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# POTASSIUM CURRENTS IN RAT TYPE II ALVEOLAR EPITHELIAL CELLS

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## SUMMARY

1. Type II alveolar epithelial cells isolated from adult rats and grown in primary culture were studied using the whole-cell configuration of the gigohm-seal voltage clamp technique.

2. The average specific capacitance of type II cells was 2·5 $\mu$F/cm$^2$, suggesting that type II cell membranes *in vitro* are irregular, with an actual area more than twice the apparent area.

3. Most type II cells have time- and voltage-dependent outward currents carried by potassium ions. Potassium currents activate with a sigmoid time course upon membrane depolarization, and inactivate during maintained depolarization. The average maximum whole-cell $K^+$ conductance was 1·6 nS.

4. Two distinct types of $K^+$-selective channels underlie outward currents in type II cells. Most cells have currents resembling delayed rectifier $K^+$ currents in skeletal muscle, nerve and immune cells. A few cells had a different type of $K^+$ conductance which is more sensitive to block by tetraethylammonium ions, has faster 'tail currents', and activates at more positive potentials.

5. In some experiments, individual type II cells were identified by staining with phosphine, a fluorescent dye which is concentrated in lamellar bodies. Both types of $K^+$ channels were seen in type II cells identified with this dye.

6. Phosphine added to the bathing solution reversibly reduced $K^+$ currents and shifted $K^+$ channel activation to more positive potentials. Excitation of phosphine to fluoresce reduced irreversibly $K^+$ currents in type II cells. The usefulness of phosphine as a means of identifying cells for study is discussed.

## INTRODUCTION

Type II alveolar epithelial cells produce and secrete surfactant, a substance which prevents alveolar collapse at low lung volumes. Within the last decade techniques have been developed for isolating and maintaining type II cells in short-term primary culture (Kikkawa & Yoneda, 1974; Mason, Williams, Greenleaf & Clements. 1977*b*; Simon, McCoy, Chu, DeHart & Goldstein, 1986). The mechanisms by which surfactant production, secretion, and re-uptake are regulated are incompletely understood. Alterations of membrane potential and transmembrane ion fluxes when

type II cells are stimulated to secrete surfactant *in vitro* provide indirect evidence that ion channels are present and play a role in the function of type II cells (Castranova, Jones & Miles, 1983; Gallo, Finkelstein & Notter, 1984). We describe here use of the whole-cell recording configuration of the 'patch-clamp' technique to discover the properties of ion channels in type II cells.

During the first few days in primary culture type II cells maintain the features which characterize them *in vivo*; they produce and secrete pulmonary surfactant, they contain lamellar bodies in which surfactant is stored and perhaps 'processed' for secretion, and they respond to physiological stimuli (see reviews: Mason, Dobbs, Greenleaf & Williams, 1977*a*; Rooney, 1985). After several days in culture type II cells transform morphologically and functionally: initially spherical, they flatten and enlarge, losing their lamellar bodies and their ability to synthesize and secrete surfactant (Diglio & Kikkawa, 1977; Mason *et al.* 1977*a*; Smith, Kikkawa, Diglio & Dalen, 1980; Messmer, Armour & Holley, 1982; Rooney, 1985). The rate of transformation depends upon culture conditions (Rannels & Rannels, 1986). In the present study most experiments were done on cells during the first week in culture.

Inherent in the study of cells in primary culture is the possibility that in spite of the purification procedures used, a fraction of the cells studied may represent contaminant cell types, e.g. macrophages in the case of type II cells. Ideally one would like to have a means of positively identifying each cell studied. For this purpose we have used the fluorescent dye phosphine (Mason, Williams & Clements, 1975) which has been used widely to identify type II cells in primary culture. This dye is concentrated in lamellar bodies, which are unique to type II alveolar epithelial cells (Weibel, Gehr, Haies, Gil & Bachofen, 1976; Mason *et al.* 1977*a*; Rooney, 1985). When studying cells identified with phosphine, we noticed that the dye itself affects membrane currents. These effects were studied systematically in order to evaluate the usefulness of phosphine as a means of identifying individual type II cells.

Voltage-gated, potassium-selective channels were present in nearly all type II cells studied. Two distinct types of $K^+$ channels were found. Both types of $K^+$ channels open during depolarizing voltage pulses, inactivate upon sustained depolarization, and are blockable by tetraethylammonium (TEA) ions. However, the two channels differ quantitatively with respect to these and other properties. In this paper the main properties of the $K^+$ channels in type II cells are described.

