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Influence of Ca^{2+} on the plasma membrane potential and electrogenic uptake of glycine by myeloma cells. Involvement of a Ca^{2+}-activated K^+ channel

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The involvement of Ca^{2+}-activated K^+ channels in the regulation of the plasma membrane potential and electrogenic uptake of glycine in SP 2/0-AG14 lymphocytes was investigated using the potentiometric indicator 3,3′-diethylthiodicarbocyanine iodide. The resting membrane potential was estimated to be -57 ± 6 mV ($n = 4$), a value similar to that of normal lymphocytes. The magnitude of the membrane potential and the electrogenic uptake of glycine were dependent on the extracellular K^+ concentration, $[K^+]_o$, and were significantly enhanced by exogenous calcium. The apparent V_{max} of Na^+-dependent glycine uptake was doubled in the presence of calcium, whereas the $K_{0.5}$ was not affected. Ouabain had no influence on the membrane potential under the conditions employed. Additional criteria used to demonstrate the presence of Ca^{2+}-activated K^+ channels included the following: (1) addition of EGTA to calcium supplemented cells elicited a rapid depolarization of the membrane potential that was dependent on $[K^+]_o$; (2) the calmodulin antagonist, trifluoperazine, depolarized the membrane potential in a dose-dependent and saturable manner with an IC_{50} of 9.4 μM; and (3) cells treated with the Ca^{2+}-activated K^+ channel antagonist, quinine, demonstrated an elevated membrane potential and depressed electrogenic glycine uptake. Results from the present study provide evidence for Ca^{2+}-activated K^+ channels in SP 2/0-AG14 lymphocytes, and that their involvement regulates the plasma membrane potential and thereby the electrogenic uptake of Na^+-dependent amino acids.

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Abbreviations diS-C_2-(5), 3,3′-diethylthiodicarbocyanine iodide, diS-C_3-(5), 3,3′-dipropylthiodicarbocyanine iodide, $TPMP^+$ methyltriphenylphosphonium, TPP^+, tetraphenylphosphonium, bis-oxonol, bis-(1,3-diethylthiobarbiturate)-trimethineoxonol, $[K^+]_o$, extracellular K^+ concentration (K^+ added), $[K^+]_i$, the intracellular K^+ concentration, IC_{50}, half-maximal inhibitory concentration, E_{K^+}, K^+ equilibrium potential

Introduction

Certain Ca^{2+}-induced changes in voltage across the plasma membrane of animal cells have been reported for a variety of tissues [1,2] which appear to be initiated by the specific gating of K^+ in a Ca^{2+}-dependent manner This phenomenon was originally described by Gardos [3] in human erythrocytes and subsequently has been shown to be regulated by calmodulin [4] Since then the presence of specific Ca^{2+}-activated K^+ conductance pathways (channels) have been demonstrated in many mammalian cell types [1,2,5–18]

In this report we provide evidence for a similar Ca^{2+}-dependent K^+ gate in cultured mouse myeloma cells We further demonstrate that the regulation of K^+ permeability may serve as an important control of the plasma membrane potential and, subsequently, the driving force for cellular uptake of Na^+-dependent amino acids, and possibly regulation of protein synthesis

Materials and Methods

The potentiometric cyanine dye, diS-C_2-(5) (laser grade) was purchased from Eastman Kodak diS-C_3-(5), was a gift from Dr Philip C Laris, trifluoperazine was a gift from Smith, Kline and French, valinomycin, EGTA, ouabain, and quinine hydrochloride were from Sigma, glycine was from General Biochemicals, DMEM (Dulbecco's modified Eagle's medium) and NCTC 135 were from Grand Island Biological Co All other chemicals were of reagent grade

