependent Ion Channels in Small-Cell Lung Cancer Cells


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/49/21/5901

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.