Since the two distinct types of $K^+$ channels found in type II cells appear to be identical with the two types of $K^+$ channels in mouse T lymphocytes (DeCoursey, Chandy, Gupta & Cahalan, 1987*a*), the same nomenclature will be used. Type *n* channels resemble delayed rectifier channels in other cells. Currents through the other channel, type *l*, compared with type *n* $K^+$ currents, (1) activate at about 30 mV more positive potentials, (2) have activation kinetics with subtly different time and voltage dependence, (3) deactivate about 10 times faster (have faster tail currents), (4) inactivate more slowly, (5) recover from inactivation much more rapidly, (6) have larger unitary conductance, (7) are 100 times more sensitive to block by external TEA ions, and (8) are less sensitive to block by $Co^{2+}$ (DeCoursey *et al.* 1987*a*). Of these, the most striking characteristics are (1), (3), (5) and (7). In the present study, classification of the type of channel in a given cell was based upon parameters (1) and (5), confirmed by either (3) or (7).

Preliminary accounts of this work have been communicated in abstract form (Jacobs, Claypool, Silver, Bone & DeCoursey, 1986; Jacobs, Silver, Bone & DeCoursey, 1987; DeCoursey & Jacobs, 1987).

## METHODS

### *Type II cell isolation*

Type II cells were isolated from rats using enzyme digestion, lectin agglutination, and differential adherence. These procedures are described elsewhere (Mason *et al.* 1977*b*; Goodman & Crandall, 1982; Simon *et al.* 1986), but since our technique is a composite of these it will be described in detail. Adult male Sprague–Dawley rats were anaesthetized with sodium pentobarbitone (50 mg/kg), and a tracheostomy performed with a 16 gauge intracatheter via cut-down. The thoracic cavity was opened, and the rat exsanguinated through a left ventricular catheter. To remove the blood the lungs were perfused with 40 ml MEM (minimum essential medium supplemented with Earle's salts and L-glutamine (cat. no. 320–1095, Gibco Laboratories, Grand Island, NY, U.S.A.), and with 10 mM-HEPES and 2% penicillin–streptomycin (penicillin base 10000 units/ml, streptomycin base 10000 $\mu$g/ml, Gibco Laboratories, Chagrins Falls, OH, U.S.A.). The lungs were then lavaged with four 10 ml aliquots of MEM, each aliquot being instilled and withdrawn three times. Non-adherent alveolar macrophages were removed with the lavage fluid, reducing macrophage contamination of the final preparation. In some instances, macrophages harvested from lavage were saved for comparison with type II cells.

The lungs were removed from the chest cavity and elastase (pancreatic elastase type II-A, Sigma E 6883, St Louis, MO, U.S.A. 0·3 mg/ml) solubilized in MEM was instilled through the trachea to total lung capacity. After incubation at 37 °C for 30 min, the lungs were dissected free of large airways and mediastinal structures, and minced into 1 mm pieces. They were stirred in the presence of DNAase (0·1 mg/ml, D 0876 Sigma, St Louis, MO, U.S.A.) solubilized in MEM for 10 min, and then filtered through 160 $\mu$m nylon mesh (Cross Wire Manufacturing Co., Belmar, NJ, U.S.A.). Ten millilitres of fetal calf serum was added to neutralize the elastase. The filtrate was centifuged for 10 min at 1500 r.p.m., and the cells resuspended in *Griffonia simplicifolia* lectin (*Griffonia simplicifolia* BS-1 Sigma L 2380, St Louis, MO, U.S.A.; 500 $\mu$g/ml) solubilized in MEM with 10% fetal calf serum (Biologos Inc., Naperville, IL, U.S.A.). Cells were incubated for 30 min at 37 °C with the lectin, and gently inverted every 10 min to promote optimal mixing. After incubation with the lectin, the cell suspension was filtered through a 15 $\mu$m nylon mesh, and pre-plated for 1 h to increase purity of type II cells. Non-adherent cells were then suspended in MEM supplemented with 10% fetal calf serum, and plated at a density of approximately $5 \times 10^5$ cells/dish in 35 mm tissue culture dishes containing several small pieces of sterile glass cover-slips. The low plating density was chosen to optimize study of isolated, non-confluent type II cells. For electrophysiological studies, cells growing on a piece of cover-slip were transferred to a small glass recording chamber and perfused with Ringer solution (Table 1).

