Transmembrane potential of isolated rat alveolar type II cells

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CASTRANOVA, V., G. S. JONES, AND P. R. MILES. *Transmembrane potential of isolated rat alveolar type II cells.* J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 54(6): 1511–1517, 1983.—Type II cells were isolated from rat lungs by elastase digestion and purified by centrifugal elutriation. The fluorescent dye, Di-S-C_3(5), was used as a probe to monitor transmembrane potential (E_m) of cells suspended in *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES)-buffered medium. With this technique, the E_m of type II cells was estimated to be -27 ± 2 mV. This resting E_m is very close to the equilibrium potential for chloride (−21 mV), which suggests that chloride is passively distributed in type II cells. The resting E_m of type II cells is more dependent on the extracellular concentration of potassium (K^+) than on external sodium (Na^+); i.e., the membrane depolarizes as external sodium is replaced by potassium, suggesting that in unstimulated type II cells the membrane is more permeable to potassium than to sodium. In addition, the resting potential appears to be due, in part, to the activity of a ouabain-sensitive, Na-K pump, which acts to hyperpolarize type II cells. Addition of a membrane perturbant, phorbol myristate acetate (PMA, 10 μg/ml), to a type II cell suspension results in an increase in oxygen consumption and membrane depolarization. Both of these responses are sodium dependent and thus appear to be linked to a PMA-induced increase in sodium permeability.

membrane potential; electrogenic pump; ouabain; Di-S-C_3(5); phorbol myristate acetate; oxygen consumption

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ALVEOLAR TYPE II PNEUMOCYTES are granular cells located in the epithelial lining of the alveoli (27). These cells are characterized by the presence of lamellar bodies which are intracellular storage sites for pulmonary surface-active materials (11). One important function of type II cells is the synthesis and secretion of surfactant which decreases the work required to inflate the lungs and prevents edema by decreasing the surface tension at the air-liquid interface of the lungs (16, 17, 26). In addition, type II cells play an important role in recovery of the lung from oxidant injury. After oxidant-induced damage to type I epithelial cells, type II cells proliferate and differentiate into type I cells (1, 9). In this manner type II cells aid in regeneration of the alveolar epithelium.

The recent development of methods for the isolation and purification of alveolar type II cells has greatly facilitated the characterization of this cell type. The majority of these investigations have been concerned with the synthesis and release of pulmonary surfactant materials (2, 3, 8). Recently studies have been conducted to characterize the ionic content, various membrane transport properties, and metabolic parameters of isolated type II cells (5, 6, 10). The objective of this investigation was to determine the resting transmembrane potential (E_m) of isolated rat alveolar type II cells and to investigate factors that alter this potential.

METHODS

Isolation of type II cells. Type II cells were isolated by elastase digestion and purified by centrifugal elutriation as described previously (13). Briefly male Sprague-Dawley rats (200–300 g) were anesthetized with pentobarbital sodium (65 mg/kg body wt), and the heart and lungs were removed en bloc. Lungs were perfused with 0.9% NaCl to remove blood cells. Free alveolar macrophages were removed by lavaging the lungs with ice-cold, calcium-free, phosphate-buffered medium [(in mM) 145 NaCl, 5 KCl, 9.35 Na_2HPO_4, 1.9 NaH_2PO_4, and 5.5 glucose; pH 7.4]. Lungs were filled with elastase solution [40 U/ml type I elastase and 0.006% DNase (Sigma Chemical, St. Louis, MO) in phosphate-buffered medium] and incubated at 37°C for 30 min to free lung cells. After enzymatic digestion, lungs were minced with a tissue chopper (slice thickness 0.5 mm), and digestion was arrested by incubation at 37°C for 10 min in 20 ml of inhibitor solution (25% fetal calf serum and 0.006% DNase in phosphate-buffered medium). The suspension was then strained through nylon mesh and loaded into an elutriator (Beckman model J-21 centrifuge equipped with a model JE-6 rotor; Beckman Instrument, Fullerton, CA) at a flow rate of 10 ml/min and a rotor speed of 2,000 rpm. Then 200 ml of phosphate-buffered medium containing 0.5% bovine serum albumin were infused through the elutriator at this flow rate and rotor speed to remove cell fragments and small pneumocytes. Type II cells were then recovered at a flow rate of 18.9 ml/min and a rotor speed of 2,000 rpm. The type II cell-enriched fraction was then centrifuged at 1,000 *g* for 5 min at 2°C and the supernatant removed by aspiration. Type II cells were suspended in *N*-2-hydroxethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES)-buffered medium [(in mM) 145 NaCl, 5 KCl, 1 $CaCl_2$, 10 NaHEPES, and 5.5 glucose; pH 7.4] for experimental investigation. In some experiments the potassium concentration of this medium was altered by the equimolar substitution of KCl for NaCl.

