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THE EFFECT OF THE FLUORESCENT PROBE, 3,3'-DIPROPYLTHIODICARBOCYANINE IODIDE, ON THE MEMBRANE POTENTIAL OF EHRLICH ASCITES TUMOR CELLS

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SUMMARY: The effects of the potential-sensitive fluorescent dye, 3,3'dipropylthiodicarbocyanine iodide, on factors establishing the membrane potential of Ehrlich ascites tumor cells have been tested. The dye itself induces membrane hyperpolarization as monitored by electrophysiological methods. In addition, the dye inhibits active (Na^++K^+)-transport and increases cell membrane permeability to K^+ by about 65% in these cells.

INTRODUCTION

The value of the membrane potential of Ehrlich ascites tumor cells has not been established unequivocally. Estimates of the potential achieved by using potential-sensitive fluorescent dyes (1-3) and intracellular microelectrodes (4-6) are not in good agreement. Application of fluorescent dyes usually yields values in the range -50 to -60 mV (1-3), although with different treatment of the cells, lower potentials (-18 to -42 mV) have been found (1). Direct measurements by microelectrodes give values in the range -20 to -30 mV in the presence of Ca^{+2} (4-6). In general, the potentials recorded with microelectrodes have not been stable. This suggests that penetration of the cells may damage the membrane sufficiently to prevent the full reflection of the potential.

Both of these methods show, however, that membrane ionic permeability and active (Na^++K^+)-transport contribute to the development of the membrane potential (1,6). The recent finding that the fluorescent dye, 3,3'-dipropylthiocarbocyanine iodide [di S-C_3-(5)] causes a rapid decline in cellular ATP levels (7) raises the possibility that the dye itself might influence the membrane potential. Furthermore, this dye alters several of the rate

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constants describing cation fluxes in human red blood cells (8), again suggesting an effect of this dye on factors establishing the potential.

We have now developed techniques which yield stable measurement of the membrane potentials in Ehrlich ascites tumor cells using intracellular microelectrodes. The stability of the recordings is in contrast to previous studies using electrophysiological methods (4-6). The experiments reported here were carried out to assess the effects of the fluorescent dye, 3,3'-dipropylthiodicarbocyanine iodide on the cell membrane potential, active (Na^++K^+)-transport and membrane cation permeability. The results indicate that this potential probe does itself hyperpolarize the membrane. The hyperpolarization reflects an increase in membrane permeability to K^+. In addition, the dye also inhibits active cation transport after 10-20 min exposure.

MATERIALS AND METHODS

Cell Suspension. Experiments were performed on Ehrlich-Lettre ascites tumor cells (hyperdiploid). Cell suspensions were prepared as previously described (6). The suspension medium contained 154 mM NaCl, 6 mM KCl, 2 mM $CaCl_2$ and 10 mM HEPES-NaOH (pH 7.4; 300 mOsm). All experiments were carried out at 22-24°C.

Chemicals. The fluorescent dye, 3,3'-dipropylthiodicarbocyanine iodide, was the kind gift of Dr. Charles Levinson, University of Texas Health Science Center at San Antonio. Stock solutions of the dye (0.5 mg/ml) were prepared in ethanol.

Membrane Potential. Membrane potential measurements were made using conventional Ling-Gerard glass microelectrodes. The apparatus and techniques applied have been described in detail (6). For this study, however, we have used 2 M potassium acetate instead of KCl to fill the electrodes.

Effect of the Dye on Cellular Na^+ and K^+. To test the effect of the dye on cellular cation contents, steady state cell suspension was split into two portions. To one portion stock dye solution was added (dye concentration = 3×10^{-6} M; ethanol = 0.35%). An equivalent amount of ethanol was added to the control cells. At specified times (5-60 min) aliquots (1.0 ml) of the cell suspensions were added to preweighed, 1.5 ml capacity polyethylene centrifuge tubes and centrifuged (1 min, 15000xg). The resulting supernatants were carefully aspirated and the cell pellet weighed. The cell pellets were mixed with 1.0 ml of 1% (V/V) perchloric acid and incubated for at least one hour. Na^+ and K^+ contents of the percloric acid extracts, cell water and dry weight were determined as previously described (6). Correction of the total Na^+ and K^+ contents for that trapped in the extracellular space of the pellets was made from regression lines relating extracellular space ($[^{14}C]$ inulin space) to pellet wet weight.