These procedures yield a purity of approximately 80–90% type II cells (Goodman & Crandall, 1982; Simon *et al.* 1986). The most frequent contaminating cell types immediately after isolation are lymphocytes and macrophages (Kikkawa & Yoneda, 1974; Mason *et al.* 1977*b*; Fisher, Furia & Berman, 1980). Since macrophages tend to be larger, and lymphocytes smaller than type II cells, cells of intermediate size were selected for study.

*Experiments using phosphine.* In some experiments individual cells were identified by adding the fluorescent dye phosphine to the bathing solution. Mason *et al.* (1975) originally described identification of lamellar bodies in type II cells with phosphine 3R, which is a mixture of phosphine and phosphine E; we used phosphine ('phosphine dye', cat. no. 0747, C.I. 46045, lot no. 975-006, Polysciences Inc., Warrington, PA 18976, U.S.A.) alone. Phosphine was diluted to 1 $\mu$g/ml in Ringer solution from a 1 mg/ml stock solution (in distilled water) kept cold and protected from light. The field was illuminated by light from a mercury lamp through an excitation filter passing 420–485 nm wavelengths (Nikon filter cassette 'B'), and observed with a Nikon Diaphot inverted microscope fitted with epifluorescence. Best visualization of lamellar bodies required exposure to the phosphine solution (1 $\mu$g/ml) for several minutes. Cells were selected which contained distinct, brightly fluorescing inclusions, presumed to be lamellar bodies. Many cells tentatively identified as type II cells on the basis of having inclusion bodies apparent under Nomarski interference contrast optics, exhibited strongly fluorescent bodies when stained with phosphine. However, occasional

cells contained cytoplasmic bodies which did not subsequently fluoresce. Thus, while it is possible to improve upon the percentage of type II cells studied by selecting cells with distinct inclusion bodies under Nomarski optics, identification of type II cells by phosphine fluorescence is a more rigorous criterion. However, phosphine itself had several effects on the properties of $K^+$ currents, which are discussed in the Results.

TABLE 1. Composition of solutions (mM)

| | $Na^+$ | $K^+$ | $Ca^{2+}$ | $Mg^{2+}$ | $Cl^-$ | $F^-$ | EGTA | HEPES | pH |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Intracellular solutions | | | | | |
| KF | 0 | 162 | 1 | 2 | 6 | 140 | 11 | 10 | 7·2 |
| KF+KCl | 0 | 162 | 1 | 2 | 76 | 70 | 11 | 10 | 7·2 |
| KCl | 0 | 162 | 1 | 2 | 146 | 0 | 11 | 10 | 7·2 |
| K aspartate* | 0 | 162 | 1 | 2 | 6 | 0 | 11 | 10 | 7·2 |
| | | | | Extracellular solutions | | | | | |
| Ringer soln. | 160 | 4·5 | 2 | 1 | 170·5 | — | — | 5 | 7·4 |
| 100 mM-TEA† | 60 | 4·5 | 2 | 1 | 170·5 | — | — | 5 | 7·4 |
| $K^+$ Ringer soln. | 0 | 160 | 2 | 1 | 166 | — | — | 10 | 7·4 |

* Contains 140 mM-aspartate$^-$.

† Made with TEA chloride.

$Rb^+$ Ringer solution is the same as $K^+$ Ringer solution, with all $K^+$ replaced by $Rb^+$. The pH of all solutions was adjusted with NaOH, except for $K^+$ Ringer solution, for which tetramethylammonium hydroxide was used. EGTA is ethylene glycol bis($\beta$-aminoethyl ether) $-N,N,N',N'$-tetraacetic acid. HEPES is 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid. Calculated free $Ca^{2+}$ concentrations of the intracellular solutions and corrections for junction potentials measured relative to Ringer solution (Methods) are: KF 2 nM, −4·5 mV; KF+KCl 8 nM, −2·4 mV; KCl 38 nM, −1·0 mV; K aspartate 38 nM, −7·0 mV.