The number of cells in the type II-enriched fraction was determined with a Coulter model ZB electronic cell counter (Coulter Instrument, Hialeah, FL). Type II cells were identified by fluorescent microscopy using phosphine 3R (18) employing a method described previously (13). Briefly phosphine 3R (final concn 0.002%) was added to cells suspended in HEPES-buffered medium. After 2 min, 40 μl of this suspension was viewed under a fluorescence microscope set at an absorption peak of 477 nm and an emission peak of 512 nm. Both type II cells (i.e., cells containing distinct yellow or yellow-green lamellar bodies) and the total number of cells were counted. Purity, calculated in this manner, was at least 85%. Minor contaminants in the type II cell preparation were polymorphonuclear leukocytes, alveolar macrophages, and some unidentified cells.

Measurement of oxygen consumption. Oxygen consumption was measured with a Gilson K-IC oxygraph equipped with a Clark electrode (Gilson Medical Electronics, Middleton, WI). Type II cells were preincubated in HEPES-buffered medium for 30 min at 37°C to equilibrate ionic content and cellular volume (13). After equilibration, 1×10^7 type II cells were suspended in 1.7 ml of HEPES-buffered medium. This suspension was transferred to a temperature-controlled chamber (37°C) equipped with a stirrer, and oxygen consumption was measured for 10 min. The oxygraph was calibrated using media equilibrated with gases of known oxygen content. The oxygen consumption of unstimulated type II cells was approximately 215 ± 43 nmol $O_2 \cdot 10^6$ $cells^{-1} \cdot h^{-1}$. Addition of sodium succinate (final concn 5 mM) to these cell suspensions had no effect on oxygen consumption. Therefore the type II cell preparations were viable, and the cellular membranes were intact.

Measurement of transmembrane potential. E_m was measured using the fluorescent dye, 3,3′-dipropylthiodicarbocyanine iodide [Di-S-C_3(5)], as originially described by Hoffman and Laris (12). Fluorescence was measured with a fluorescence spectrophotometer (model MPF-3L, Perkin-Elmer, Norwalk, CT) fitted with a magnetic stirrer. Excitation and emission wavelengths were set at 622 and 665 nm, respectively. All fluorescence measurements were made at 22°C. We have used this method previously to measure the E_m of other lung cells, e.g., alveolar macrophages (4, 20). With this probe, an increase in fluorescence indicates membrane depolarization while hyperpolarization results in a decrease in fluorescence (4, 12).

Type II cells were preincubated for 30 min at 37°C in HEPES-buffered medium to equilibrate ionic content and cellular volume (13). After equilibration, type II cells (5.75×10^6) were suspended in 3 ml of HEPES-buffered media containing various concentrations of potassium. Di-S-C_3(5) (final concn 0.66 μg/ml) was added to each cell suspension, and the samples were allowed to equilibrate at 22°C until the fluorescence level was steady. To measure resting E_m, the potassium ionophore, valinomycin (final concn 1×10^{-6} M), was added to each sample and the change in fluorescence was monitored for cells suspended at each extracellular potassium concentration. In this manner, the potassium null point (i.e., the extracellular potassium concentration at which there was no fluorescence change in response to valinomycin) was determined. At the potassium null point E_m can be estimated as the potassium equilibrium potential using the Nernst equation as follows

$$E_m = 58 \log[K]_o^*/[K]_i$$

where $[K]_o^*$ is the extracellular potassium concentration (mmol/l) at the null point and $[K]_i$ is the intracellular potassium concentration for type II cells. The value used for $[K]_i$, i.e., 107 mmol/l of cell water, has been reported previously (13). We have used the potassium null point method to estimate E_m for various cellular systems, such as alveolar macrophages, granulocytes, and red blood cells (4, 14, 23). In these cellular systems, Di-S-C_3(5) had little effect on ionic content, cellular volume, metabolism, and membrane permeability (4, 14, 23). In addition, we find that Di-S-C_3(5) has no significant effect on oxygen consumption by type II cells. Therefore our evidence suggests that this fluorescent probe can be used safely to measure E_m in type II cells.