Effect of the Dye on Passive Na^+ and K^+ Movements. To assess the effect of the dye on the leak fluxes of Na^+ and K^+, we incubated cell suspension with ouabain (2 mM) for 45 min prior to addition of the dye. Following this pre-treatment, the inhibited cell suspension was split. Dye was added to one half (final concentration = 3.0×10^{-6} M), while the carrier ethanol was added to the control. After 30 min and 60 min aliquots of both portions were taken for analysis of Na^+, K^+, wet and dry weights as described above.

RESULTS AND DISCUSSION

Direct measurements of the membrane potential of Ehrlich ascites tumor cells using microelectrodes filled with potassium acetate (2M) yield recordings which rapidly attain stable values. The average stable potential is -23.3 ± 1.8 mV (n=14). This is in good agreement with peak values of unstable recordings previously reported (4-6). To test the effect of the dye, 3-3'-dipropylthiodicarbocyanine iodide, on the potential, a stable recording from a cell was achieved and followed for at least 1 min to establish the control value. Dye was then added to the suspension medium and the potential followed until a new stable value was reached. The results of a typical experiment are illustrated in Fig. 1. Addition of dye to the cell induces membrane hyperpolarization. The hyperpolarization

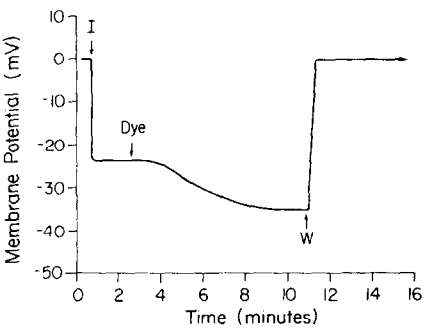
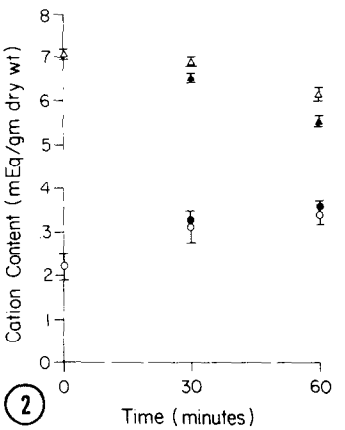


Figure 1: Representative membrane potential recording from an Ehrlich ascites tumor cell. The potential shown is that of the microelectrode relative to an indifferent electrode in the environment. The cells used for potential measurements were placed in glass Petri plates (diameter = 10 cm) containing 10 ml environment. The microelectrode was inserted into the cell at I and withdrawn at W. Dye for addition to the test cell was prepared by adding 42 µl stock solution to 2 ml of the environment.

After the cell had been impaled and the stable potential recording obtained (-24 mV), 2 ml of environment containing the dye (final concentration = 3×10^{-6} M) was added at the periphery of the Petri plate. Following a delay of about 1 min (diffusion time?), the membrane slowly hyperpolarized to a new stable level (-35.2 mV). Fresh Petri plates were used for each cell tested.

begins approximately 1 min after dye addition and develops slowly (4-10 min) to the new stable potential. In 14 cells tested by this protocol, 12 cells hyperpolarized in response to the dye (mean hyperpolarization = 6.0 ± 1.5 mV; range, 2.8 to 11.2 mV). Two of the cells tested depolarized after exposure to the dye. The character of the depolarization was similar to that found in cells which spontaneously depolarized prior to dye addition. Thus, we feel it is likely that these two cells were damaged during the impalement and did not respond normally.