SP 2/0-AG14 mouse myeloma cells were cultured in medium containing, in a final volume of 1 liter, 10 7 g DMEM, 0 94 g NCTC 135, 10 9 mmol L-glutamine, 1 4 mmol L-cysteine, 1 0 mmol sodium pyruvate, 50 μmol β-mercaptoethanol, 14 3 mmol $NaHCO_3$, 12 6 mmol Hepes, 50 mg gentamycin sulfate, 10% horse serum, and 5% fetal bovine serum The pH was adjusted to 7 2 Cells were grown in monolayer at 37°C in an atmosphere of 95% air and 5% CO_2 Log phase cells were harvested by gentle agitation, washed twice and resuspended to 10^7 cells/ml in Na^+-Ringer buffer (154 mM NaCl, 6 2 mM KCl, 1 6 mM $MgSO_4$, 1 2 mM $CaCl_2$, and 3 mM sodium phosphate (pH 7 4)), unless otherwise specified Prior to assay, aliquots of cells were diluted with buffer to a final concentration of 6 7 10^5/ml and preincubated for 20 min at 37°C in an orbital water bath shaker Without preincubation, a progressive increase in dye fluorescence was observed, which leveled off within 20 min (data not shown) A similar phenomenon was reported for Ehrlich ascites tumor cells [19] and was shown subsequently to be due to electrogenic loss of intracellular Na^+-coupled amino acids [20] In K^+-free Na^+-Ringer buffer, NaCl was substituted for KCl K^+-Ringer and choline-Ringer buffers were prepared by substituting equivalent concentrations of KCl and choline chloride for NaCl

Plasma membrane potential of cells suspended in Na^+-Ringer buffer (unless otherwise specified) was monitored continuously in the presence of 1 7 μM cyanine dye The final volume in the cuvette was 3 ml and the temperature was maintained at 37°C Fluorescence was monitored at excitation and emission wavelengths of 622 and 670 nm, respectively, using a Perkin-Elmer MPF-3 fluorescence spectrophotometer equipped with a thermostated cell compartment and electronically controlled stirrer

Quinine and trifluoperazine at the concentrations used enhanced dye steady-state fluorescence To correct for this interference, changes in fluorescence were monitored in the absence of a membrane potential (disrupted cell suspensions) Aliquots of cells were subjected to three cycles of freezing and thawing and then homogenized in a Dounce homogenizer Complete dissipation of the membrane potential was confirmed by adding valinomycin and observing no change in fluorescence under conditions where large changes in fluorescence were observed with intact cells (see below) The changes in fluorescence observed upon addition of quinine and trifluoperazine to homogenates in the presence of dye were subtracted from changes obtained under similar conditions using intact cells

Results

Effect of $[K^+]_o$ on plasma membrane potential

The addition of the K^+-specific ionophoretic antibiotic valinomycin to cell suspensions containing diS-C_2-(5) resulted in rapid changes in the total steady-state fluorescence (Fig 1) The direction and degree of fluorescence changes were dependent on $[K^+]_o$, decreasing at low $[K^+]_o$ (hyperpolarized, inside more negative) and increasing at high $[K^+]_o$ (depolarized, inside less negative or positive) (Fig 1A) These observations are similar to those with erythrocytes [21] and Ehrlich ascites tumor cells [20] The use of this and other related potentiometric cyanine dyes has been reviewed recently in detail for other cellular systems [22] A plot of the steady-state fluorescence after addition of valinomycin versus $\log[K^+]_o$ resulted in a linear