RESULTS

The E_m of rat alveolar type II cells was determined using the fluorescent probe, Di-S-C_3(5). With this technique, fluorescence decreases as E_m becomes more negative (hyperpolarization) and increases as E_m changes in the positive direction (depolarization). The data in Fig. 1 indicate a shift in fluorescence, and thus E_m, when type II cells are made permeable to potassium by the addition of valinomycin (1×10^{-6} M). At external K^+ concentrations below 25 mM, valinomycin elicits membrane hyperpolarization, while depolarization occurs at higher extracellular K^+ concentrations. These data can be plotted as fluorescence changes in response to valinomycin vs. the extracellular K^+ concentration to calculate the potassium null point; i.e., the external K^+ concentration where there is no fluorescence change in response to valinomycin (Fig. 2). At the potassium null point, E_m can be estimated as the equilibrium potential for K^+ calculated using the Nernst equation as described in METHODS. The resting E_m of rat alveolar type II cells obtained in this manner is −27 ± 2 (SE) mV (for 3 different cell preparations).

In most mammalian cell types, chloride ions move across the plasma membrane by passive transport. For this reason, the equilibrium potential for chloride is a reflection of E_m. Therefore the effectiveness of Di-S-C_3(5) as an E_m probe in type II cells can be verified by measurement of the distribution of chloride across the plasma membrane. We have reported previously (15) that when suspended in HEPES-buffered medium the intracellular concentration of chloride in type II cells is 70 mM. Therefore the equilibrium potential for chloride is −21 mV for type II cells. This estimate of E_m is in good agreement with a measurement using Di-S-C_3(5) and suggests that Di-S-C_3(5) is an accurate probe of E_m in type II cells.

Data from Fig. 1 can also be used to gain information concerning the relative permeabilities of sodium and potassium in resting type II cells. These data indicate that base-line fluorescence intensity (i.e., the steady-state

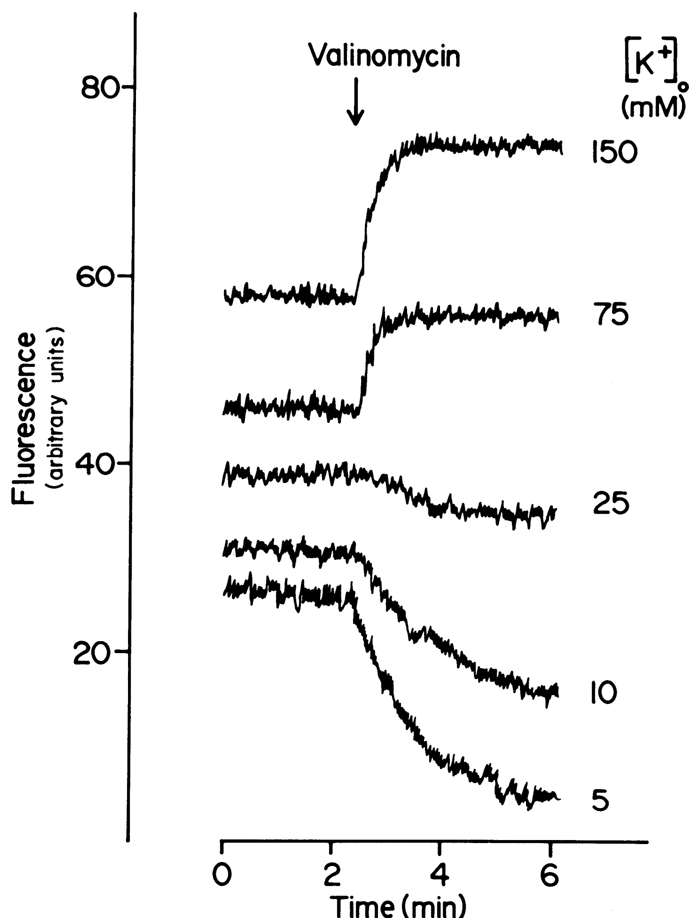

FIG. 1. Time course of fluorescence shifts resulting from addition of valinomycin (1×10^{-6} M) to type II cells suspended in media of various potassium concentrations. Type II cells were preincubated in HEPES-buffered medium for 30 min at 37°C to equilibrate ionic content and cellular volume. After equilibration type II cells (5.75×10^7 cells) were centrifuged and resuspended in 3 ml of medium of a given potassium concentration. Di-S-C_3(5) was added to the cell suspensions at a final concentration of 0.66 μg/ml and the fluorescence level recorded at 22°C with an excitation wavelength of 622 nm and an emission wavelength of 665 nm. Increase in fluorescence indicates depolarization; decrease indicates hyperpolarization. These data are from single determination typical of 3 experiments.

fluorescence level before the addition of valinomycin) increases (i.e., the membrane depolarizes) as the external K^+ concentration is increased and external Na^+ concentration is decreased. Data concerning the ionic dependence of base-line fluorescence are summarized in Fig. 3 and indicate that when external NaCl is replaced with KCl the resting E_m of type II cells varies directly with the equilibrium potential for K^+ (which becomes more positive) and is inversely dependent on that for Na^+ (which becomes more negative). Thus the data indicate that unstimulated type II cells are more permeable to K^+ than to Na^+.