*Recording techniques.* Micropipettes were pulled in two or three stages, coated with Sylgard 184 (Dow Corning Corporation, Midland, MI, U.S.A.), and heat-polished. The following glass types which reportedly have excellent noise properties (Rae & Levis, 1984) were found to produce microelectrodes with a shape appropriate for whole-cell recording, and to form tight seals (often 10–50 GΩ) with type II cell membranes: KG–12, 0010, and EG–6 (Garner Glass Co., Claremont, CA, U.S.A.). In some experiments non-heparinized capillary tubing was used (Fisher Scientific, Pittsburg, PA, U.S.A.). Electrical contact with the pipette solution was achieved by a small sintered Ag–AgCl pellet (In Vivo Metric Systems, Healdsburg, CA, U.S.A.) attached to a silver wire covered by a Teflon tube. The solutions used to fill the pipettes are given in Table 1. In most experiments the KF+KCl solution was used as a compromise between KF, which tended to clog the pipette tip in the bath before a seal could be achieved, and KCl, which seemed to have deleterious effects on the cells. Pipette tip resistances measured in Ringer solution with KF+KCl solution in the pipette generally ranged from 2 to 5 MΩ.

Measured values of junction potentials between the pipette solutions and Ringer solution used both in the bath and in the agar in the bath electrode, are given in Table 1. Derived data (e.g. Fig. 3) were corrected according to the values in Table 1, but since the correction for the most frequently used pipette solution, KF+KCl, is only −2·4 mV, raw current records are shown without correction.

## RESULTS

### *Whole-cell voltage clamp experiments in type II cells*

Type II cells from rats were studied in the whole-cell configuration to determine the types and numbers of ion channels in their plasma membranes. Regardless of time in culture, almost all cells studied had voltage-dependent outward currents carried by $K^+$ ions. In some cells evidence was found for other kinds of channels which have not been fully characterized.

Type II cells in primary culture are at first spherical, but as they transform they gradually flatten and enlarge. The diameter of cells studied during the first few days in culture ranged from about 7 to 15 $\mu$m. Whole-cell input capacity was measured upon rupturing the patch of membrane at the tip of the pipette to achieve whole-cell

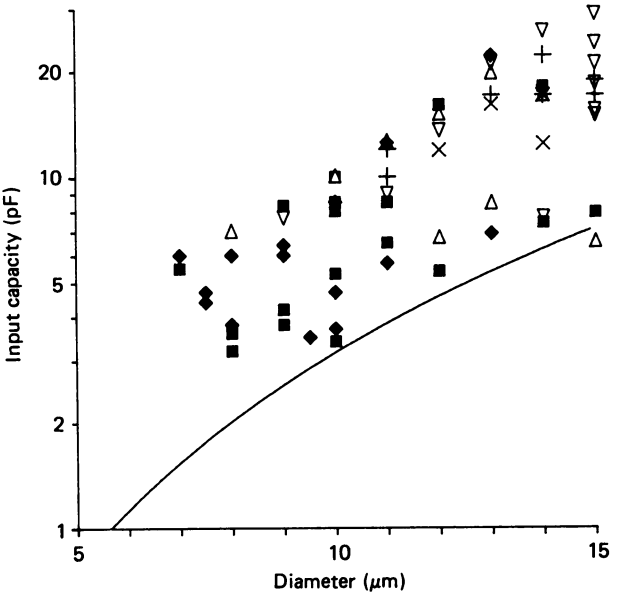


Fig. 1. Input capacity of type II cells as a function of diameter. Only cells which appeared to be approximately spherical are included. Symbols indicate time in culture: ◆, day 0 (the day of isolation): ■, day 1; △, day 2; ▽, day 3; +, day 4; ×, day 5 or 6. The curve shows the theoretical input capacity of a smooth spherical cell of the indicated diameter with a specific capacitance of 1 $\mu$F/cm$^2$. Input capacity was estimated immediately after the transition from on-cell patch to whole-cell configuration, by integrating the capacity transient for a small (*ca.* 20 mV) voltage step, and subtracting any capacity not cancelled before patch rupture. Reading the capacity from the capacity compensation dial gave comparable results.

configuration. Input capacity is plotted as a function of cell diameter in Fig. 1 in cells with approximately spherical shape. The curve shows the capacity expected if the cells are smooth spheres with a specific capacitance of 1 $\mu$F/cm$^2$, which is probably an upper limit for biological membranes (e.g. Hodgkin & Nakajima, 1972; Dulhunty & Franzini-Armstrong, 1977). The input capacity of nearly all cells falls well above this curve, corresponding to a mean specific capacitance of 2·5 $\mu$F/cm$^2$ in sixty-nine cells, indicating that the true membrane area of type II cells in culture is more than twice the apparent area. Type II cells studied within 1 day after isolation (filled symbols) tended to be smaller and to have slightly lower capacitance. The apparent specific capacitance was increased in cells cultured for 2 days or more, but the largest increase (at day 3), while statistically significant, was only 36%.

