Thermosensitivity of the Membrane Potential of Normal and Simian Virus 40-transformed Hamster Lymphocytes

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ABSTRACT

The effects of temperature in the fever range (37°–42°) on the membrane potentials of normal and simian virus 40-transformed hamster lymphocytes were analyzed. The transmembrane distributions of radiolabeled triphenylmethylphosphonium and thiocyanate were measured, and they provided upper and lower limits for the normal cell membrane potential at 37° of −48 ± 6 (S.D.) and −31 ± 5 mV and for the tumor cells, −36 ± 4 and −19 ± 2 mV. The mitochondrial contribution to the triphenylmethylphosphonium-measured membrane potential, 5 to 10 mV for both splenocytes and simian virus 40-transformed lymphocytes, was estimated by utilizing antimycin A and carbonylcyanide-m-chlorophenylhydrazone to inhibit generation of a mitochondrial membrane potential.

Incubation for 1 to 2 hr at 38-42° resulted in a 6- to 15-mV depolarization of normal cells and a 2- to 6-mV hyperpolarization of tumor cells. Both depolarization and hyperpolarization were fully reversible by subsequent incubation at 37° and insensitive to antimycin A and carbonyl-cyanide-m-chlorophenylhydrazone.

The membrane potential of normal splenocytes when measured with triphenylmethylphosphonium at 37° was depolarized by 35% with 1 mM ouabain and thermally induced depolarization was blocked. The membrane potential of tumor cells at 37° was insensitive to ouabain; however, the hyperpolarization at 40° was inhibited. The membrane potential of normal lymphocytes stimulated with phytohemagglutinin was depolarized relative to that of nonstimulated control cells and assumed the thermal response characteristics of tumor cells.

INTRODUCTION

Most cancer chemotherapeutic drugs act intracellularly, but depend on either passive membrane permeability or specific transport processes for access to their sites of action. Experimentation has shown that treatment of cells with membrane-active agents such as dimethyl sulfoxide (23) or the detergent Tween 80 (33) enhances the cytotoxicity of antitumor drugs, presumably by increasing membrane permeability. Perhaps of more clinical relevance is the proposal that membrane permeability and thus drug cytotoxicity can be enhanced with thermotherapy in the fever range (17).

A structural-functional property of cell membranes that reflects their permeability to different solutes is the transmembrane electrochemical potential. The magnitude of the cell membrane potential is a function of ion gradients, the relative permeabilities of cations and anions and the activities of enzymatic ion pumps (e.g., Na⁺-K⁺-ATPase). The energy derived from the membrane potential is used in part by cells to drive solute uptake (e.g., amino acids, 18). Moreover, the accumulation of drugs such as melphalan which are transported by energy-requiring processes (3, 43) may also be dependent on the electrochemical potential.

Previous studies from our laboratory have shown that the membrane potential of human erythrocytes is sensitive to temperatures in the fever range (27–29°). With erythrocytes starved of glucose, there is an abrupt decrease in membrane potential centered at 38–39° which is reversible up to 41° and irreversible at higher temperatures. With erythrocytes supplemented with glucose, the thermal transition is centered at 41° and is completely reversible up to the highest temperature tested, 45°. These thermally induced changes in erythrocyte membrane potential can be correlated with alterations in membrane structure including the unfolding of membrane proteins that occur within the fever temperature range (4, 5, 19, 37, 42).

For small cells such as lymphocytes, electrophysiological methods for measuring membrane potential are difficult to apply (e.g., Refs. 30 and 41). However, measurement of the transmembrane distributions of radiolabeled lipophilic cations and anions has been successfully utilized with bacterial membrane vesicles (35), isolated mitochondria (2), and nucleated cells (e.g., Refs. 3 and 24) including lymphocytes (14, 21). The membrane potential at 37° is calculated from cation or anion transmembrane distributions according to the equation

\[ E_m (mV) = -61 \log \frac{[C]}{[C_0]} = -61 \log \frac{[A]}{[A_0]} \]  

where \( E_m \) is the membrane potential in mV, \([C]\) and \([C_0]\) are intracellular and extracellular cation concentrations, and \([A]\) and \([A_0]\) are extracellular and intracellular anion concentrations.