We have shown previously that type II cells possess a highly active ouabain-sensitive Na-K pump (13). To determine whether this pump is electrogenic, we studied the effects of ouabain on the fluorescence of type II cells suspended in the presence or absence of potassium (5 mM). These data are shown in Fig. 4. In the presence of potassium, ouabain (5 mM) results in a 16 ± 0 (SE) % increase in fluroescence, i.e., depolarization (for 3 different cell preparations). Therefore in type II cells the Na-K pump is electrogenic and contributes to the electronegativity of E_m. In contrast, ouabain does not alter the

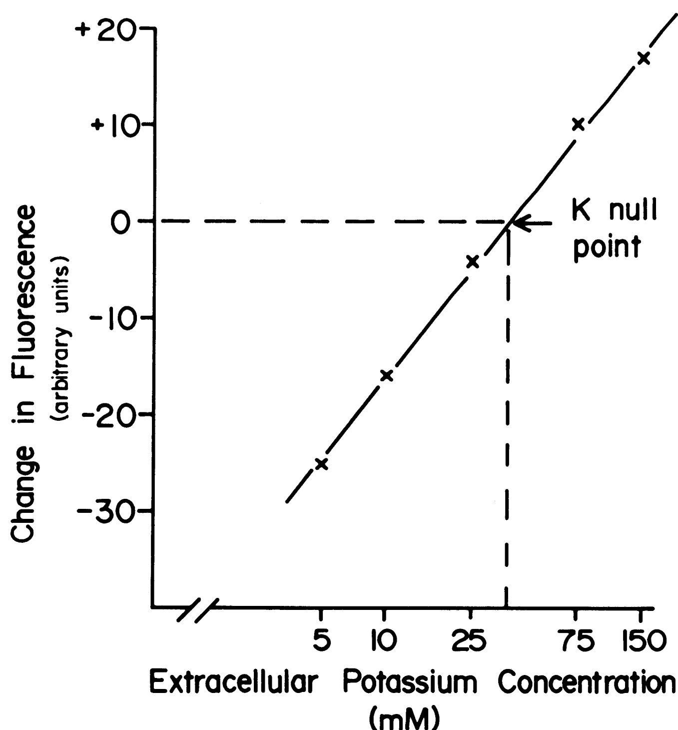

FIG. 2. Determination of potassium null point, i.e., the external potassium concentration at which there is zero change in fluorescence of Di-S-C_3(5) after addition of valinomycin (1×10^{-6} M) to a suspension of type II cells. Potassium null point obtained from this graph is 36 mM, and calculated E_m is −27 mV. These data are from single determination typical of 3 experiments.

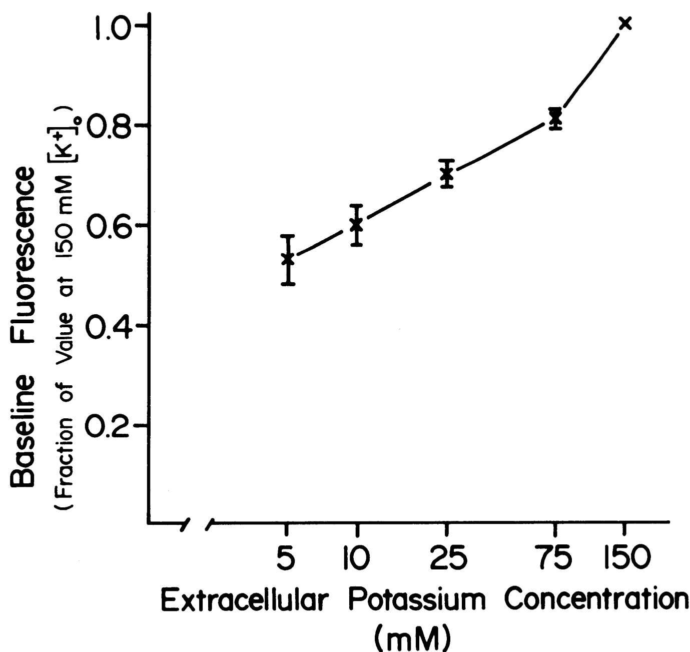

FIG. 3. Relative changes in base-line fluorescence intensity of Di-S-C_3(5), i.e., steady-state fluorescence level before addition of valinomycin, as a function of extracellular potassium level. Data (means ± SE for 3 experiments) were obtained as described in Fig. 1.

