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Validation of the Use of the Lipophilic Thiocyanate Anion for the Determination of Membrane Potential in Ehrlich Ascites Tumor Cells

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Summary. The utility of the lipophilic anion thiocyanate (SCN^-) as a probe for the indirect estimation of the cell membrane potential (V_m) in Ehrlich ascites tumor cells has been evaluated by comparison to direct electrophysiological measurements. SCN^- accumulation is consistent with first-order uptake into a single, kinetically-identifiable cellular compartment, achieving steady-state distribution in 20–30 min at 22°C. The steady-state distribution ratio ($[SCN^-]_c/[SCN^-]_e$) in physiological saline is 0.44 ± 0.02. Treatment of the cells with propranolol (0.13 mM), an activator of Ca^{2+} dependent K^+ channels, reduces the steady-state distribution ratio to 0.19 ± 0.02. Conversely, treatment with $BaCl_2$ (10 mM), an antagonist of the pathway, increases the SCN^- distribution ratio to 0.62 ± 0.01. The equilibrium potentials (V_{SCN}) calculated under these conditions are virtually identical to direct electrophysiological measurements of the V_m made under the same conditions. The effect of varying extracellular $[K^+]$ ($[K^+]_e$) in the presence of constant $[Na^+]_e$ = 100 mM has also been tested. In control cells, elevation of $[K^+]_e$ from 6 to 60 mM reduces V_{SCN} from −20.6 ± 1.0 to −13.2 ± 1.2 mV. Again, microelectrode measurements give excellent quantitative agreement. Propranolol increases the sensitivity of the cells to varying $[K^+]_e$, so that a 10-fold elevation reduces V_{SCN} by approximately 31 mV. $BaCl_2$ greatly reduces this response; a 10-fold elevation in $[K^+]_e$ yielding only a 4-mV reduction in V_{SCN}. It is concluded that the membrane potential of Ehrlich cells can be estimated accurately from SCN^- distribution measurements.

Key Words Ehrlich ascites tumor cell · membrane potential · thiocyanate · K^+ channels · barium · propranolol · lipophilic anions

Introduction

Ehrlich ascites tumor cells actively extrude Na^+ and accumulate K^+ and as a consequence maintain these ions away from their electrochemical equilibrium (Hempling, 1958; Maizels, Remington & Truscoe, 1958). The passive movements of these ions down their respective electrochemical gradients, along with active Na^+/K^+-transport, contribute to the development of the cell membrane potential (Laris, Pershadsingh & Johnstone, 1976; Smith & Vernon, 1979; Smith & Robinson, 1981*b*). In addition, it is now widely recognized that the cation gradients, in particular that for Na^+, can be utilized to energize a number of cotransport systems, including amino acid accumulation (Eddy, Mulcahy & Thomson, 1967; Eddy, 1968; Schultz & Curran, 1970), $Na^+/K^+/Cl^-$-cotransport (Geck et al., 1980; Levinson, 1985) and Na^+/H^+ antiport (Wiener, Dubyak & Scarpa, 1986).

One of the consistent insights from the study of Na^+-dependent transport systems is that the membrane potential (V_m) plays an important role in their function. Through its contribution to the ion electrochemical gradients, the potential serves as a source of energy for the uptake of certain amino acids (Laris et al., 1976; Philo & Eddy, 1978; Hacking & Eddy, 1981; Dawson & Smith, 1987). Conversely, activation of amino acid transport leads to membrane depolarization, consistent with stimulation of rheogenic Na^+ entry through the cotransport system (Henius & Laris, 1979; Hacking & Eddy, 1981; Dawson & Smith, 1987). This depolarization is transient unless active Na^+/K^+ transport is inhibited, suggesting that the subsequent activation of the Na^+ pump is electrogenic (Dawson & Smith, 1987). Besides serving simply as a source of energy, however, the potential also affects the kinetic characteristics of the rheogenic transport processes (Geck & Heinz, 1976). Depending on the nature of the interaction between the transported species and the membrane transport system, the effects may be reflected by changes in maximum flux, binding affinity or both. Clearly, an understanding of the factors involved in establishing V_m is important in elucidating the mechanisms serving to regulate and energize Na^+ cotransport systems. In turn, these evaluations require accurate and reliable methods for measurement of V_m.

Unfortunately, the value of membrane potential

in Ehrlich ascites tumor cells has not been established unequivocally. In general, two different methods have been applied to estimate V_m: direct electrophysiological measurements utilizing intracellular microelectrodes, and indirect determinations utilizing passively distributed ions. The estimates achieved by these approaches are not always in good agreement (*cf.* Johnstone, Laris & Eddy, 1982; Smith, 1982). Typically, direct measurements by microelectrodes give values in the range −20 to −30 mV (Lassen et al., 1971; Smith & Vernon, 1979; Smith & Robinson, 1981*b*; Dawson & Smith, 1986), while indirect estimates are significantly more elevated (−40 to −60 mV; Laris et al., 1976; Hoffmann & Lambert, 1983; Valdeolmillos, Garcia-Sancho & Herreros, 1986). However, it should be noted that prolonged incubations of dilute cell suspensions with the concomitant depletion of cellular amino acid content leads to indirect estimates of V_m which more closely approximate those obtained using microelectrodes (Laris et al., 1976, 1978). Simple differences in incubation conditions cannot account completely for the observed disparity, however, since direct measurements of the steady-state V_m in the presence and absence of 2-aminoisobutyric acid (AIB) show no sustained depolarization (Dawson & Smith, 1987). At present the basis for the differences in the results from these approaches remains unresolved.