Deutsch et al. (14), in studies of human peripheral lymphocytes, demonstrate that the transmembrane distributions of the cation probe, TPMP, and the anion probe, SCN, provide, respectively, upper and lower limit estimates for the membrane potential. For cation probes, both intracellular compartmentation (e.g., mitochondria) and probe binding contribute to an overestimate of the cell membrane potential. When corrections for these factors are applied (zero voltage at high extracellular K⁺), agreement between electrophysiological and ion distribution methods are achieved (13, 24). For anionic probes such as SCN, intracellular compartmentation is minimal, since SCN is excluded from the intracellular space at negative membrane potentials (in contrast to enhanced cation probe uptake; cf. Equation A). For this reason, however, anion probes may be less sensitive to changes in membrane potential. When the change in equilibrium distribution of SCN between low- and
high-K⁺ medium (to correct for probe binding at zero membrane potential) has been used to calculate the membrane potential for surface-attached mouse neuroblastoma cells, a value of −48 mV is obtained that is consistent with −41 mV measured electrophysiologically (8, 23).

Here, we present our initial studies comparing the membrane potential thermal sensitivities of normal and SV40-transformed hamster lymphocytes. To measure the membrane potential of these cells, we have determined the transmembrane distributions of TPMP and SCN.

MATERIALS AND METHODS

Materials

Reagents and their respective suppliers are: ³H₂O, [³H]polyethylene glycol, and [³H]TPMP bromide, New England Nuclear, Boston, Mass.; sodium [¹⁴C]SCN, ICN Pharmaceuticals, Irvine, Calif.; sodium tetraethylboron, Aldrich Chemical Co., Milwaukee, Wis.; PHA, Wellcome, Great Britain; Dulbecco’s phosphate-buffered saline with Ca²⁺ and Mg²⁺, McCoy’s 5A culture medium, and fetal calf serum, Grand Island Biological Co., Grand Island, N. Y.; wheat germ agglutinin, Miles Laboratories, Inc., Elkhart, Ind.; fluorescein-labeled rabbit anti-hamster IgG, Cappel Laboratories, Cochranton, Pa.; ouabain, Sigma Chemical Co., St. Louis, Mo.; and silicone oil SF1250, General Electric Co., West Point, N. Y.

SV40-transformed GD248 cells (15) are propagated aseptically as s.c. tumors in 6- to 8-week old outbred male Syrian hamsters (Lakeview Hamster Colony, Newfield, N. J.). Normal lymphocytes are obtained from the spleens of hamsters of the same age. Hamsters are maintained at 22°C on a 12-hr dark-light cycle of 6 a.m. to 6 p.m. (light) and 6 p.m. to 6 a.m. (dark).

Methods

Cell Isolation. Hamsters are sacrificed between 8 and 9 a.m. Anesthetized hamsters are killed by heart puncture exsanguination, and tumors or spleen are removed. The tumors are freed of necrotic and hemorrhagic tissue, cut into small pieces, and minced in Dulbecco’s phosphate-buffered saline with Ca²⁺ and Mg²⁺. McCoy’s 5A culture medium, and fetal calf serum, Grand Island Biological Co., St. Louis, Mo., and silicone oil SF1250, General Electric Co., West Point, N. Y.

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Cell Cultures. Both normal splenocytes and GD248 lymphocytes are cultured at a cell density of 10⁶ cells/ml in McCoy’s 5A culture medium plus 10% fetal calf serum. Maximal PHA stimulation of splenocytes as defined by [³H]thymidine incorporation into trichloroacetic acid-precipitable material is obtained with PHA (2 µg/ml) after 72 hr of culture. Cell viability after culture for 72 hr with or without PHA was greater than 80% as determined by dye exclusion.