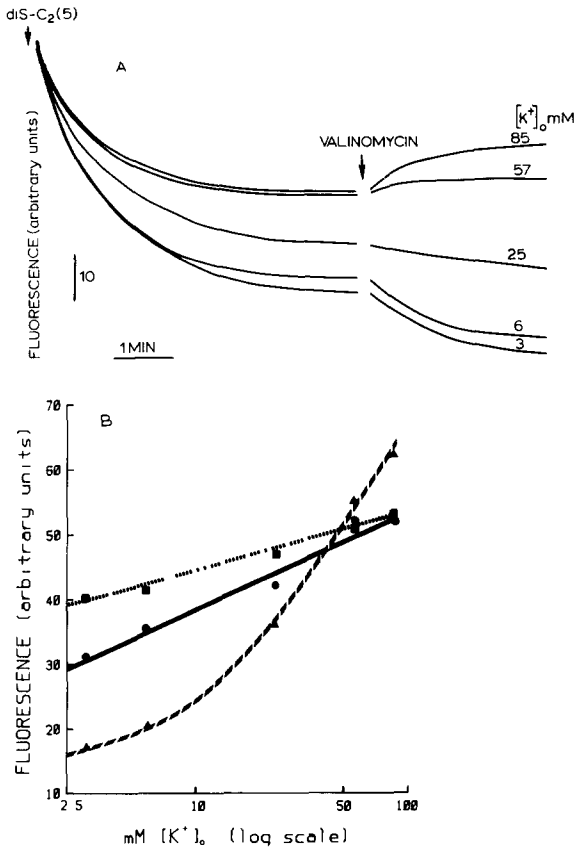

Fig 1 Effect of $[K^+]_o$ on the plasma membrnae potential Cells at a final concentration of 6 7 10^5/ml were prepared as described in Methods and Materials (A) diS-C_2-(5) (1 7 μM) and valinomycin (0 85 μM) were added where indicated to cells preincubated in the presence of varying $[K^+]_o$ (3–85 mM) $CaCl_2$ (1 2 mM) was present in the buffer (B) Steady-state fluorescence of diS-C_2-(5) observed in A plotted as the log of $[K^+]_o$ before (●———●) and after (▲------▲) the addition of valinomycin The relationship obtained in the absence of added Ca^{2+} and valinomycin is shown by (■ ■) When this study was initiated, pilot experiments were carried out with the cyanine dye analog diS-C_3-(5) Thereafter, diS-C_2-(5) was employed since, unlike the former, it was commercially available with high purity Both compounds were essentially indistinguishable in terms of relative changes in fluorescence and response time The same excitation and emission wavelengths were employed for both

relationship above 10 mM K^+, and limiting below this value (Fig 1B)

The steady-state fluorescence achieved after addition of dye to cells (prior to addition of valinomycin) also was noted to be related linearly to $\log[K^+]_o$, over the entire range studied (Fig 1B) It should be noted that this linearity occurred within the range where valinomycin-dependent changes in fluorescence were also a linear function of $\log[K^+]_o$ (i e at $K^+ > 10$ mM) One may employ these data to estimate the plasma membrane potential difference (E_R) under 'resting' or steady-state conditions corresponding to those of cells in culture at the time of harvest This is accomplished by (1) assuming that, in the presence of valinomycin, the electrochemical gradient for K^+ is the major contributor to the plasma membrane potential, and may be approximated by the Nernst equation for K^+ only [19], and, (2) utilizing only the linear portion of the fluorescence versus $\log[K^+]_o$ curve (with valinomycin) which encompasses the data points for fluorescence in the absence of valinomycin (Fig 1B) In the presence of valinomycin, the K^+ permeability far exceeds that of any other ion therefore,

$$E_R \approx E_{K^+} = RT/F \ln[K^+]_o/[K^+]_i$$

where E_{K^+} is the K^+ equilibrium potential Using this relationship one may convert fluorescence units to millivolts over the linear portion of the valinomycin curve (▲------▲, Fig 1B) and interpolate the membrane potential value on the Ca^{2+} curve (———, Fig 1B) at $[K^+]_o = 5$ mM (culture conditions) Assuming $[K^+]_i$ to be 136 mM [23], one may, therefore calculate the E_R under culture conditions Using this approach, E_R was calculated to be -57 ± 6 mV ($n = 4$), which is in close agreement to values obtained for lymphocytes using [^{3}H]TPMP$^+$ [13] and ^{3}H-TPP$^+$ (see Table I, Ref 24 for review)