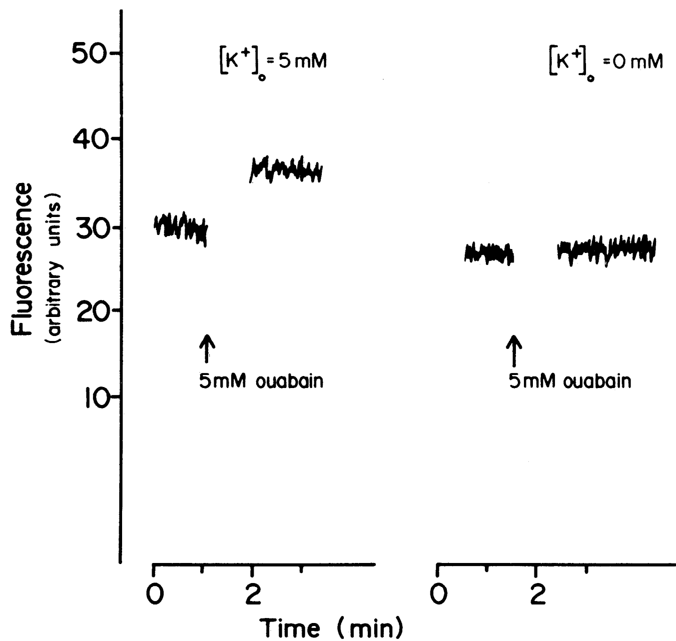

FIG. 4. Effect of ouabain (5 mM) on membrane potential (E_m) of type II cells suspended in presence or absence of potassium. E_m was monitored using fluorescent probe, Di-S-C_3(5), as described in Fig. 1. Increase in fluorescence indicates depolarization. Ouabain was added to cell suspensions as a solid. Breaks in signal represent time required for ouabain to dissolve. These tracings are result of typical experiment. At 5 mM potassium, ouabain induces a 16 ± 0 (SE) % (for 3 experiments) increase in fluorescence, while there is no change in fluorescence signal of cells suspended in potassium-free medium.

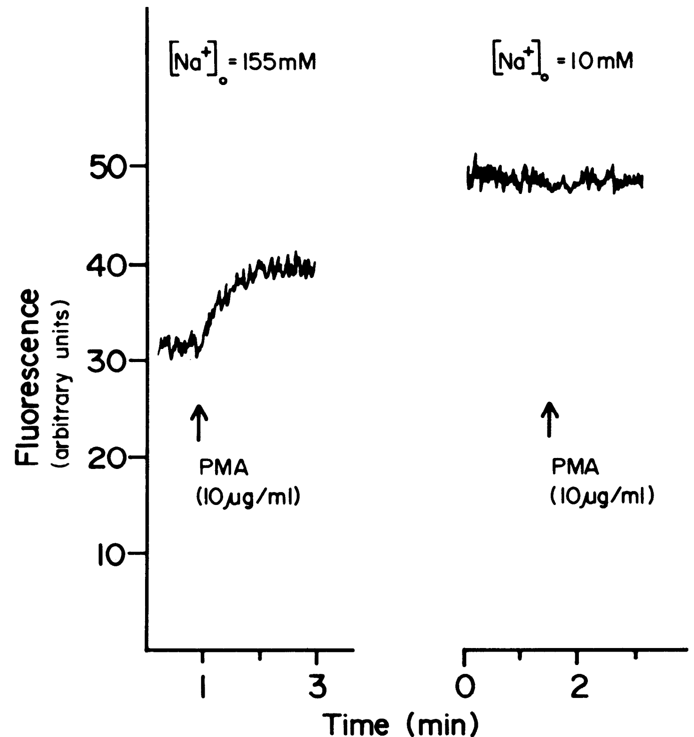

FIG. 5. Effect of phorbol myristate acetate (PMA, 10 µg/ml) on membrane potential (E_m) of type II cells suspended in HEPES-buffered media containing high or low sodium. E_m was monitored using fluorescent probe as described in Fig. 1. Increase in fluorescence indicates depolarization. PMA was added as a liquid. Stock solutions were made by dissolving PMA in 50 µl of dimethyl sulfoxide before bringing solution up to volume with HEPES-buffered medium. These data are from single determination typical of 3 experiments.

fluorescence of type II cells suspended in potassium-free medium. This is to be expected, since the Na-K pump does not function in the absence of external K^+.