In neuroblastoma cells, the passive distribution of the lipophilic anion SCN^- has been validated as a measure of V_m by comparison to direct microelectrode determinations (Catterall, Ray & Morrow, 1976; Lichtschtein, Kaback & Blume, 1979). This anionic probe offers the advantage of limited uptake by negatively charged cellular compartments. Its accumulation in the cytoplasm is also restricted by its charge. Thus, in highly polarized cells low cellular concentration give a low "signal-to-noise" ratio and provides less sensitivity than do cationic probes. However, the V_m of Ehrlich cells is sufficiently low to permit accurate measurement of its cellular uptake. The present studies were undertaken to evaluate critically the use of SCN^- distribution as an alternative method for measurement of V_m in Ehrlich cells. Our results show: (i) SCN^- transport into Ehrlich cells is characteristic of passive, diffusional transport; (ii) low concentrations of SCN^- ($[SCN^-] < 10$ mM) are without effect on the ability of the cell to maintain normal ion electrochemical gradients and amino acid accumulation capacity; (iii) the equilibrium potential for SCN^- (V_{SCN}) provides an accurate measure of V_m as determined by microelectrodes; and (iv) at the K^+ "null point" the intracellular K^+ activity agrees well with that found using K^+-selective microelectrodes.

Materials and Methods

Cell Suspension

Ehrlich ascites tumor cells (Lettre strain; hyperdiploid) were maintained by weekly peritoneal transplantation in Ha/ICR male mice. Tumor-bearing animals with growths between 8 and 11 days were used. Cells were removed by aspiration and washed free of ascitic fluid. The wash medium was physiological saline ($[Na^+] = 154$ mM; $[K^+] = 6$ mM; $[Cl^-] = 150$ mM; $[Ca^{2+}] = 2$ mM; and $[Mg^{2+}] = 0.2$ mM) containing 10 mM HEPES buffer to yield a final pH of 7.4 and 290–300 mosM. Cells were resuspended in physiological saline or in saline media in which $[K^+]$ was varied by partially replacing NaCl with equivalent amounts of KCl and/or choline chloride. The total monovalent cation content of all media was 160 mM. The cell suspensions were incubated in Erlenmeyer flasks under an air atmosphere on a gyrorotary shaker set for 48 oscillations per min. Incubations were continued for at least 30 min to insure steady-state conditions with respect to cellular ion and water contents (Levinson, 1970). All experiments were performed at 21–23°C.

Reagents

Radioisotopes were obtained from Amersham (Arlington Heights, IL). Stock Na[^{14}C]SCN (0.33 Ci/mol) was prepared as 150 mM NaSCN and diluted to a final concentration of 0.5 mM in the cell suspensions. DL-propranolol HCl was obtained from Sigma. All other reagents were of the highest purity available.

Intracellular Cation Contents and Radioisotope Uptake

The methods used to determine intracellular Na^+ and K^+ contents by flame photometry have been described previously (Smith & Vernon, 1979). Briefly, 1.0-ml aliquots of cell suspensions (50 mg cell wt/ml) were centrifuged at 15000 × *g* for 1.0 min; the pellets were aspirated carefully and extracted with 1.0 ml of 1% perchloric acid. The Na^+ and K^+ contents of the perchloric acid extract were determined with a Beckman KLiNa flame photometer using Li^+ as an internal standard. The cellular accumulation of SCN^- was followed using [^{14}C]SCN as a tracer. The radioisotope was added to the cell suspensions, and the radioactivity associated with the perchloric acid extracts and supernatant were determined as a function of time. All estimates of intracellular ion contents and radioactivity were corrected for trapped extracellular fluid using a regression line relating [^{3}H]methoxyinulin space to the wet weight of the cell pellet. Cellular water content was determined from wet and dry weights of the pellets (Smith & Vernon, 1979).

The SCN^- distribution ratio $([SCN^-]_c/[SCN^-]_e$, where the subscripts *c* and *e* identify the cellular and environmental compartments, respectively, was taken as activity of [^{14}C]SCN per ml cell water/activity [^{14}C]SCN per ml medium.

Measurement of the Membrane Potential

The membrane potential of Ehrlich ascites tumor cells was measured using glass microelectrodes filled with 300 mM potassium acetate. The apparatus and techniques have been described in

detail (Smith, Mikiten & Levison, 1972; Smith & Vernon, 1979; Dawson & Smith, 1986).

Glass dishes were prepared to promote adherence of the cells to the glass surface and to facilitate impalement. Briefly, glass petri plates (diameter = 10 cm) were soaked in NaOH-saturated methanol for 24 hr at 21–23°C. The plates were rinsed thoroughly with distilled water and with physiological saline prior to use. The tumor cells were prepared by adding 0.2 ml cell suspension to 10 ml of test solution and were placed in the petri plate. The majority of cells adhered to the prepared surface, and impalements were possible. The potential difference between the cell cytoplasm and medium was recorded as previously described (Smith & Vernon, 1979). Our criteria for the validity of potential measurements have been discussed in detail (Dawson & Smith, 1986). These include consideration of the characteristics of the recordings upon insertion and withdrawal of the electrode, as well as attainment of a stable recording. Approximately 60% of our attempts at cell impalement yield responses consistent with these criteria.

Corrections of the experimentally determined values of the membrane potential for the influence of junction potentials were made by applying a modified form of the Henderson equation (Barry & Diamond, 1970). For these calculations we used the ion mobility ratios $U_{Na}:U_K:U_{Cl} = 0.682:1.0:1.038$. Ionic activity coefficients were taken as 0.73 for K^+ and Cl^- and 0.71 for Na^+ in the medium, while in the cytoplasm they were 0.41, 0.67 and 0.18 for K^+, Cl^- and Na^+, respectively (Dawson & Smith, 1986). The maximum correction was −2.1 mV.

Statistical Evaluation

All values are expressed as the mean ± SE. Student's *t* test was used to evaluate statistical significance.