In the absence of valinomycin, plots of fluorescence versus $\log[K^+]_o$ (Fig 1B) yielded straight lines both in the presence (●———●) and absence of Ca^{2+} (■ ■), however, the difference in slopes (expressed as Δ fluorescence/Δ log $[K^+]_o$) was significant The increase in fluorescence as a function of $\log[K^+]_o$ was steeper (slope = 10 1 ± 2 8) in the presence of 1 2 mM Ca^{2+} than in its absence (slope = 3 3 ± 1 8), suggesting that addition of exogenous Ca^{2+} further increased K^+ permeability If the plasma membrane was impermeable to K^+ a similar plot would yield a straight line with zero slope The data represent the mean ± S E of four separate determinations with sig-

nificance estimated by paired Student's t-test, $P < 0.01$

Effect of EGTA

The divalent cation chelator EGTA was employed to demonstrate that the hyperpolarized state obtained in the presence of low $[K^+]_o$ is a Ca^{2+}-activated phenomenon which was independent of Mg^{2+} EGTA has a high affinity ($10^{7.63}$ M^{-1}, pH 7.4) for Ca^{2+} and a low affinity ($10^{1.84}$ M^{-1}, pH 7.4) for Mg^{2+} [25], so that in the presence of the chelator, approx 99% of the Mg^{2+} present remains unchelated whereas more than 99% of the Ca^{2+} present is associated with chelator [25] Addition of EGTA to hyperpolarized cells (Ca^{2+} present and low $[K^+]_o$) resulted in a rapid depolarization (Fig 2) This is taken to represent significant inactivation of a Ca^{2+}-dependent increase in K^+ permeability since the EGTA-induced change in fluorescence was dependent on $[K^+]_o$ and became progressively smaller at increasing $[K^+]_o$ (Fig 2) These observations could not be explained by loss of integrity of the plasma membrane permeability barrier since subsequent addition of valinomycin resulted in fluorescence changes in the direction of the E_{K^+} in both cases (Fig 2)

Effect of ouabain

When cells were incubated in Na^+-Ringer buffer (Ca^{2+} present) at varying $[K^+]_o$ in the presence of ouabain, no perceptible difference was observed as

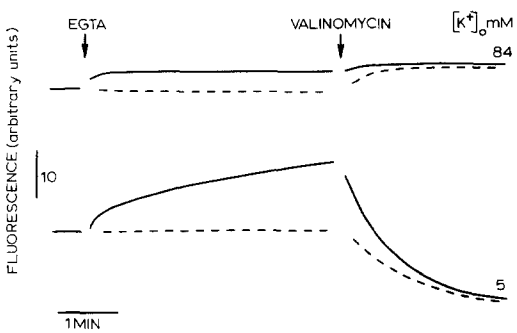

Fig 2 Effect of EGTA on the plasma membrane potential EGTA (1 mM) and valinomycin (0.85 μM) were added, where indicated, to cells preincubated in the presence of low $[K^+]_o$ (5 mM) or high $[K^+]_o$ (84 mM) after achieving steady-state dye fluorescence Dashed line represents fluorescence of the dye in the absence of EGTA Other conditions were the same as those described in the legend to Fig 1(A)

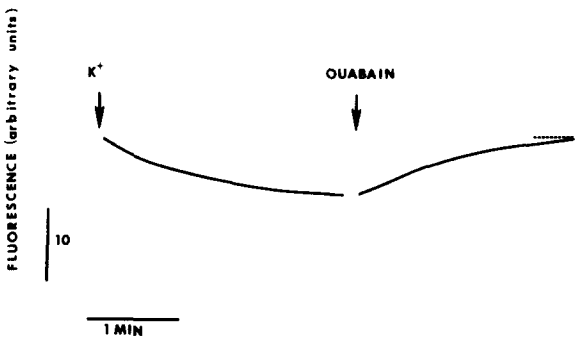

Fig 3 K^+-dependent ouabain-sensitive alteration in the plasma membrane potential Cells were prepared as described in Methods and Materials, washed, resuspended (136.9 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.5 mM KH_2PO_4, and 0.5 mM $MgCl_2$ (pH 7.4) and incubated at 4°C for approx 1 h prior to use A 15-fold dilution of the cells was then made into K^+-free Ringer buffer supplemented with 10 mM D-glucose K^+ (6 mM) and ouabain (1 mM) were added, where indicated, after achieving steady-state dye fluorescence Dashed line represents steady-state dye fluorescence in the absence of added K^+