We have previously reported that phorbol myristate acetate (PMA) is a membrane perturbant that depolarizes alveolar macrophages and granulocytes and activates the respiratory burst and secretory mechanisms in these phagocytes (15, 20). In addition, phorbol esters induce proliferation of human fibroblasts (22) and the release of disaturated phosphatidylcholines, a component of pulmonary surfactant materials, from isolated alveolar type II cells (7). Therefore it was of interest to study the actions of PMA on type II cells. The effects of PMA on the E_m of type II cells are shown in Fig. 5. PMA (10 µg/ml) causes an increase in fluorescence, i.e., membrane depolarization, when added to type II cells suspended in medium containing normal levels of sodium (155 mM). However, PMA has no effect on E_m when extracellular NaCl is replaced by KCl (Fig. 5). The sodium dependence of the PMA-induced potential has also been demonstrated using choline as a sodium substitute (data not shown). Thus the PMA-induced depolarization seems to be the result of an increase in sodium permeability. These data are summarized in Table 1. The effect of PMA on oxygen consumption by type II cells is shown in Fig. 6. These data indicate that oxygen consumption is increased by PMA. However, as with E_m, this effect is sodium dependent and occurs only at high concentrations of extracellular sodium (Table 1). Whether the effects of PMA on membrane potential and oxygen consumption are directly related is not known.

Experiments were conducted to determine whether enhanced oxygen consumption is a generalized response to depolarization of type II cells. In these experiments valinomycin was added to cells suspended in high potassium medium to cause a membrane depolarization larger than that induced by PMA. However, this potassium-dependent depolarization does not affect oxygen consumption (data not shown). Therefore it appears that enhanced oxygen consumption is associated only with sodium-dependent depolarization of type II cells. Since PMA-induced depolarization is apparently the result of enhanced sodium influx, it is possible that an increase in intracellular sodium would stimulate the Na-K pump and thus metabolism. However, this is not the case, since the PMA-induced increase in oxygen consumption is unaffected by ouabain (data not shown). The absence of an ouabain effect in this case is not surprising, since intracellular sodium in type II cells is normally 51 mM (13). Such a value is much higher than $K_{1/2}$ values reported for Na-K pumps. Thus the pump in type II cells is probably unaffected by the addition of intracellular sodium beyond normal levels. Therefore the data suggest that stimulation of oxygen consumption by PMA is directly associated with an increase in sodium permeability resulting in enhanced sodium influx and is not an indirect response to an increase in the activity of the Na-K pump.

DISCUSSION

In this investigation, we have estimated the E_m of

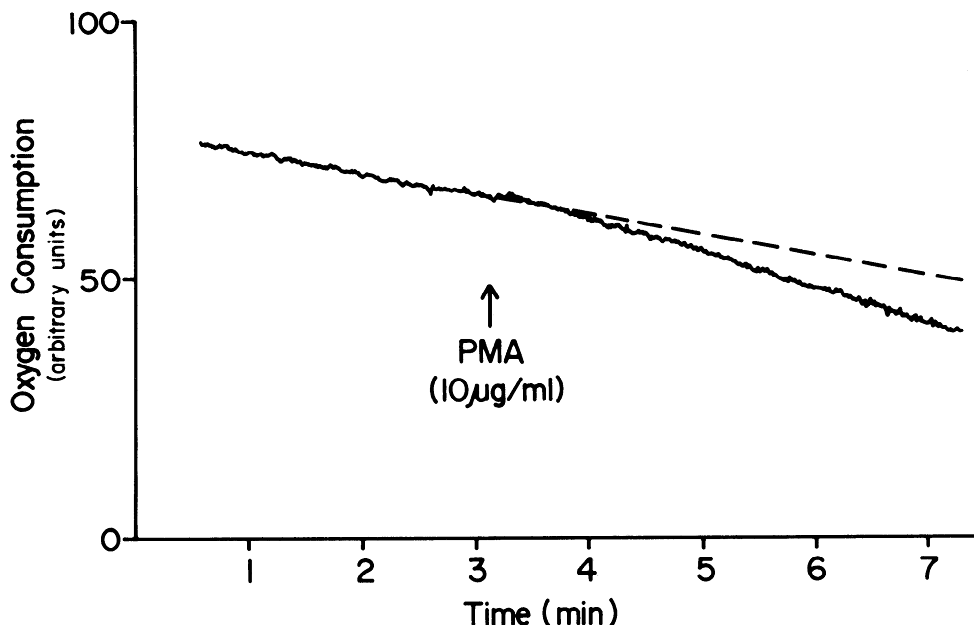

FIG. 6. Effect of phorbol myristate acetate (PMA, 10 µg/ml) on oxygen consumption of type II cells. Type II cells were preincubated in HEPES-buffered medium for 30 min at 37°C to allow equilibration of ionic content and cellular volume. After equilibration type II cells (1×10^7 cells suspended in 1.7 ml of HEPES-buffered medium) were added to temperature-controlled chamber (37°) equipped with stirrer. Oxygen consumption was measured with oxygraph equipped with Clark electrode. These data are from single determination typical of 3 experiments. Oxygen consumption of unstimulated type II cells is approximately 215 ± 43 nmol $O_2 \cdot 10^6$ $\text{cells}^{-1} \cdot h^{-1}$. In this experiment, PMA increased oxygen consumption by 50%.