Results

Uptake and Accumulation of $[^{14}C]SCN^-$ by Ehrlich Cells

Thiocyanate (SCN^-) transport *per se* has not been previously characterized in Ehrlich ascites tumor cells. However, its effects as a replacement anion for Cl^- are consistent with the view that SCN^- is unreactive with the well-described cation-dependent Cl^- transport system. Instead it utilizes a non-selective, conductive anion channel (Kramhoft et al., 1986). In this case, the steady-state transmembrane distribution of SCN^- should be governed exclusively by the chemical and electrical driving forces (Barts et al., 1980; Midgley & Thompson, 1985) and provide a measure of V_m. We have tested this expectation in a series of experiments designed to characterize the transport and effects of $[^{14}C]SCN^-$ in Ehrlich cells.

Ehrlich ascites cells suspended in physiological saline take up $[^{14}C]SCN^-$ rapidly for 5 to 10 min and reach steady-state accumulation within 20 to 30 min (Fig. 1*A*). The steady-state level is maintained for at least 2 hr (*data not shown*), suggesting that cell viability is not compromised by chronic exposure to the probe. The average steady-state $[^{14}C]SCN^-$ distribution ratio under these experimental conditions is 0.45 ± 0.02. The sensitivity of uptake to alterations in V_m was tested by treating the cells with propranolol (0.13 mM), an agent postulated to activate Ca^{2+}-dependent K^+ channels in Ehrlich cells (Laris et al., 1976; Valdeolmillos et al., 1986). Chronic exposure to propranolol causes a 55–65% reduction in the steady-state accumulation capacity.

The time-course of the accumulation of $[^{14}C]SCN^-$ (Fig. 1*A*) is suggestive of uptake in a closed, two-compartment system: one compartment represented by the intracellular phase; the other by the extracellular medium. In these experiments the extracellular phase is large (compared to the intracellular phase), so that its $[^{14}C]SCN^-$ activity can be considered to remain constant. Under these conditions the uptake of $[^{14}C]SCN^-$ into the cellular compartment is described by (Solomon, 1949)

$$\log[1 - \{SCN(t)/SCN(\infty)\}] = -(k_e/2.303)t + A \quad (1)$$

where $SCN(t)$ is the activity (CPM/ml cell water) at time t, and $SCN(\infty)$ is the activity after the steady-state distribution has been achieved (taken as that after 45 min). The slope of the relationship provides a measure of the efflux rate coefficient (k_e) for $[^{14}C]SCN^-$, while the intercept, A, provides a measure of the correction of cellular $[^{14}C]SCN^-$ activity for that trapped in the extracellular fluid of the cell pellet.

We have evaluated the kinetics of the uptake in terms of this model (Fig. 1*B*). The linearity of the plot for uptake in control cells is consistent with accumulation into a single kinetically-identifiable cellular compartment. The efflux rate coefficient is $k_e = 0.28 \pm 0.02\ min^{-1}$, giving a half-time for equilibration of 2.5 min. The intercept is not different from unity, confirming the adequacy of the correction applied for activity in trapped extracellular space. $[^{14}C]SCN^-$ uptake kinetics in cells treated with propranolol are bi-phasic. At early times ($t < 3$ min), the approach to equilibrium follows that of control cells. However, at later times the slope of the semi-logarithmic plot is reduced. Pre-incubation (10 min) of the cells in the presence of propranolol prior to $[^{14}C]SCN^-$ addition converts the uptake kinetics to a single phase with $k_e = 0.14\ min^{-1}$ (*data not shown*).

The analysis of the concentration dependence (range: 0.5 to 10 mM) of $[^{14}C]SCN^-$ transport in steady-state Ehrlich cells is summarized in Fig. 2.