compared with the control (i e data shown in Fig 1B) Therefore, Ca^{2+}-dependent changes in membrane potential as a function of $[K^+]_o$ appeared to be independent of the ouabain-sensitive Na^+,K^+ pump Nevertheless, it was possible to demonstrate ouabain-sensitive Na^+,K^+ pump activity with these cells After approx 1 h at 4°C, the cells were diluted 15-fold into warm K^+-free Na^+-Ringer buffer Dye was added and fluorescence allowed to reach a steady state (Fig 3) Under these conditions, the Na^+,K^+ pump was inhibited due to insufficient $[K^+]_o$ since addition of exogenous K^+ to initiate pump activity produced a hyperpolarization which was reversed by addition of ouabain (Fig 3) Thus, these findings indicate the presence of a functional electrogenic Na^+,K^+ pump in the plasma membrane of these cells

Effect of trifluoperazine

Trifluoperazine [10,11,14,15,26] and related phenothiazines [27] have been reported to block the Ca^{2+}-dependent increase in K^+ permeability in other tissues A similar inhibitory effect of trifluoperazine also was observed in the present study The addition of trifluoperazine reversed the hyperpolarized state induced in cells suspended in Na^+-Ringer buffer (Ca^{2+} present) at low $[K^+]_o$ (Fig 4) The inhibitory effect of trifluoperazine was dose-dependent and saturable between 2 to 30 μM The

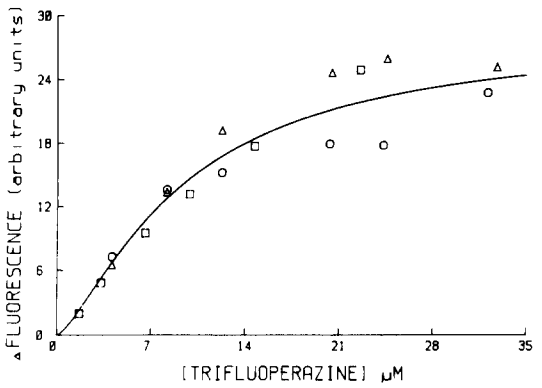

Fig 4 Trifluoperazine-dependent depolarization of the plasma membrane potential Changes in the steady-state dye fluorescence were monitored upon the addition of trifluoperazine to cells preincubated in the presence of low $[K^+]_o$ (5 mM) Conditions were the same as those described in the legend to Fig 1(A) The means from three separate preparations are shown The curve was obtained by fitting the data to the Hill equation using our adaptation (Long, J W , Jr and Vorbeck, M L unpublished data) of the method of Atkins [28]

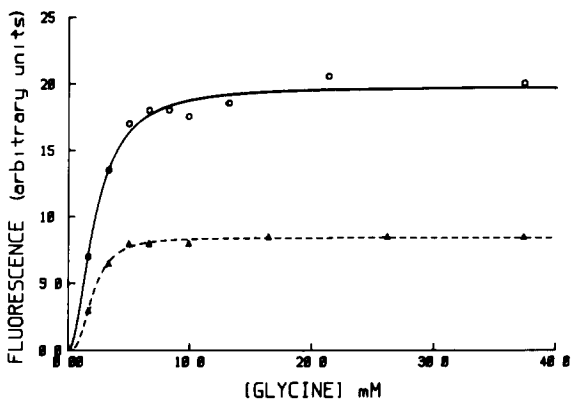

Fig 5 Ca^{2+}-enhanced glycine-dependent depolarization of the plasma membrane potential Changes in steady-state dye fluorescence were measured following the addition of glycine to cells preincubated in the presence (○———○) and absence (▲------▲) of added Ca^{2+} (1 2 mM) Experimental details were as described in the legend to Fig 1 with $[K^+]_o = 10$ mM, and $[Na^+]_o = 80$ mM

IC_{50} was determined to be 9 4 μM trifluoperazine Data from the present studies suggest (1) the presence of a Ca^{2+}-activated K^+ channel in the plasma membranes of these cells, and (2) that a Ca^{2+}-dependent regulatory protein, for example calmodulin, may play an important role in activation of this K^+ specific permeability