Membrane Potential Measurements. The transmembrane potential is determined with [³H]TPMP and [¹⁴C]SCN essentially as described by Deutsch et al. (14). GD248 or normal splenocytes (1 to 2 × 10⁶ cells/ml) are incubated in 500 µl McCoy’s medium plus 10% fetal calf serum containing 1 µCi [³H]TPMP (or [¹⁴C]SCN). At designated times, 200-µl aliquots are withdrawn and immediately pipetted into polypropylene microcentrifuge tubes containing silicone oil (200 µl) overlaying 10% formic acid (50 µl). After centrifugation for 1 to 2 min at 12,000 × g in a Beckman microfuge, an aliquot of the supernatant is removed to determine extracellular TPMP concentration, and the remaining supernatant and silicone oil are aspirated off. The tip of the centrifuge tube containing the cell pellet is cut off directly into a scintillation vial. Radioactivity is determined with a Packard 3000 scintillation counter with National Diagnostics Hydrofluor as scintillation fluid.

Simultaneously, cell volume measurements are performed with [³H]polyethylene glycol (M.W. 4000; 1 µCi/10⁷ cells) as marker for trapped extracellular space marker and [³H₂O (1 µCi/10⁷ cells) for total pellet volume. As has been reported (14, 46), trapped extracellular space is approximately 25% of total pellet volume for 10⁷ normal or transformed cells and does not vary over the temperature range 25–43°C.

The final concentration of ethanol used as solvent for TPMP and polyethylene glycol did not exceed 0.2% (v/v). Temperature of all incubations is maintained at the set temperature ±0.01°C (S.D.) with Lauda K4r incubators. Careful control of temperature and other experimental variables allows for a maximum standard deviation of ±1 mV for triplicate samples within any one particular cell preparation.

RESULTS

Core Body Temperature of Hamsters. To provide a set point for judging the physiological significance of temperature-induced changes in cell membrane potential, we determined the core body temperatures of both normal and GD248-infected hamsters. At the time of sacrifice, the body temperature of normal hamsters measured with a rectal thermometer averages 35.4 ± 0.5°C. Body temperature of GD248-infected animals at time of sacrifice is 36 ± 0.6°C.
Cell Water Volume of Splenocytes and GD248 Lymphocytes. The average cell water volume for normal splenocytes (7 cell preparations) over the temperature range of 32–41° is 2.1 ± 0.4 μl/10^7 cells. At the highest temperature used (43°), cell volume increases slightly (15%). At temperatures lower than 32°, cell water volumes decreases to a minimum of 1.5 ± 0.25 μl/10^7 cells at 24°.

With GD248 cells, average cell volume calculated for 6 cell preparations is 3.5 ± 0.5 μl/10^7 cells and is independent over the temperature range of 24–43°. The qualitative difference in average cell volumes of the transformed and normal cells is also readily apparent by light microscopy.

Transmembrane Electrical Potential. A time course for uptake of TPMP by GD248 cells and normal splenocytes at 37° is shown in Chart 1. For both cell types, uptake is slow with final equilibrium values being achieved after 40 min incubation. During initial experiments, we attempted to accelerate TPMP uptake by the addition of low concentrations of tetraphenyl boron (10^-6 to 10^-5 M) as described by Deutsch et al. (14). Contrary to their observations, we found that tetraphenyl boron increases not only the rate of TPMP equilibration but also the final TPMP concentration equilibrium value. Moreover, in the presence of tetraphenyl boron and at temperatures greater than 37°, cell death is appreciable with both normal and GD248 cells. Reasons for the discrepancy between our results and those of Deutsch et al. (14) are not apparent, other than the use of different cells and incubation medium. In the experiments to be described, tetraphenyl boron is not included during the incubations.

Also apparent in Chart 1 is the relative difference between TPMP-measured membrane potentials of normal splenocytes and GD248 tumor cells at 37°. The membrane potential averaged over 7 splenocyte cell preparations is -48 ± 6 mV. For 5 tumor cell preparations, the average membrane potential is -36 ± 4 mV.