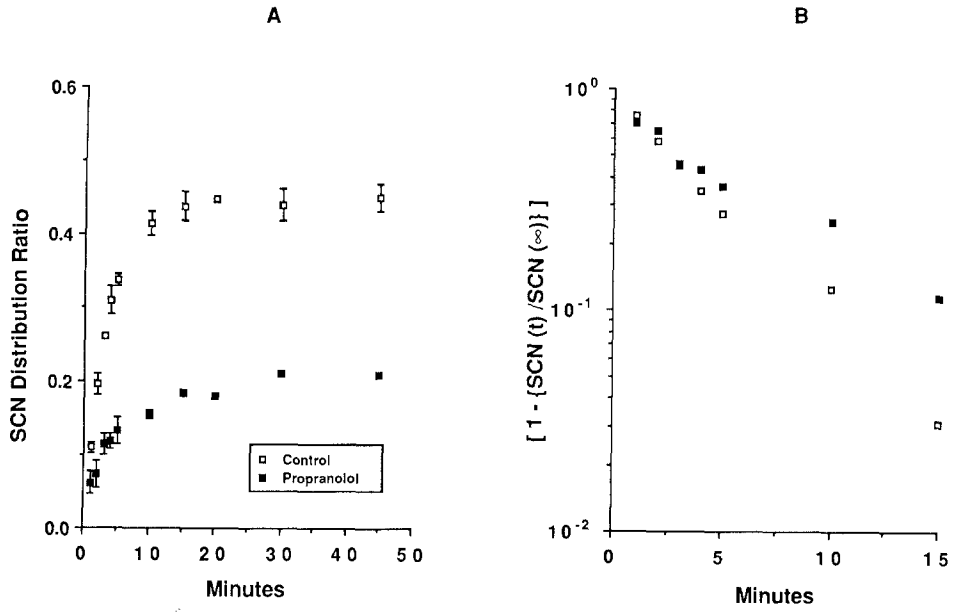

Fig. 1. [^{14}C]SCN$^-$ uptake by Ehrlich ascites tumor cells. (*A*) Time course of [^{14}C]SCN$^-$ accumulation. Ehrlich ascites tumor cells were suspended in physiological saline containing 0.5 mM [^{14}C]SCN$^-$ (0.33 Ci/mol) in the absence (open symbols) or presence (closed symbols) of propranolol (0.13 mM). Duplicate aliquots of the cell suspensions were removed at the specified times and analyzed for [^{14}C]SCN$^-$ activity. The distribution ratio was determined as ([^{14}C]SCN$^-$ activity per ml cell water/[^{14}C]SCN$^-$ activity per ml medium). Each point represents the mean value from eight experiments. Standard errors of the mean are given unless smaller than the symbols. (*B*) Compartmental analysis of [^{14}C]SCN$^-$ uptake. The data from *A* have been replotted as the semi-logarithmic approach to isotopic equilibrium in the absence (open symbols) and presence (closed symbols) of propranolol. The equilibrium activity [SCN(∞)] was taken as the [^{14}C]SCN$^-$ activity/ml cell water at 45 min. SCN(*t*) represents the [^{14}C]SCN$^-$ activity/ml cell water at the specified times

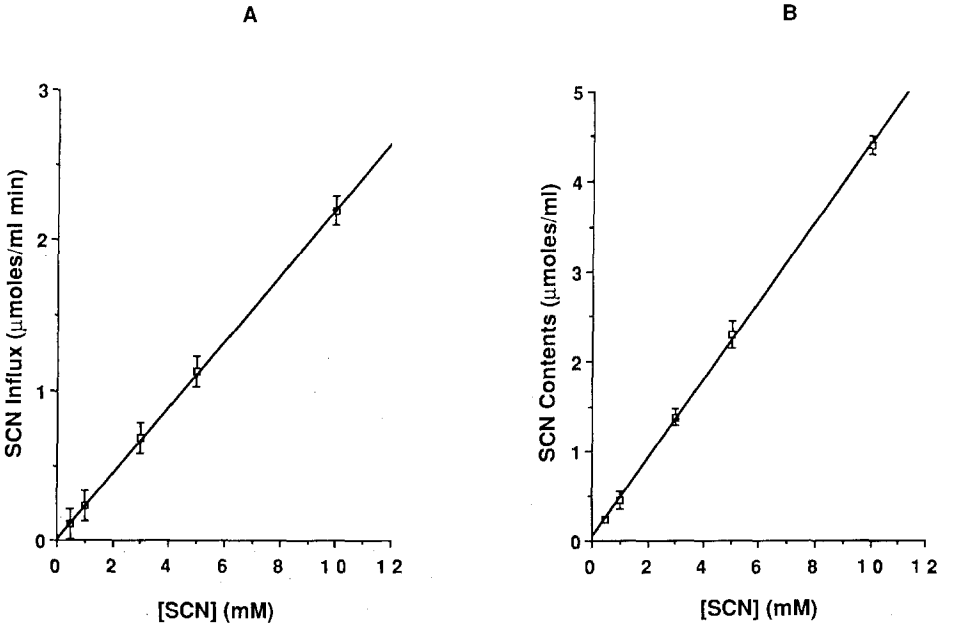

Fig. 2. Concentration dependence of [^{14}C]SCN$^-$ uptake by Ehrlich ascites tumor cells. Cells were incubated in physiological saline to establish steady-state contents with respect to electrolytes and water. SCN$^-$ uptake was initiated by addition of appropriate volumes of 150 mM NaSCN containing tracer [^{14}C]SCN$^-$. The influx (*A*) was determined as a function of [SCN$^-$] (range: 0.5 to 10 mM) from the uptake of radioactivity 1 min after the addition. The [^{14}C]SCN$^-$ content (*B*) was determined after 45 min as [^{14}C]SCN$^-$ activity per ml cell water/specific activity of [^{14}C]SCN$^-$ in the medium. Each point is the mean of four experiments. Standard errors of the mean are shown unless they are smaller than the symbols

The cellular uptake of $[^{14}C]SCN^-$ is essentially linear for at least 1 min, with steady-state accumulation being achieved after approximately 20 min (Fig. 1). Consequently, we have taken the initial 1 min accumulation as a measure of $[^{14}C]SCN^-$ influx and the cellular contents after 45 min as the equilibrium uptake. The initial $[^{14}C]SCN^-$ influx (Fig. 2*A*) is directly proportional to extracellular $[SCN^-]$. Furthermore, the steady-state level of accumulation is also a linear function of $[SCN^-]$ over this concentration range (Fig. 2*B*). Under these conditions the steady-state distribution ratio is 0.44 ± 0.02. This behavior is consistent with the view that SCN^- transport is by passive, nonsaturable mechanisms with equilibration across the membrane being sensitive to V_m.

The possibility that $[^{14}C]SCN^-$ might compromise cellular function or alter factors that contribute to the development of V_m has also been explored. Ehrlich cells maintain nonequilibrium distributions of Na^+ and K^+ across the cell membrane by coupling their transport to cellular metabolism (Hempling, 1958; Maizels et al., 1958). In addition, the energy available from the ion gradients is utilized to permit cellular accumulation of the Na^+-dependent amino acid α-methylaminoisobutyric acid (MeAIB) (Philo & Eddy, 1978; Hacking & Eddy, 1981; Dawson & Smith, 1987). The ability of the cells to maintain the normal cation gradients and the capacity to concentrate MeAIB thus provide a sensitive measure of normal cell function. We have tested the effects of SCN^- on cellular Na^+, K^+ and MeAIB over the range $[SCN^-] = 0.5$ to 10 mM (Fig. 3). Steady-state cells in physiological saline solution and the absence of $[^{14}C]SCN^-$ have $[Na^+]_c = 23.2 \pm 2.1$ mM, $[K^+]_c = 166.7 \pm 2.3$ mM and concentrate MeAIB 29.5 ± 0.3-fold from medium containing 1 mM MeAIB. The addition of $[^{14}C]SCN^-$ is without effect on any of the parameters for $[SCN^-] \leq 5$ mM. Only at the highest level tested, $[SCN^-] = 10$ mM, is there a significant effect. $[Na^+]_c$ is unaltered (24.3 ± 4.0 mM). However, $[K^+]_c$ is reduced to 151.7 ± 6.5 mM, while the accumulation capacity for MeAIB increases to 32.5 ± 0.5-fold. Consequently, we have performed all remaining studies at $[SCN^-] = 0.5$ mM.