Effect of Na^+-dependent amino acid uptake on membrane potential

The addition of glycine to cells suspended in Na^+-Ringer buffer (Fig 5) produced a concentration-dependent increase in fluorescence (depolarization) similar to observations made with Ehrlich ascites tumor cells [19] Similar results were obtained with L-proline (data not shown) Both these amino acids are cotransported with Na^+ (system A) and the driving force for mediated uptake of glycine is derived from the electrochemical gradient for Na^+ [29] Substitution of choline for Na^+ resulted in no change in fluorescence upon addition of glycine Furthermore, the addition of ϵ-aminocaproic acid, which enters the cell via a Na^+-independent facilitated transport pathway, also gave no change in fluorescence These observations indicate that uptake of Na^+-dependent amino acids in these myeloma cells is an electrogenic process

Assuming that increases in fluorescence with addition of increasing concentrations of glycine reflect the rate of uptake of the amino acids, one may evaluate the data using standard kinetic analysis and obtain values for the $K_{0.5}$ and the V_{max} These are given in Table I The presence of 1 2 mM exogenously added Ca^{2+} had a highly significant effect on the apparent electrogenic uptake velocity of glycine (see also Fig 5) The apparent maximum velocity was doubled in the presence of Ca^{2+} whereas the $K_{0.5}$ was not significantly affected

Effect of $[K^+]_o$ and Ca^{2+} on glycine-dependent membrane potential changes

If the ionic basis of the membrane potential is

TABLE I

EFFECT OF Ca^{2+} ON KINETIC PARAMETERS FOR THE ELECTROGENIC UPTAKE OF GLYCINE

Data obtained from Hill analysis of the data See legends to Fig 5 and 6 for experimental details Data expressed as the mean ± S E ($n = 4$)

Kinetic parameter	Exogenous Ca^{2+} added		Significance
	None	1 2 mM	
$K_{0.5}$ (mM)	4 4±1 9	3 5±0 6	n s
V_{max} (arbitrary fluorescence units)	9 3±1 2	18 7±2 7	$P < 0.01$

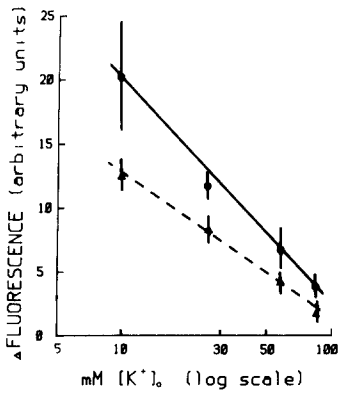

Fig 6 Influence of Ca^{2+} on glycine-induced K^+-dependent depolarization of the plasma membrane potential Changes in K^+-dependent steady-state dye fluorescence were measured following the addition of glycine (13 1 mM) and plotted as the log of $[K^+]_o$ in the presence (●———●) and absence (▲------▲) of added Ca^{2+} (1 2 mM) Experimental details were as described in the legend to Fig 1 $[Na^+]_o$ was maintained at 80 mM

primarily due to K^+ permeability in the presence of extracellular (exogenously added) Ca^{2+}, then the driving force for Na^+-dependent amino acid uptake, an electrogenic process, should be directly related to the magnitude of the plasma membrane K^+ gradient Furthermore, the glycine-dependent depolarization (ΔF_{gly}) should be a linear function of $\log[K^+]_o$ as predicted by the Nernst equation for K^+

As predicted, the extent of depolarization was a linear function of $\log[K^+]_0$ both in the presence and absence of added Ca^{2+} (Fig 6) However, an enhancement of glycine-induced depolarization was obtained as evidenced by a steeper slope in cells preincubated and suspended in Ca^{2+}-containing buffers

The rate and degree of depolarization by glycine was greatest when Ca^{2+} was present, conditions under which the Ca^{2+}-dependent K^+ channels were presumably maximally activated This suggests that Ca^{2+} had a direct effect on the plasma membrane potential and consequently the electrical component of the Na^+ electrochemical gradient since $[Na^+]_o$ was held constant at 80 mM The presence of Ca^{2+} in the extracellular mileau therefore modified the driving force for entry of Na^+-dependent amino acids to a greater extent simply by increasing the plasma membrane's permeability to K^+