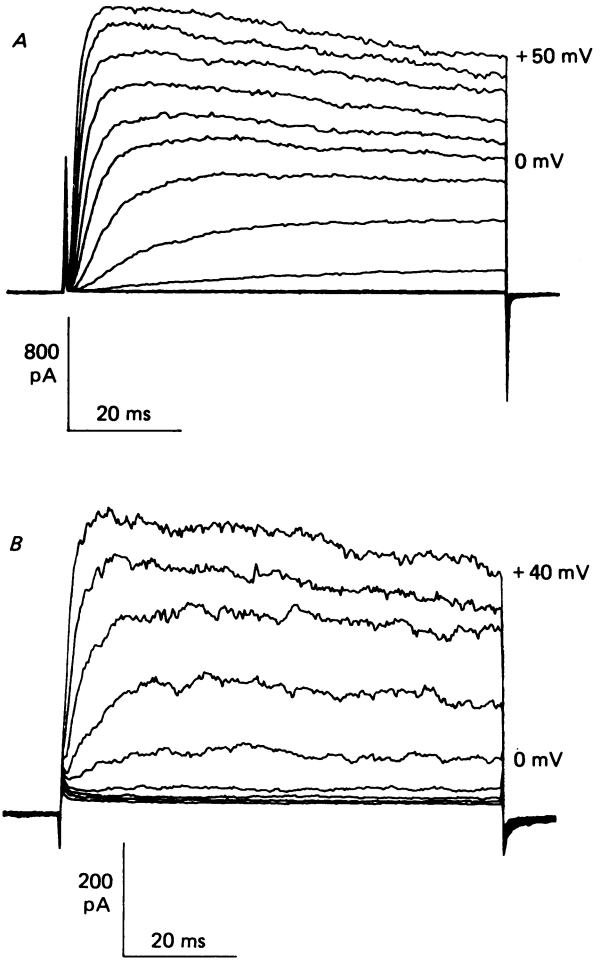


Fig. 2. Whole-cell current families in a rat type II cell with mainly type $n$ $K^+$ channels ($A$) and in another cell with type $l$ $K^+$ channels ($B$). $A$, currents during voltage pulses to $-70$ mV in 10 mV increments up to $+50$ mV, from a holding potential of $-80$ mV, with a 20 s interval between pulses. The $K^+$ currents were exceptionally large in this cell; it was selected for clarity of illustration. The currents in this cell exhibited typical type $n$ properties, which are illustrated in other Figures, including its voltage dependence (◆, Fig. 3), tail current kinetics (Fig. 4$A$), TEA sensitivity (△, Fig. 5), and sensitivity to phosphine (Fig. 6$A$). This cell was cultured for 3 days, was 13 $\mu$m in diameter and under Nomarski optics appeared loaded with inclusion bodies. The cell was not exposed to phosphine. Series resistance compensation 60%; run started after 24 min equilibration in the whole-cell configuration; pipette solution KF+KCl (Table 1); input capacity 19 pF. $B$, a family of whole-cell currents in a freshly isolated type II cell with type $l$ $K^+$ currents. Currents are illustrated for voltage pulses to $-40$ mV in 10 mV increments up to $+40$ mV from a holding potential of $-80$ mV. Properties of the currents in this cell which identify the channels as type $l$ include relatively positive voltage dependence of activation (◇, Fig. 3), lack of accumulation of inactivation with repeated depolarization, rapid tail current kinetics (Fig. 4$B$), and high TEA sensitivity (■, Fig. 5). Observed in the presence of phosphine 1 $\mu$g/ml, this cell contained numerous brightly fluorescent bodies. Diameter 8 $\mu$m; input capacity 3·8 pF; run started 7 min after achieving whole-cell configuration; pipette solution KF+KCl.