The membrane potential can also be calculated from [14C]-SCN distribution experiments. Uptake of SCN by splenocytes and GD248 lymphocytes is biphasic with initial uptake characterized by first-order kinetics and a later phase exhibiting zero order kinetics (data not shown). Similar uptake kinetics is observed with human peripheral lymphocytes by Deutsch et al. (14). These authors suggest that the zero order process reflects metabolism of SCN and intracellular accumulation of metabolized SCN (e.g., SO_3^-; Ref. 44). Utilizing this latter assumption and subtracting the zero order contribution, we calculate membrane potentials of -31 ± 5 mV for splenocytes (3 cell preparations) and -19 ± 2 mV for GD248 lymphocytes (2 cell preparations). As in the TPMP experiments, the membrane potential of tumor lymphocytes is lower than that of normal splenocytes.

Temperature Sensitivity of the Membrane Potential. A thermal titration curve from 24–42° for GD248 lymphocytes membrane potential is shown in Chart 2 with TPMP as probe. Over this temperature range, there is an increase in the membrane potential of GD248 cells with the largest increase occurring at 24–34°. At temperatures greater than 37°, a slight hyperpolarization (2 to 6 mV) is observed with all tumor cell preparations. When cells are incubated at temperatures equal to or higher than 43°, large variability in measured membrane potential between different cell preparations is found. However, at these elevated temperatures, cell viability is reduced by at least 40% as judged by trypan blue dye exclusion and [14C]-thymidine incorporation during cell culture. Chart 2, inset, displays an Arrhenius plot which summarizes the thermal titration of the GD248 lymphocyte membrane potential.

A thermal titration curve for normal splenocytes in which 3 phases can be distinguished is shown in Chart 3. Over the temperature range of 24–30° a hyperpolarization is observed that parallels that found with GD248 lymphocytes and at higher temperatures (30–37°), the membrane potential remains relatively constant. As the temperature is elevated into the fever range (>37°), a marked depolarization of the cell membrane potential occurs in contrast to the hyperpolarization associated with tumor cells in this temperature region. Temperatures greater than 42° are lethal.

The extent of depolarization induced by temperatures above 37° varies between different cell preparations. The range of values for net depolarization induced by shifting temperature from 37 to 41° for 5 normal cell preparations is 6 to 15 mV with TPMP as membrane potential probe.

Similar results are obtained in the fever range with SCN as membrane potential probe (Chart 4). Both the hyperpolarization transition of tumor cells and the depolarization of normal cells...
commence at temperatures within the fever range. For 5 normal cell preparations, a net depolarization upon shifting temperature from 37 to 41 ° varied between 5 and 20 mV. Thermally induced hyperpolarization of GD248 cells varied between 3 and 11 mV (5 cell preparations).

In the experiments described in Charts 2 to 4, cells are incubated for 1 hr at the designated temperatures. As seen in Chart 5, the depolarization at 41 ° with splenocytes can also be observed within 40 min incubation as a reduced TPMP uptake, and is maintained for at least 120 min of incubation at 41 °. The effect of elevated temperatures on the splenocyte membrane potential probably occurs before 40 min but is obscured by the enhanced diffusion rate of TPMP at these higher temperatures. For similar reasons, it is not possible with the present methodology to determine the onset of hyperpolarization of the tumor cell membrane. However, the hyperpolarization is sustained for at least 2 hr at 41 ° (data not shown).

An additional feature of the membrane potential thermosensitivity of normal and SV40-transformed cells which we investigated is the reversibility of both the depolarization and the hyperpolarization processes. As seen in Table 1, a 1-hr 37 ° incubation, following a 1-hr 41 ° incubation, reverses the temperature-induced hyperpolarization of the tumor cell membrane. Under similar conditions, a 41 °-induced depolarization of splenocytes is also reversed by subsequent 37 ° incubation.

**Effect of Temperature on T- and B-Cells.** The GD248 lymphocyte is of B-cell origin on the basis of cell surface IgG (9), whereas the spleen is composed of approximately equivalent amounts of T- and B-lymphocytes. Thus, to make a fair comparison between tumor and normal cells, we fractionated splenocytes into T- and B-cell-enriched cell populations by the method of Bourguinon et al. (9). As shown in Table 2, the membrane potentials of T- and B-lymphocytes are both thermosensitive, exhibiting equivalent depolarizations as the temperature is raised from 37 to 41 °.