Effect of quinine

Preincubation of cells in the presence of quinine, a known Ca^{2+}-activated K^+ channel antagonist [30], resulted in a small depolarization of the membrane potential (13 9 ± 4 3% depolarization with 0 75 mM quinine) Such a reduction in the transmembrane potential also would be expected to result in a decrease in the uptake of glycine by hyperpolarized cells As predicted, glycine induced changes in steady-state dye fluorescence of hyperpolarized cells were inhibited by quinine (25 4 ± 6 8% inhibition with 0 75 mM quinine)

Discussion

Plasma membrane K^+ channels whose activity is regulated by intracellular Ca^{2+} have obtained considerable attention recently for several reasons First, they may serve as the basis for explaining well known K^+ transients linked to stimulus secretion coupling such as those observed in exocrine glands [2] and other cellular activation events such as concanavalin A stimulation of capping and volume regulation in lymphocytes [13,15] and chemotactic peptide stimulation of phagocytosis in macrophages [9] Second, they may provide a fundamental rectification current, thus maintaining the plasma membrane in an electrically receptive steady-state analogous to the K^+-dependent rectification of the action potential in nerve and muscle Third, regulation of these K^+ channels may play a pivotal role in the transduction of humoral signals [1,2,16] Finally, as addressed in the present study, their activation may directly modify Na^+-dependent amino acid uptake by increasing the electrochemical gradient (through plasma membrane hyperpolarization) for Na^+, thereby suggesting a mechanism whereby the composition of cellular amino acid pools may be modulated by Ca^{2+}-dependent control of membrane permeability Interestingly, the majority of essential amino acids are accumulated in a Na^+-dependent manner

A discussion concerning the efficacious use of cyanine dyes for monitoring membrane potentials has been reviewed recently [22] Also, the fact that, under conditions employed in this study, the fluorescence signal reports the plasma membrane potential rather than that across the inner

mitochondrial membrane, has been discussed elsewhere [22] diS-C_3-(5) had been shown to block the Ca^{2+}-activated K^+ channel of the erythrocyte plasma membrane under conditions of zero $[K^+]_0$ [31] However, this complication was obviated by including 0 5 mM KCl which would overcome the inhibitory effect diS-C_3-(5) also has been reported to be toxic to cells by lowering ATP levels via inhibition of mitochondrial oxidative phosphorylation at site I [32] In an effort to address this issue, Rink et al [23] assessed the membrane potential in lymphocytes using diS-C_3-(5), and the relatively non-toxic fluorescent probe, bis-oxonol They determined that results with bis-oxonol supported those obtained with diS-C_3-(5), and calibrated the lymphocyte membrane potential at -60 mV using the K^+/valinomycin null point method of Laris et al [19] Myeloma cells are neoplastic lymphocytes and our value for the plasma membrane potential is virtually identical to that of normal lymphocytes, being -57 ± 6 mV, obtained by using the same calibration technique Interestingly, Rink et al [23], who included 1 25 mM $CaCl_2$ in their buffer, noted a depolarization both when lymphocytes were placed in high $[K^+]_0$ medium and when cells were placed in low $[K^+]_0$ medium but in the presence of 1 mM quinine On the basis of these observations, they inferred the presence of a Ca^{2+}-activated K^+ channel in lymphocytes [23]