TABLE 1. *Effect of phorbol myristate acetate on transmembrane potential and O_2 consumption of isolated alveolar type II cells*

External Sodium, mM	PMA-Induced Increase in Fluorescence, %	PMA-Induced Increase in O_2 Consumption, %
155	24.0 ± 8.0	43.3 ± 6.7
10	0.3 ± 0.3	0.0 ± 0.0

Values are means ± SE for 3 expts. Rat type II cells were isolated by elastase digestion and purified by centrifugal elutriation. Cells were preincubated in HEPES-buffered medium for 30 min at 37°C to allow equilibration of ionic content and cellular volume. Transmembrane potential (E_m) was then monitored using the fluorescent probe, Di-S-C_3(5), and oxygen consumption measured using a Clark electrode. Data represent phorbol myristate acetate (PMA)-induced changes from resting levels for type II cells suspended in HEPES-buffered media containing high or low sodium.

isolated rat alveolar type II epithelial cells using the fluorescent probe, Di-S-C_3(5). At rest the E_m of type II cells is approximately −27 mV and is dependent on the extracellular potassium concentration; i.e., the membrane depolarizes as external potassium is increased. Type II cells possess a highly active Na-K pump which is electrogenic and contributes to the electronegativity of the E_m. This electrogenic pump is inhibited by ouabain or the removal of extracellular potassium. Type II cells can be stimulated by the membrane perturbant, PMA. This activation is characterized by membrane depolarization and an increase in oxygen consumption. Both of these responses are dependent on extracellular sodium.

One way to determine the effectiveness of Di-S-C_3(5) as an E_m probe in type II cells is to compare the value obtained with the fluorescent probe with that obtained from the distribution of chloride across the plasma membrane. In a previous study, we measured the ionic content of type II cells (13). The intracellular concentration of chloride in type II cells is 70 mM, and the equilibrium potential for chloride is −21 mV. Therefore the equilibrium potential for Cl^- agrees well with the estimate of E_m using Di-S-C_3(5), i.e., −27 mV. This suggests that Di-S-C_3(5) is an accurate probe of E_m in type II cells.

It is possible that our estimate of E_m in type II cells is incorrect due to cellular contamination. In the present investigation, the cellular preparation contained 85% type II cells. The major contaminating cells were alveolar macrophages and leukocytes, each representing less than 5% of the cellular sample. We have previously employed Di-S-C_3(5) to estimate the E_m of alveolar macrophages and leukocytes and reported values of −37 and −100 mV, respectively (4, 14). Therefore our estimate for the E_m of type II cells can be easily corrected for the contribution of these contaminating cells. After correction, our estimate of E_m is −24 mV for type II cells. Therefore cellular contamination has only a minor effect on the results.

We have reported previously that type II cells possess a highly active Na-K pump (13). The present investigation indicates that this Na-K pump contributes to the electronegativity of the resting E_m; i.e., ouabain results in a 16% increase in fluorescence. In a previous study, we have shown that the rate of sodium transport is high in type II cells (13). Data from the present investigation (Fig. 3) suggest that type II cells are more permeable to potassium than to sodium. Therefore it is possible that this ouabain-induced depolarization results from inhibition of a nonelectrogenic pump, which results in the collapse of transmembrane gradients for Na^+ and K^+ and thus depolarization. However, this is unlikely for the following reasons. First, the ouabain-induced depolarization rapidly (i.e., within 1 min) reaches a steady potential rather than slowly going to zero potential. Second, using flux values previously reported (13), we estimate that the concentrations of cations would change by less than 4 mM when the Na-K pump is blocked for 1 min, i.e., the time required to monitor the ouabain-induced depolarization. Such concentration changes would result in 1- to 2-mV changes in the equilibrium potentials for Na^+ and K^+ which are in opposite directions and thus would tend to cancel each other. Thus, ouabain-induced depolarization seems to result from inhibition of an actual electrogenic Na-K pump.

It is possible to quantitate the electrogenicity of the

Na-K pump in millivolts if the following assumptions are made: *1*) the Na-K pump is inactivated at 2°C but is reactivated when type II cells are rewarmed to 37°C; *2*) the movement of chloride ions is by passive transport; and *3*) chloride ions attain equilbrium within 30 min, i.e., by the time cellular volume has attained the steady state. If these assumptions are correct, the contribution of the Na-K pump to E_m can be estimated as the difference between the equilibrium potential for chloride at 37 and 2°C. We have previously measured the intracellular concentration of chloride in type II cells incubated at 37 and 2°C (13). From these values, the equilibrium potentials for chloride are −21 mV at 37°C and −18 mV at 2°C. Therefore our estimate of the electrogenicity of the Na-K pump is −3 mV.