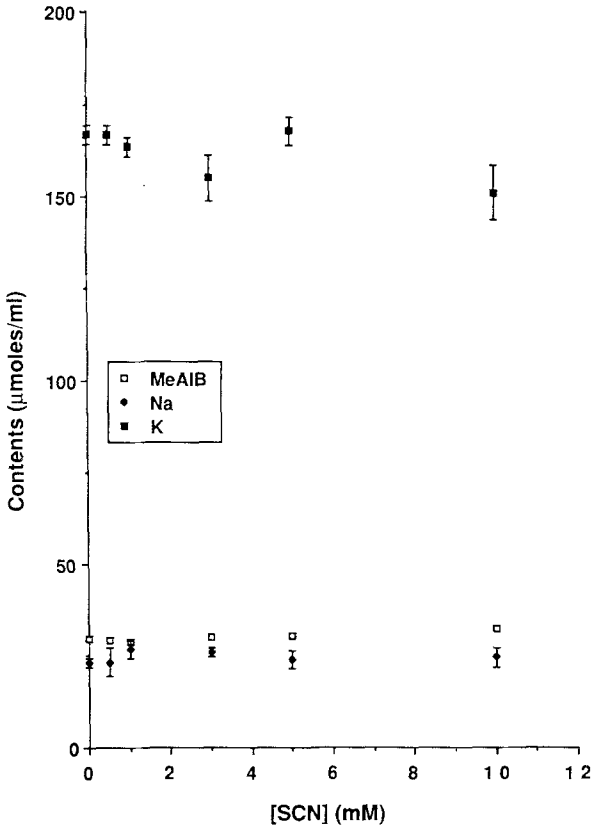

Fig. 3. Effect of [SCN] on cation and amino acid contents of Ehrlich ascites tumor cells. Cells were incubated in the presence of SCN^- ($[SCN^-]$ range: 0 to 10 mM) for 1 hr to permit establishment of steady-state contents with respect to cations and the test amino acid α-methylamino[^{14}C]isobutyric acid (MeAIB; initial [MeAIB] = 1 mM). Each point is the mean of four values. Standard errors of the mean are given unless smaller than the symbols

$[^{14}C]SCN^-$ Accumulation as a Probe of Membrane Potential

The results described in the preceding section are consistent with the conclusion that $[^{14}C]SCN^-$ transport in Ehrlich cells occurs by passive, nonmediated pathways. Furthermore, kinetic analyses suggest there may be only a single intracellular compartment. Both the steady-state accumulation and the efflux rate coefficient are sensitive to treatment expected to alter V_m. Together these findings support the view that the $[^{14}C]SCN^-$ equilibrium distribution is governed by V_m. In this case it is expected that V_m can be accurately estimated from

$$V_{SCN} = (RT/F) \ln\{a_{SCN}(\text{cell})/a_{SCN}(\text{env})\} \quad (2)$$

where V_{SCN} is the SCN^- equilibrium potential (cytoplasm with respect to environment) and a_{SCN}(cell) and a_{SCN}(env) are the electrochemical activities for SCN^- in the cytoplasm and environment, respectively. We have assumed that the ion activity coefficients for SCN^- are identical in the two compartments. Thus $[SCN^-]$ rather than a_{SCN} has been used for the computation of V_{SCN}.

We have compared V_{SCN} to measurements of V_m under conditions chosen to alter the contribution of the K^+ electrochemical potential gradient to V_m (Fig. 4). Direct measurements of V_m were obtained

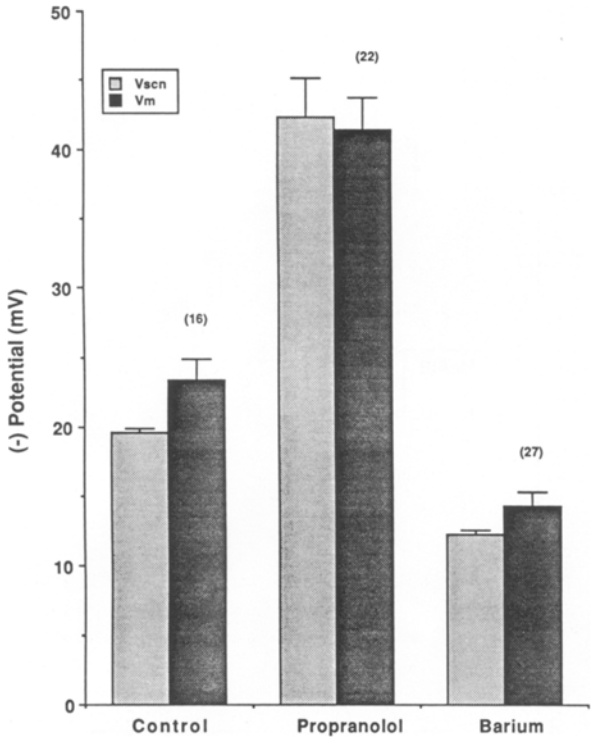

Fig. 4. Comparison of the SCN^- equilibrium potential (V_{SCN}) and the membrane potential (V_m). Cells were incubated in physiological saline (control) and physiological saline containing propranolol (0.13 mM) or $BaCl_2$ (10 mM). V_{SCN} was determined from the Nernst potential using the steady-state accumulation of $[^{14}C]SCN^-$ ($[SCN^-]_e$ = 0.5 mM). V_m was measured using glass microelectrodes for impalement of the cells. The columns represent the mean values ± SE for V_{SCN} (eight determinations) and V_m (number of impalements in parentheses)