The data presented in this communication provide evidence for the presence of a Ca^{2+}-activated K^+ channel in the plasma membrane of the mouse myeloma cell These channels appear to impose a fundamental influence on the plasma membrane potential which depends upon a critical Ca^{2+} threshold of cytoplasmic Ca^{2+} Evidence which supports this contention is obtained from four observations First, the membrane potential, as reflected by fluorescence, was shown to be a linear function of $\log[K^+]_o$, becoming more progressively depolarized at increasing $[K^+]_0$ The rate and degree of depolarization was significantly enhanced in the presence of exogenous Ca^{2+} suggesting a Ca^{2+}-dependent increase in K^+ permeability Second, the Ca^{2+} chelator, EGTA, reversed the hyperpolarization in low $[K^+]_o$, but had little or no effect at high $[K^+]_0$ Third, quinine, a drug showing inhibition of different K^+-channel types including the Ca^{2+} activated variety, depolarized the cell and inhibited the glycine-dependent increase in fluorescence However, the drug for unknown reasons appeared to be less effective as compared with other cell systems [33] and maybe related to its lack of specificity Nevertheless, this also suggests a chose relationship between function of the K^+ channels and electrogenically coupled amino acid transport Finally, the calmodulin antagonist, trifluoperazine, reversed the Ca^{2+}-dependent hyperpolarization in low $[K^+]_o$ medium in a dose-dependent fashion with an IC_{50} in the micromolar range, in agreement with observations made in both adipocyte plasma membrane [26] and sarcolemmal preparations [10] where the IC_{50} values for trifluoperazine were 8 μM Interestingly, calmodulin directly stimulates Ca^{2+}-activated K^+ permeability in plasma membrane vesicles of erythrocytes [4] and adipocytes [26] It therefore appears that this ubiquitous Ca^{2+}-dependent regulatory protein also controls Ca^{2+}-dependent gating of K^+ in the myeloma cells

We next explored the effect of Na^+-cotransported amino acids, particularly glycine, on the plasma membrane potential Glycine addition depolarized the cells in a concentration-dependent fashion, yielding a $K_{0\,5}$ of 3–4 mM, if one assumes that glycine induced depolarization is proportional to uptake rate of the amino acid This is a reasonable assumption since, in a similar study with Ehrlich ascites tumor cells, a $K_{0\,5}$ of 3–4 mM was obtained for glycine utilizing results from diS-C_3-(5) fluorescence [19] Furthermore, this value agreed with the $K_{0\,5}$ obtained from [^{14}C]glycine uptake studies in the same cell type [34]

Since Na^+-dependent amino acid uptake was demonstrated to be electrogenic, we tested the hypothesis that glycine induced depolarization may be changed in a predictable fashion in response to manipulation of the membrane potential via changes in $[K^+]_0$ in the presence of Ca^{2+} The data presented demonstrate that the plasma membrane potential of myeloma cells may be altered in a K^+-dependent manner in the presence of Ca^{2+} In conjunction, the extent of depolarization by the Na^+-cotransported amino acid glycine, which represents electrogenic uptake, may also be altered in a K^+-dependent manner both in the presence and absence of exogenously added Ca^{2+} Apparently,

the K^+ channels are active in both cases, but are activated to a greater degree upon exogenous addition of Ca^{2+}. Ordinarily, endogenous Ca^{2+} present in standard 'Ca^{2+} free' buffers is in the micromolar range. Intracellular Ca^{2+} in these cells is probably in the submicromolar range. Therefore, even in the absence of added Ca^{2+}, the electrochemical gradient for Ca^{2+} is still inwardly directed with a total driving force of approx. 80 mV, as compared with that of approx. 160 mV in the presence of Ca^{2+} (1.2 mM). Passive entry of Ca^{2+} may therefore activate the Ca^{2+}-dependent K^+ channels, but more so in the latter case. Furthermore, the $K_{0.5}$ for Ca^{2+} activation of the K^+ channels may be as low as 30 nM free Ca^{2+}, suggesting that they may be spontaneously active to some degree [26].

There have been reports that changes in plasma membrane potential result in changes in thymidine incorporation into DNA of Chinese hamster ovary cells [35,36]. Interestingly, the changes in membrane potential were induced simply by modifying the $[K^+]_o$ in the medium bathing the cells. Increases in $[K^+]_o$ resulted in a depolarization and a reduced rate of cell proliferation. Our observations provides a mechanism whereby changes in membrane potential could regulate amino acid uptake and content of cellular amino acid pools. Availability of substrate for protein synthesis could conceivably provide a means of growth regulation perhaps through control of the cell cycle. Although these suggestions are clearly hypothetical, modulation of the Ca^{2+}-activated K^+ channel by hormonal or other humoral factors could be a fundamental link to their ultimae control of intracellular amino acid metabolism.

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