**Effect of Temperature on the Membrane Potential of Splenocytes with and without PHA.** Table 3 summarizes the results from 4 experiments where the thermosensitivity of the membrane potential of splenocytes cultured for 72 hr in the presence and absence of PHA is analyzed. Cells stimulated to divide with PHA are characterized by a low membrane potential at both 37 and 41 °. Importantly, the membrane potential of cells cultured with PHA are no longer depolarized in the fever range and, in fact, a slight hyperpolarization is observed. With 4 different cell preparations, the hyperpolarization upon raising the temperature from 37 ° to 41 ° varied between 4 and 20 mV.

Tissue culture of tumor cells with or without PHA did not alter the thermal response of the membrane potential; i.e., hyperpolarization is observed at temperatures above 37 ° with or without PHA.

**Effect of Ouabain on Thermosensitivity of the Lymphocyte Membrane Potential.** Chart 6 presents data concerning the effects of ouabain, an inhibitor of Na+-K+-ATPase, on the thermosensitivity of the membrane potential studied. With normal splenocytes, the addition of 1 mM ouabain reduces membrane potential at 37 ° from −48.9 mV to −31.8 mV and abolishes the thermally induced depolarization. In contrast, the membrane potential of GD248 lymphocytes is insensitive to 1 mM ouabain at 37 °. At temperatures of 40 ° or greater, the membrane potential is decreased approximately 30% by 1 mM ouabain.

**Intracellular Compartmentation and Binding of Membrane Potential Probes.** Although the ouabain experiments suggest that the membrane potential thermal responses of GD248 cells and splenocytes originate at the plasma membrane, information on the mitochondrial contributions to membrane potential estimates and thermosensitivities is lacking. We have attempted...
Membrane Potential Thermosensitivity

Table 1
Reversibility of thermal transitions of normal and GD248 cells

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Membrane potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GD248</td>
<td></td>
</tr>
<tr>
<td>37° (1 hr)</td>
<td>-34.2 ± 0.6*</td>
</tr>
<tr>
<td>37° (2 hr)</td>
<td>-35.1 ± 0.3</td>
</tr>
<tr>
<td>41° (1 hr)</td>
<td>-39.1 ± 0.9</td>
</tr>
<tr>
<td>41° (2 hr)</td>
<td>-40.5 ± 0.7</td>
</tr>
<tr>
<td>41° (1 hr) to 37° (1 hr)</td>
<td>-35.5 ± 0.2</td>
</tr>
<tr>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>37° (1 hr)</td>
<td>-49.6 ± 1.0</td>
</tr>
<tr>
<td>37° (2 hr)</td>
<td>-49.5 ± 0.5</td>
</tr>
<tr>
<td>41° (1 hr)</td>
<td>-37.8 ± 0.5</td>
</tr>
<tr>
<td>41° (2 hr)</td>
<td>-39.0 ± 0.6</td>
</tr>
<tr>
<td>41° (1 hr) to 37° (1 hr)</td>
<td>-50.1 ± 0.3</td>
</tr>
</tbody>
</table>

* Mean ± S.D.

Table 2
Thermosensitivity of membrane potential of splenocytes and fractionated T- and B-cells

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Membrane potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of capping with anti-IgG</td>
<td>37°</td>
</tr>
<tr>
<td>Control</td>
<td>25</td>
</tr>
<tr>
<td>T-enriched</td>
<td>4</td>
</tr>
<tr>
<td>B-enriched</td>
<td>46</td>
</tr>
</tbody>
</table>

* Mean ± S.D.

to account for probe compartmentation and binding by establishing a transmembrane potential of zero volts and utilizing inhibitors of mitochondrial metabolism.