*Two types of potassium channels*

Families of whole-cell currents predominated by $K^+$ currents with type *n* and type *l* properties are shown in Fig. 2*A* and *B*, respectively. Both types of $K^+$ currents activate during depolarizing voltage pulses and then slowly inactivate. Both currents activate faster at more positive potentials. The currents thus appear superficially similar, but consistent quantitative differences exist. Type *n* channels activate at more negative potentials, with sizeable current apparent at $-30$ mV in Fig. 2*A*; type *l* channels in Fig. 2*B* are activated only at $-10$ mV.

Like delayed rectifier currents in other cells, type *n* currents in type II cells characteristically activate with a delay, giving rise to a sigmoid time course. For large depolarizing pulses, the currents could be fitted with a Hodgkin–Huxley (1952) type parameter raised to the fourth power ($n^4$). For small depolarizations, a smaller exponent sometimes gave a better fit (data not shown). Sigmoid activation was less pronounced in cells with type *l* channels, and in some instances these currents were adequately fitted by a simple exponential rise. In other cells with type *l* channels a distinctly better fit was achieved with exponents greater than one. In these cells a pattern like that in cells with type *n* currents was observed; larger exponents were required to fit currents recorded during larger depolarizations. In addition, type *l* currents tended to have a slow final approach to their peak which could not be fitted adequately with any exponent at the same time that the rapidly rising phase of the current was fitted.

During maintained depolarization, currents through both channels inactivate with a time constant of several hundred milliseconds. Recovery from inactivation is characteristically quite slow for type *n* currents, but rapid for type *l* currents. This difference is apparent experimentally in the rate at which depolarizing pulses can be repeated without progressive decreases in the $K^+$ current amplitude. In some type II cells $K^+$ current inactivation was obscured, especially at more positive potentials, by a slowly increasing outward current. These slowly activating currents reversed near 0 mV and were not further studied.

*Voltage dependence.* The peak $K^+$ conductances, $g_K$, for the currents from both cells in Fig. 2 are plotted together in Fig. 3. The curves show the best fit of the data in each cell to a Boltzmann function:

$$\frac{g_K}{g_{K,max}} = \frac{1}{1+\exp\{(V-V_n)/k_n\}} \quad (1)$$

in which the potential at which $g_K$ is half-activated, $V_n$, the slope factor, $k_n$, and the maximum $K^+$ conductance, $g_{K,max}$, are allowed to vary. In the cell with type *n* channels (filled symbols), $V_n$ is $-23$ mV, compared with $+3$ mV in the cell with type *l* channels (open symbols). Because the $K^+$ currents in many type II cells were small, it was not always possible to identify which type of $K^+$ channels were present. In cells in which the channel type was identified, $V_n$ averaged $-23{\cdot}1 \pm 6{\cdot}2$ mV (mean $\pm$ S.D.) in twenty-one cells with type *n* channels, and $+7{\cdot}4 \pm 6{\cdot}8$ mV in nine cells with type *l* channels. The voltage dependence of activation of $g_K$ was steeper in the cells with type *n* channels, with $k_n$ $-5{\cdot}5 \pm 0{\cdot}8$ mV, compared with $-8{\cdot}3 \pm 1{\cdot}5$ mV in the cells with type *l* channels.

*Tail current kinetics.* One characteristic difference between the two types of $K^+$ channels is in their tail current kinetics, illustrated in Fig. 4. After the $K^+$ conductance was maximally activated by a brief depolarizing pulse, the cell