by using intracellular microelectrodes filled with 300 mM potassium acetate. Stable membrane potentials from steady-state Ehrlich cells can be recorded for as long as 15 min using techniques previously described (Smith & Robinson, 1981*a*). The average V_m for cells maintained in physiological saline solution is −23.3 ± 1.5 mV. This value is in excellent agreement with those from previous electrophysiological studies (Smith & Vernon, 1979; Smith & Robinson, 1981*a*; Dawson & Smith, 1986). Exposure of the cells to propranolol leads to membrane hyperpolarization, V_m = −41.4 ± 2.3 mV, while Ba^{2+}, an antagonist of Ca^{2+}-sensitive K^+ channel activation (Schwartz & Passow, 1983), induces depolarization to V_m = −14.3 ± 1.0 mV. The $[^{14}C]SCN^-$ distribution ratios were measured under identical conditions. V_{SCN}, calculated from Eq. (2), is −19.6 ± 0.3 mV in physiological saline. Treatment of the cells with propranolol or Ba^{2+} gives V_{SCN} values of −42.3 ± 2.8 and −12.3 ± 0.3 mV, respectively. It is noteworthy that Ba^{2+} is without effect on propranolol-induced hyperpolarization (*data not shown*). In no case is the difference between V_m and V_{SCN} significant.

We have extended the comparison to explore the effect of altered extracellular $[K^+]$ ($[K^+]_e$) on $[^{14}C]SCN^-$ accumulation in the presence and absence of propranolol and Ba^{2+} (Fig. 5*A*). In control cells, increasing $[K^+]_e$ from 6 to 60 mM is accompanied by a monotonic increase in the $[^{14}C]SCN^-$ distribution ratio from 0.45 ± 0.02 to 0.59 ± 0.04. This corresponds to an alteration in V_{SCN} from −20.6 ± 1.0 to −13.2 ± 1.2 mV for the 10-fold increase in $[K^+]_e$ (Fig. 5*B*). Direct measurements of V_m over the same concentration range show that the responses of V_{SCN} and V_m to changes in $[K^+]_e$ are not different.

Treatment of the cells with propranolol reduces the $[^{14}C]SCN^-$ distribution ratio to 0.17 ± 0.01 in the presence of 6 mM $[K^+]_e$ (corresponding to V_{SCN} = −45.5 ± 1.6 mV) and increases the dependence of the ratio on $[K^+]_e$. In this case a 10-fold increase in $[K^+]_e$ induces approximately a 31-mV depolarization. On the other hand, Ba^{2+} treatment leads to increased uptake of $[^{14}C]SCN^-$ at the lowest $[K^+]_e$ (distribution ratio = 0.55 ± 0.01; V_{SCN} = −15.2 ± 0.2 mV) and renders its distribution nearly insensitive to $[K^+]_e$ (corresponding to a 4-mV depolarization for a 10-fold increase). Concomitant determinations of intracellular K^+ ($[K^+]_c$) were performed in these experiments. In control and Ba^{2+} treated cells $[K^+]_c$ increases from 155.4 ± 1.2 to 169.1 × 0.4 mM as extracellular $[K^+]$ is elevated from 6 to 60 mM. In propranolol-treated cells, $[K^+]_c$ increases from 152.6 ± 6.7 to 166.0 ± 3.1 mM over the same range of $[K^+]_e$. Thus, comparison of the effects of the experimental treatments are not complicated by differences in the transmembrane K^+ concentration gradient.

It is important to note that as $[K^+]_e$ is increased, the $[^{14}C]SCN^-$ distribution ratios (and V_{SCN}) for the three experimental conditions converge, so that at $[K^+]_e$ = 60 mM, there is no significant difference (Fig. 5*A* and *C*). Neither is there a difference in $[K^+]_c$. If it is assumed that the only effects of propranolol and Ba^{2+} are to alter the membrane conductance to K^+, then the point of convergence defines the "null point," the $[K^+]_e$ at which K^+ is in electrochemical equilibrium (Hoffman & Laris, 1974; Laris et al., 1976). At the "null point" for K^+ (60 mM), the average $[^{14}C]SCN^-$ ratio is 0.60 ± 0.01, yielding V_{SCN} = −13.0 ± 0.3 mV.

Membrane Permeability to SCN^-

The characteristics of SCN^- transport which we have described are those of a passive, nonmediated