One approach to generate a transmembrane potential of zero volts is to reduce the K+ diffusion potential by substituting extracellular K+ for Na+. In experiments with splenocytes, this proved unsuccessful, since cell viability at 37° or 40° was less than 50% at extracellular K+ concentrations greater than 100 mM (Table 4). The residual membrane potential observed with SCN (~7.0 mV) at high extracellular K+ is probably a consequence of electrogenic ion pumps or other ion diffusion potentials. The difference (11 mV) between membrane potentials at 140 mM K+ measured with TPMP and SCN results from mitochondrial TPMP uptake and TPMP binding. Parallel experiments at 40° and elevated K+ levels proved unsuccessful due to low cell viability at these conditions.

Mitochondrial respiration inhibitors (e.g., antimycin A) or uncouplers (e.g., CCCP) can be used to measure mitochondrial TPMP uptake in intact cells if mitochondrial-derived energy is unnecessary for maintenance of plasma membrane ion gradients. Philo and Eddy (31) have demonstrated that Na+ gradient-dependent methionine transport and the Na+ and K+ levels of mouse ascites tumor cells are maintained under conditions of maximal respiration inhibition with 2.5 μM antimycin A. In experiments with splenocytes or GD248 cells, we cannot demonstrate swelling of either cell type in the presence of 5 μM antimycin A or 1 μM CCCP, suggesting no alterations in transmembrane Na+ or K+ gradients. In contrast, osmotic swelling of GD248 cells and splenocytes was observed at extracellular K+ concentrations greater than 20 mM (See also Ref. 14).

Control experiments with GD248 cells and splenocytes indicate maximal inhibition of O2 consumption at 5 μM antimycin A (>90%) and maximal stimulation of cell oxygen uptake at 1 μM CCCP. With 3 GD248 and 2 splenocyte preparations, decrements of 5 to 10 mV in TPMP-measured membrane potential were observed at 37° and 41° with either antimycin A or CCCP. No effect of these metabolic inhibitors on thermosensitive membrane potential transitions was demonstrable.

DISCUSSION

An assumption common to many investigations of cancer thermoderapy utilizing laboratory animals and cells isolated therefrom is that the animal core body temperature is 37°. However, a large body of information from work with pyrogens is available, indicating that the core body temperature is variable depending on the time of day, amount of handling, animal's past history (raised indoors or out), environmental temperature and body mass (e.g., Refs. 20, 26, and 40). In general, rodents maintained at normal laboratory temperatures of 25° or below
Complete depolarization with SCN as probe was not observed, GD248 at 37°. Maximal depolarization with either TPMP or estimates of probe binding and compartmentation. An important conclusion from their work is the necessity associated with ion distribution methods, including intracellular discrepancy between SCN- and TPMP-measured membrane potentials measured for lymphocytes with microelectrodes for potentials measured for experimental animals may be important.

A critical question for evaluating our experimental results is whether the transmembrane distributions of TPMP and SCN accurately report the membrane potential across the cell plasma membrane. This is of particular importance since the range of values that we obtain for normal splenocytes (∼31 to −48 mV with SCN and TPMP, respectively) is higher than that for potentials measured for lymphocytes with microelectrodes (−11 to −25 mV; Refs. 30 and 41), and there is an apparent discrepancy between SCN- and TPMP-measured membrane potentials. Deutsch et al. (14) have discussed the problems associated with ion distribution methods, including intracellular probe binding and compartmentation as they apply to lymphocytes. An important conclusion from their work is the necessity to use both cation and anion membrane potential probes to provide upper and lower limits to the membrane potential and estimates of probe binding and compartmentation.

In our studies, we have attempted to reconcile the differences between TPMP- and SCN-determined membrane potentials by reducing the K⁺ diffusion potential at high extracellular K⁺. As noted in "Results," this approach was applicable only to GD248 at 37°. Maximal depolarization with either TPMP or SCN was observed as K⁺ concentrations greater than 100 mM. Complete depolarization with SCN as probe was not observed, suggesting the presence of electrogenic ion pumps or other ion diffusion potentials. The 11-mV differential between TPMP- and SCN-measured membrane potential at high extracellular K⁺ does account for the different membrane potentials for GD248 cells with TPMP and SCN and results from intracellular compartmentation and binding of TPMP.