We next tested the effect of the dye on net, passive Na^+ and K^+ movements in cells inhibited by ouabain (2 mM). In one hour, cells treated with ouabain plus the dye lose 64.6% more K^+ than the pump-inhibited cells (Fig. 2). There is no significant alteration in Na^+ gain by the dye-treated cells. This reflects a dye-induced increase in membrane perme-



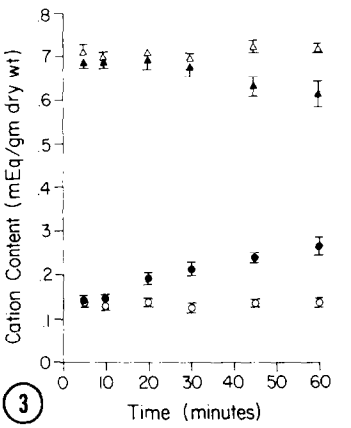


Figure 2. Effect of 3,3'-dipropylthiodicarbocyanine iodide on ion contents of transport-inhibited Ehrlich ascites tumor cells. The cells were pre-treated with ouabain (2mM) for 45 min prior to initiation of the experiment. The inhibited cells were then exposed to the dye ($3x10^{-6}$ M; closed symbols) or ethanol (0.35%; open symbols). Na^+ (circles) and K^+ (triangles) contents of the cells were determined after 30 and 60 min. Each point is the mean of eight values. Standard errors of the means are shown.

Figure 3. Effect of 3,3'-dipropylthiodicarbocyanine iodide on cellular Na^+ and K^+ contents of Ehrlich ascites tumor cells. Steady state cells were exposed to the dye (final concentration = $3x10^{-6}$ M; closed symbols) or an equivalent volume of ethanol (0.35%; open symbols) and the cellular Na^+ (circles) and K^+ (triangles) contents determined periodically for 60 min. Each point is the mean of eight measurements. Standard errors of the mean are given unless smaller than the symbol.

bility to K^+. A 65% increase in K^+ permeability would be expected to hyperpolarize the membrane by approximately 11 mV (6). This slightly exceeds the hyperpolarization we have measured.

It has been previously shown that this dye depletes the cells of ATP (7), suggesting the possibility that inhibition of active (Na^++K^+)-transport may also occur. To assess this possibility, we exposed steady state cells to the dye and determined its effects on cellular Na^+ and K^+ concentrations. The dye induces loss of cellular K^+ and accumulation of cellular Na^+ (Fig. 3). These alterations in cellular cation content are apparent 10-20 min after addition of the dye. Since it is generally conceded that electrogenic cation transport contributes to membrane potential development in these cells (1,6), the apparent inhibition of cation transport might be expected to result in membrane depolarization. However, this prediction must be viewed in light of the hyperpolarizing effect of increased K^+ permeability on the potential. The response of the membrane potential will depend upon the resultant of these two factors. The finding (Fig. 1) that the membrane does hyperpolarize in response to the dye infers that at least in the time-course of our measurements, the dominant effect is the increased membrane permeability. This is consistent with the delayed alteration in cellular cation contents (Fig. 3). However, the concurrent inhibition of active (Na^++K^+)-transport may reduce the hyperpolarization even though the cation contents do not measurably change in this period. This net effect would account for our finding that the measured hyperpolarization (6 mV) is less than that predicted from the increase in K^+ permeability (11 mV).

CONCLUSIONS

The minimum requirement for a satisfactory method of measuring membrane potential is that the method itself does not alter the potential. It is generally agreed that in Ehrlich ascites tumor cells the cell membrane potential is established by membrane ionic permeability and active (Na^++K^+)-

transport (1,2,6). The evidence presented above clearly demonstrates that the fluorescent dye, 3,3'-dipropylthiodicarbocyanine iodide, which has been extensively used to estimate the membrane potential in these cells (1,7) alters both of these factors. Indeed, exposure of steady state cells to this dye results in membrane hyperpolarization (Fig. 1).

The hyperpolarization induced by the dye occurs within the time-course necessary for steady fluorescent levels to be established in these cells (1-3). Taken together, the results of this study clearly demonstrate that this fluorescent dye is not a satisfactory monitor of the membrane potential.

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