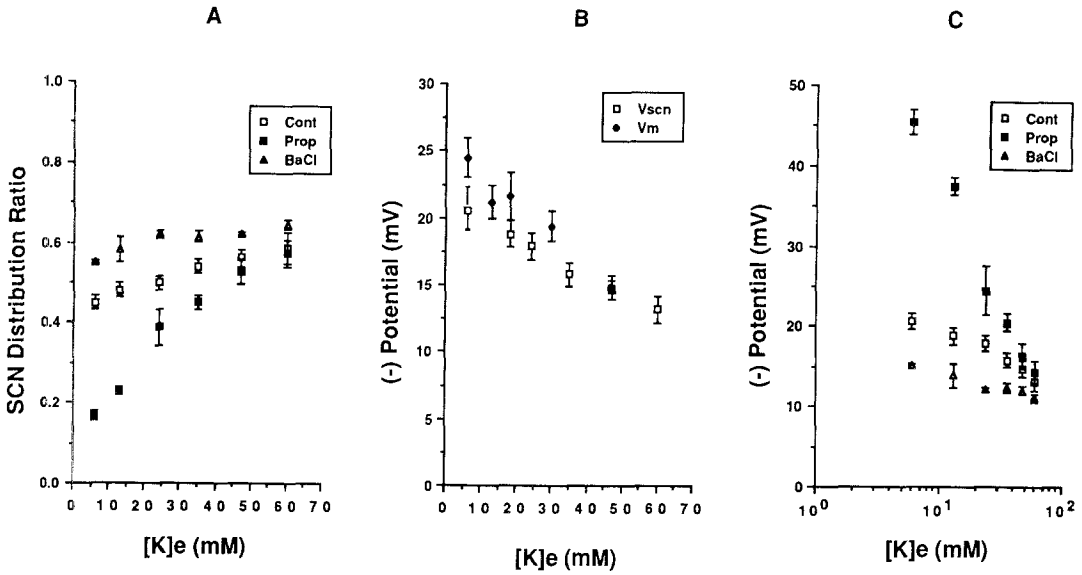

Fig. 5. Effects of external K^+ and altered K^+ permeability on [^{14}C]SCN^- uptake by Ehrlich ascites tumor cells. (*A*) Dependence of [^{14}C]SCN^- accumulation on extracellular K^+ ($[K^+]_e$). Cells were incubated in media containing constant [Na^+] (100 mM) with [K^+] varied by equivalent replacement by choline ([K^+] + choline = 60 mM). The [^{14}C]SCN^- distribution ratio was determined from the cellular accumulation of radioactivity ([SCN^-] = 0.5 mM). The effects of added propranolol (0.13 mM) and $BaCl_2$ (10 mM) are also shown. Each point represents the means from eight determinations. Standard errors of the mean are given unless smaller than the symbols. (*B*) Comparison of the effects of extracellular K^+ on V_{SCN} and V_m. V_{SCN} was calculated from the Nernst relationship using the distribution ratio achieved by the control cells described in *A*. V_m was measured directly under similar conditions using glass microelectrodes. Each point represents the mean from at least 13 impalements. Standard errors of the mean are given. (*C*) Effects of extracellular K^+ on V_{SCN}. V_{SCN} was calculated from the Nernst relationship using the distribution ratios found for control cells and cells treated with propranolol (0.13 mM) or $BaCl_2$ (10 mM). Note the semi-logarithmic scale

system. This finding is consistent with the studies of Kramhoft and co-workers (1986). These authors have demonstrated that SCN^- cannot substitute for Cl^- in cation-dependent systems, but uses a conductive anion channel for transport. In this case the diffusional permeability of the membrane to SCN^- (P_{SCN}) is given by (Hodgkin & Katz, 1949)

$$P_{SCN} = J_e RT[1 - \exp(FV_m/RT)]/FV_m[SCN^-]_c \quad (3)$$

where J_e is the steady-state unidirectional efflux of SCN^- corresponding to the steady-state cytoplasmic $[SCN^-]_c$. For this evaluation, we have estimated $J_e = k_e[SCN^-]_c$ from the data of Fig. 1. The flux was converted from units of μmol/(ml·min) to pmol/(cm^2 · sec), assuming that the cells are spherical with average radius = 7×10^{-4} cm. For steady-state cells ($V_m = -23.3 \pm 1.5$ mV), the apparent $P_{SCN} = (1.6 \pm 0.3) \times 10^{-6}$ cm/sec. Thus, the Ehrlich cell membrane permeability to SCN^- exceeds that estimated for the conductive movement of Cl^- by about 40-fold (Hoffmann, Simonsen & Sjoholm, 1979), in good agreement with expectations based on cell volume responses to anion substitution (Kramhoft et al., 1986).

Discussion

The results of these studies provide convincing evidence that the membrane potential of Ehrlich ascites tumor cells can be monitored by measuring the equilibrium distribution of the lipophilic anion SCN^-. This conclusion is supported by several lines of evidence. First, the transport of [^{14}C]SCN^- can be characterized as a passive, nonmediated process, with the steady-state accumulation being sensitive to experimental conditions that alter the cell membrane potential. The kinetics of [^{14}C]SCN^- uptake are consistent with its uniform distribution within all intracellular compartments. Furthermore, the steady-state accumulation is achieved within minutes of addition, so that its use is both convenient and compatible with maintaining cell viability. Second, exposure of cells to low concentrations of SCN^- ([SCN^-] < 10 mM) does not compromise their ability to maintain normal, nonequilibrium Na^+ and K^+ contents or to accumulate MeAIB actively. Since both the cation and amino acid transport systems rely on cellular metabolism, their lack of sensitivity to [^{14}C]SCN^- attests to its lack of toxicity. Finally, comparison of the SCN^- equilibrium

potential (V_{SCN}) to direct measurements of the membrane potential (V_m) using intracellular microelectrodes shows excellent quantitative agreement. There are no significant differences between V_{SCN} and V_m in steady-state cells maintained in physiological saline, in cells treated to alter membrane K^+ conductance, or in cells incubated in media of varying extracellular [K^+]. Thus, [^{14}C]SCN should serve as a convenient, accurate, noninvasive and nontoxic probe of the Ehrlich cell membrane potential. The primary limitation to its use derives from the time necessary for equilibrium. The half-time is approximately 2.5 min. Consequently, rapid transient changes in V_m may not be followed precisely by the redistribution of [^{14}C]SCN^-. However, steady-state or slowly changing values of V_m can be followed accurately, and qualitative reflections of rapid events should still be possible.