For lymphocytes, TPMP uptake by the nuclear and mitochondrial compartments requires consideration. We note, as have Deutsch et al., that although lymphocytes are characterized by a large nuclear-cytoplasm volume ratio the transnuclear membrane potential, if existent, is small, relative to that at the plasma membrane (Ref. 14 and references therein). In contrast, mitochondria exhibit electrical potentials within the range of −48 to −74 mV (13, 22). We have determined the mitochondrial contribution to TPMP uptake as 5 to 10 mV in both splenocytes and GD248 cells by utilizing antimycin A as an inhibitor of respiration and CCCP as an uncoupler at oxidative phosphorylation. These results are consistent with theoretical calculations. Assuming a mitochondrial volume of 5% of total cell volume (14), a transmitochondrial electrical potential of −48 to −74 mV (13, 22), and a 2-compartment system (13), we calculate a mitochondrial fraction of −10 to −12 mV to the TPMP distribution-derived membrane potential.

The extent of potential independent TPMP or SCN binding cannot be determined for these cells. However, it would appear unlikely that, at the relatively high extracellular and intracellular ionic strengths, differential binding of μM levels of TPMP or SCN account for the difference between normal and tumor cells. We emphasize that experiments with both cation and anion probes qualitatively support the same conclusions, namely, that the membrane potential of normal cells is higher than that of tumor cells and that their respective thermosensitivities are different. Moreover, ouabain, an inhibitor of plasma membrane Na⁺-K⁺-ATPase, blocks the thermal transitions of both cell types whereas antimycin A and CCCP are without effect.

The observation that the membrane potentials of GD248- and PHA-stimulated splenocytes are lower than that of quiescent normal cells at 37° correlates with other investigations demonstrating a decreased membrane potential for rapidly proliferating cells (6, 10). However, the exact relationship between a depressed membrane potential and cell division is uncertain (Ref. 34). In the specific case of lymphocytes, agents known to alter transmembrane ion gradients including ouabain and the K⁺ ionophores, valinomycin and nigericin, inhibit lectin-stimulated DNA synthesis of lymphocytes (11, 12, 32). Other studies have shown that tumor cells and mitogenically stimulated lymphocytes are characterized by elevated intracellular Na⁺ levels and, as a probable consequence, enhanced Na⁺-K⁺-ATPase activity (1, 36, 38).

Kiefer et al. (21) have shown that the membrane potential changes associated with lectin-activated mitogenesis may be considerably more complicated. Addition of lectin to mouse splenocytes resulted in a transient membrane potential depolarization (measured with tetraphenylphosphonium) centered at 2 hr, followed by a hyperpolarization after 24 hr. The origins of the discrepancy between these results (after 24 hr) and our own experimental findings (Table 3) are not known.

Potentially important to an understanding of the relationship between membrane potential and cell growth are the observed differences in thermosensitivity of normal and tumor cell membrane potentials. The thermosensitive processes underlying these differences remain to be established and possibly include changes in ion leak fluxes and/or the coupling ratio of Na⁺⁻K⁺-ATPase. Concerning the former, Yi (45) has shown that incubation of mouse mastocytoma P815 cells at 43° for 30 min results in a 40% decrease in intracellular K⁺ without complete compensation by an increase in Na⁺ levels. The membrane potential or ion fluxes were not determined; however, assuming no other thermal effects, these results suggest an enhanced K⁺ conductance and thus hyperpolarization at elevated temperatures.

The Na⁺-K⁺-ATPase of cell membranes can express both electrogenic and nonelectrogenic activities, depending on whether the Na:K coupling ratio is 3:2 or 2:2. We have shown here that ouabain-inhibitable activity contributes at least 35% of the splenocyte membrane potential when the TPMP is used as membrane potential probe. Thus, slight changes in the Na:K coupling ratio can also have significant effects on the transmembrane potential.
Membrane Potential Thermosensitivity


Acknowledgments

We gratefully acknowledge the assistance of Dr. Peck-Sun Lin in the immunofluorescence experiments, Dr. Charles Dinarello for discussions on the core body temperature of laboratory animals, and Dr. Donald F. H. Wallach for helpful suggestions during preparation of the manuscript.

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