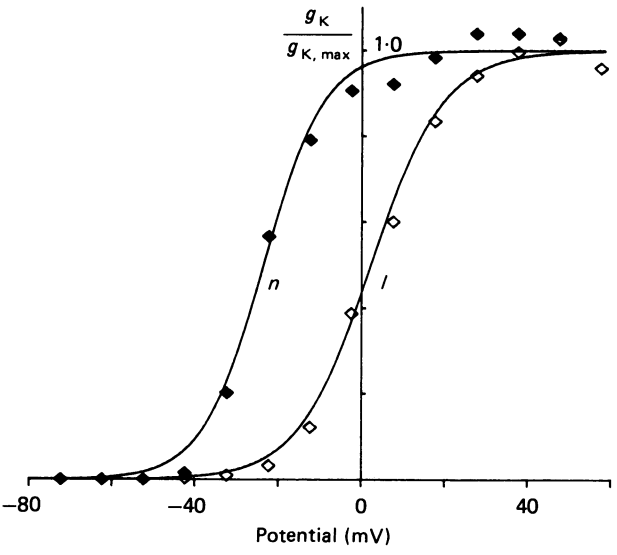


Fig. 3. Normalized $K^+$ chord conductance–voltage relationships for the two cells in Fig. 2. For both cells $g_K$ was calculated from the peak $K^+$ current at each potential after subtraction of a leak current estimated by linear extrapolation of the current during subthreshold depolarizing pulses, assuming a $K^+$ reversal potential of −80 mV. The data points were fitted with eqn (1) as described in the text. Fitted parameters for the cell with type *n* channels (◆) are $V_n$ −23 mV, $k_n$ −7·3 mV, and $g_{K,max}$ 14·7 nS; for the cell with type *l* channels (◇) $V_n$ +3 mV, $k_n$ −8·8 mV, and $g_{K,max}$ 4·0 nS. Instantaneous current–voltage relationships within the voltage range at which $K^+$ channels are activated were approximately linear in cells with either type of $K^+$ channel.

membrane was stepped to various potentials at which the channels eventually close, or deactivate. The relaxation kinetics at each potential reflect the rate of channel closing at that potential. Type *n* channels (*A*) close about an order of magnitude slower than type *l* channels (*B*). Both types close faster at more negative potentials. Type *l* tail currents are so fast at negative potentials that they are not clearly resolved from the capacity transient.

*Selectivity.* Type *n* currents generally reversed near −80 mV with Ringer solution in the bath and 162 mM-$K^+$ in the pipette solution (calculated $K^+$ equilibrium potential −90·6 mV). Type *l* tail currents were not clearly resolved at potentials more negative than about −50 mV, at which potentials their kinetics were comparable with those of the capacity transients (cf. Fig. 4*B*). The reversal potential estimated from outward tail currents in four cells was roughly −60 mV, a value likely to be too positive due to contamination by capacity currents. Calculated using the Goldman (1943)–Hodgkin & Katz (1949) equation, the relative $Na^+$ permeability of type *l* channels ($P_{Na}/P_K$) is therefore < 0·07, and that of type *n* channels is < 0·02. When the bathing solution was replaced by $K^+$ Ringer solution (Table 1), the average reversal potential of type *n* currents was +4·6±5·6 mV (mean±S.D., ten cells), compared with a calculated $K^+$ equilibrium potential of −0·3 mV. Replacement of $K^+$ in the Ringer solution with $Rb^+$ (Table 1) shifted the reversal potential to more negative potentials by 5·3±2·4 mV (mean±S.D., five cells), for a relative per-