The ionic basis for the membrane potential of Ehrlich cells has not been established unequivocally. A number of previous studies have established the relative insensitivity of V_m of Ehrlich cells to changes in $[K^+]_e$ (Heinz et al., 1977; Smith & Robinson, 1981*b*; Valdeolmillos et al., 1986), a finding consistent with the view that V_m is not a K^+ diffusion potential. Our evaluation of the effects of variations in $[K^+]_e$ on the [^{14}C]SCN^- distribution in control cells and in the presence of propranolol or Ba^{2+} (Fig. 5*A*) supports this view and provides fresh insights into the underlying mechanisms. The replots of the responses in terms of potential (Figs. 5*B* and *C*) better emphasize the quantitative relationships. In control cells, a 10-fold increase in $[K^+]_e$ gives approximately 7 to 10 mV depolarization as measured by either V_{SCN} or microelectrodes (Fig. 5*B*). This represents only 11–16% of the response expected for a pure K^+ diffusion potential. Even activation of the Ca^{2+}-dependent K^+ channels fails to completely convert the sensitivity to that of a K^+ diffusion system (31 mV depolarization measured versus 59 mV expected; Fig. 5*C*). It is clear that additional electrogenic mechanisms must contribute importantly to the development of V_m in Ehrlich cells. We have previously suggested that a major contribution derives from active Na^+/K^+ transport (Smith & Robinson, 1981*b*). More extensive studies will be needed to completely describe the ionic bases of V_m in these cells. Toward this end, it is important to note that Ba^{2+} virtually abolishes the contribution of K^+ diffusion (Fig. 5*C*). In the presence of Ba^{2+}, a 10-fold increase in $[K^+]_e$ gives only a 4-mV depolarization. Ba^{2+} should provide a powerful agent for isolating the electrogenic processes involved in establishing V_m.

Validation of V_{SCN} as a noninvasive reporter of V_m permits us to seek a resolution to the disagreement between previous estimates of the potential (*cf.* Johnstone et al., 1982; Smith 1982). Estimates of V_m based on indirect methods generally give values greater than those obtained by direct electrophysiological techniques (Laris et al., 1976, Philo & Eddy, 1978; Smith & Robinson, 1981*b*). This difference can be appreciated by comparing the V_m obtained in the present studies (-23.3 ± 1.5 mV in physiological saline) to the estimates under similar experimental conditions obtained using fluorescent probes (-40 to -60 mV) (Laris et al., 1976; Philo & Eddy, 1978). We have previously suggested that the discrepancy may result from an incorrect calibration (Dawson & Smith, 1986).

The indirect methods must be calibrated against a "known" value of the membrane potential. Universally this "known" value is established from the "null point" method, which identifies an $[K^+]_e$ such that alterations in membrane K^+ conductance give no change in distribution of the indirect probe (Hoffman & Laris, 1974; Laris et al., 1976; Valdeolmillos, 1986). The V_m at the "null point" is the K^+ equilibrium potential (V_K). Clearly values for V_m obtained from this approach are intimately dependent on accurate estimates of V_K. At the "null point"

$$V_m = V_K = -(RT/F) \ln\{a_K(\text{cell})/a_K(\text{env})\} \quad (4)$$

where a_K(cell) and a_K(env) are the electrochemical activities for K^+ in the cytoplasm and environment, respectively. All previous studies have evaluated a_K in both compartments from chemical measurements of [K^+]. This assumes that the ion activity coefficients for K^+ (γ_K) are identical in the two compartments. We have recently determined a_K(cell) using ion-selective electrodes (Dawson & Smith, 1987) and found it to be only about 56% that expected from chemical estimates ($\gamma_K^{cell} = 0.41 \pm 0.05$ versus $\gamma_K^{env} = 0.73$). In this case, it is expected that estimates of V_K based on chemical measurements will significantly overestimate the true value.

The data presented in Fig. 5 provide a test of the "null point" expectations in the present studies. The [^{14}C]SCN^- distribution ratios in control cells and in cells treated with either propranolol or Ba^{2+} converge to the same value for $[K^+]_e = 60$ mM. The convergence corresponds to the "null point" and occurs with $V_{SCN} = -13.0 \pm 0.3$ mV (Fig. 5*C*). Under these conditions, K^+ must also be in electrochemical equilibrium. For these cells, $[K^+]_c = 168.1 \pm 3.2$ mM and $[K^+]_e = 60$ mM. If we follow the usual assumption that the chemical measurements of [K^+] can be used instead of a_K, then $V_K = -26.4 \pm 0.5$ mM (Eq. (3)). Clearly, the "null point" V_m estimated from V_K significantly exceeds that esti-

mated from V_{SCN}. *However, recognition that the ion activity coefficients for K^+ are not equivalent in the cellular and extracellular compartments leads to a different conclusion.* In this case

$$V_K = -(RT/F)\ \ln\{\gamma_K^{cell}[K^+]_c/\gamma_K^{env}[K^+]_e\}$$
$$= -11.6 \pm 1.5 \text{ mV}$$

compared to $V_{SCN} = -13.0 \pm 0.3$ mV. *Thus, the "null point" determination of V_m based on V_K is not different from that reported by V_{SCN} if a_K is correctly estimated.*

Taken together, the agreement between V_{SCN} and V_K at the "null point" and our finding that V_{SCN} agrees in all cases with direct microelectrode measurements of V_m strongly support the conclusion that the [^{14}C]SCN^- distribution ratio provides an accurate and convenient monitor of the membrane potential in Ehrlich cells. Furthermore, it is probable that the disagreement between direct and indirect evaluations of V_m can be resolved by recalibration of V_K at the "null point." V_{SCN} could serve for the calibration.

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