Data from Fig. 3 indicate that type II cells depolarize as extracellular Na^+ is replaced by K^+, i.e., E_m varies directly with the equilibrium potential for K^+ and inversely with that for Na^+. This suggests that the permeability of type II cells to potassium (P_K) is greater than to sodium (P_{Na}). Since we have shown previously that the transport rate for sodium is high in these pneumocytes (13), the movement of potassium across the type II cell membrane must be very rapid, i.e., having a half time of minutes. If one assumes that chloride is in equilibrium, the E_m of type II cells can be calculated as follows

$$E_m = \left[58 \log \frac{([K]_o + P_{Na}/P_K[Na]_o)}{([K]_i + P_{Na}/P_K[Na]_i)} \right] + E_{pump}$$

Type II cells contain 107 mM K^+ and 51 mM Na^+ (13). In the present study, we have estimated E_m to be −27 mV and E_{pump} (i.e., the potential due to the Na-K pump) to be −3 mV. Therefore we can solve for the ratio of P_{Na} to P_K. This analysis indicates that P_{Na}/P_K is approximately 0.27; i.e., in type II cells P_K is approximately fourfold greater than P_{Na}.

We have found that PMA induces sodium-dependent membrane depolarization, i.e., an increase in the fluorescence of Di-Si-C_3(5). It is unlikely that this fluorescence change can be an artifact due to a change in the membrane environment and thus a decrease in the lipid solubility of Di-S-C_3(5), because the fluorescence change is sodium dependent, i.e., it does not occur when extracellular Na^+ is replaced by K^+. Such ionic dependence suggests that PMA causes depolarization of type II cells, which is the result of a PMA-induced increase in sodium permeability. It is possible to estimate the magnitude of this PMA-induced depolarization if the relationship between percent change in fluorescence and millivolts is known. We estimated this relationship by using data for the effect of ouabain on fluorescence data (Fig. 4) and the calculation that $E_{pump} = -3$ mV. From this relationship and the PMA data from Table 1, the PMA-induced depolarization would be approximately 4.5 mV. This value can then be used to calculate the ratio of P_{Na} to P_K in PMA-treated type II cells in a manner similar to that described above. The data indicate that treatment of type II cells with PMA increases P_{Na}/P_K from a resting level of 0.27 to 0.33, i.e., PMA causes an increase of 22% in P_{Na}.

Data from Table 1 indicate that PMA causes a 43% increase in oxygen consumption, i.e., an increase of 92 nmol $O_2 \cdot 10^7$ $cells^{-1} \cdot h^{-1}$. PMA also stimulates the oxygen consumption of phagocytes by 100% (data from our laboratory). In the present investigation the type II cell preparation contained less than 5% alveolar macrophages and 5% leukocytes. Therefore these contaminating cells probably contribute to only 6.5% of the increase in oxygen consumption reported in this study, i.e., approximately 6 nmol $O_2 \cdot 10^7$ $cells^{-1} \cdot h^{-1}$. Therefore the PMA-induced increase of oxygen consumption by type II cells is not an artifact due to cellular contamination.

It is intriguing to speculate on the possible physiological role of depolarization of type II cells. To date no studies have been conducted in this area. However, some interesting correlations exist. Our data indicate that PMA activates type II cells; i.e., PMA induces sodium-dependent membrane depolarization and increased oxygen consumption. Dobbs and Mason (7) have shown that phorbol esters enhance the secretion of disaturated phosphatidylcholines (DSPC) by type II cells. Is it possible that PMA-induced secretion of surfactant could be triggered by membrane depolarization or sodium influx? In addition, phorbol esters have also been shown to induce proliferation in cultured fibroblasts (22). This mitogenic activity, i.e., cell proliferation and DNA synthesis, has been related to an increased sodium influx (19, 24, 25) and membrane depolarization (5, 21). Is it possible that sodium-dependent depolarization could trigger the proliferation of type II cells that occurs after oxidant injury to the lungs (1, 9)? There is no doubt that these questions will stimulate future investigation.

In summary, we have estimated the E_m of isolated type II cells and have investigated factors that alter this potential. At this time, the relationship between E_m and known functions of type II cells is yet to be determined.

Di-S-C_3(5) was a gift from Dr. Alan Waggoner, Dept. of Chemistry, Amherst College, Amherst, MA.

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