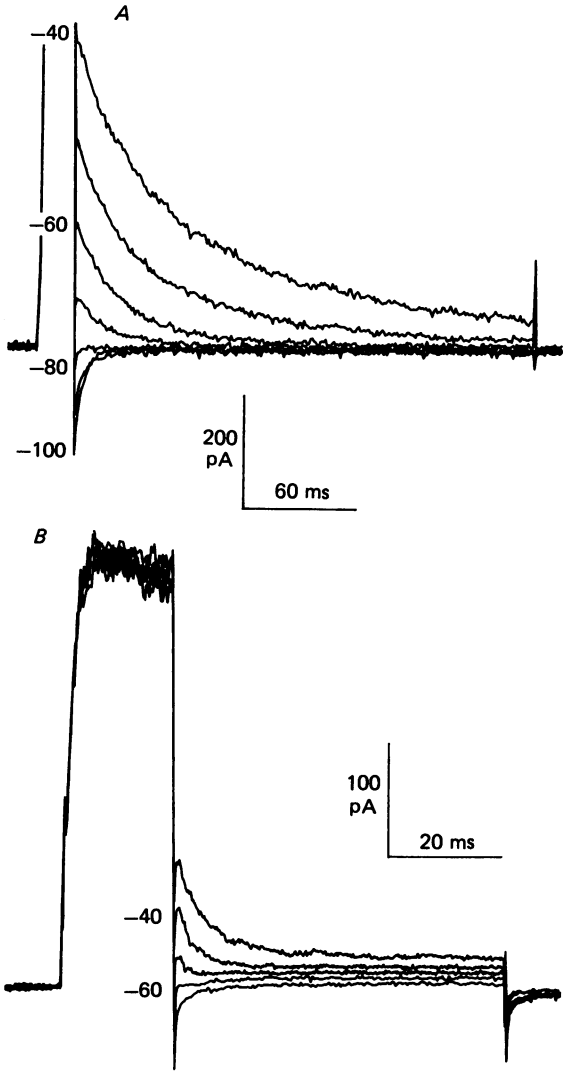


Fig. 4. Tail currents in a cell with type $n$ $K^+$ channels ($A$), and in a cell with type $l$ $K^+$ channels ($B$). Note the different time calibrations. In both experiments a brief pre-pulse to +20 mV opened most of the $K^+$ channels, and the subsequent current relaxation during pulses to various potentials reflects the rate of channel closing or deactivation at that potential. The test pulses in both are in 10 mV increments; the current during the pre-pulse in $A$ is off-scale. Type $l$ channels close so rapidly at negative potentials that the kinetics of inward currents are obscured due to contamination by capacity currents.

meability of $Rb^+$ to $K^+$ of 0·81 ($P_{Rb}/P_K$). Similar measurements were completed in one cell with type $l$ currents: the currents reversed at +1·6 mV in $K^+$ Ringer solution and about 4 mV more negative in $Rb^+$ Ringer solution. In summary, both channels are strongly selective for $K^+$ over $Na^+$, and pass $Rb^+$ current less readily. Further experiments are required to determine whether the selectivity of type $l$ channels differs from that of type $n$ channels.

*Block by TEA.* Another distinctive difference between the two types of $K^+$ channels is their sensitivity to block by externally applied TEA. Plotted in Fig. 5 is the ratio of the peak $K^+$ current during a depolarizing pulse in the presence of TEA to the current in the absence of TEA. Open symbols represent measurements in cells

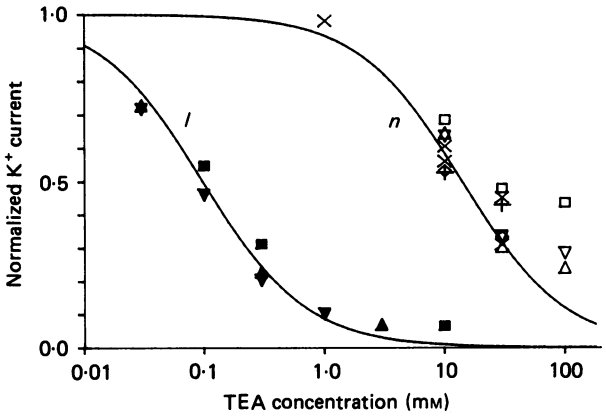


Fig. 5. Dose–response curves for the reduction of peak whole-cell $K^+$ current by externally applied TEA. Cells were classified as having predominantly type *l* channels (filled symbols) or type *n* channels (open symbols) on the basis of the voltage dependence of activation, tail current kinetics, and rate of recovery from inactivation. The cells were depolarized briefly (to +20 or +40 mV, usually for 80 ms) from a holding potential of −80 mV every 30 s in the absence or presence of TEA. The $K^+$ currents in the presence of TEA were normalized to the current in its absence, after correction for a leak conductance assumed to be linear and reverse at 0 mV. Control measurements were bracketed around the measurements in TEA-containing solutions to minimize the effect of run-down. For concentrations up to 30 mM, TEA chloride was added to Ringer solution; the 100 mM-TEA solution is described in Table 1. The smooth curves illustrate dose–response relations calculated on the assumption that one TEA molecule blocks one $K^+$ channel, with half-block at 100 $\mu$M and 15 mM for type *l* and *n* channels respectively.

with type *n* channels, filled symbols measurements in cells with type *l* channels. Dose–response curves are drawn assuming 1:1 stoichiometry between TEA and $K^+$ channels, with half-block of type *l* currents at 100 $\mu$M, and half-block of type *n* currents at 15 mM. The data points for 100 mM-TEA fall consistently above the curve. This deviation may reflect a component of outward current not blocked by TEA. Fitting the data with the assumption of an unblockable component of outward current reduces the half-block concentration in these cells to about 10–12 mM. Block of both types of $K^+$ channels by TEA was rapidly reversible.

*Channel types in different cells.* About 100 rat type II cells were studied before the existence of type *l* $K^+$ channels was noticed. The currents in most of these cells were not sufficiently characterized to allow retrospective classification of the $K^+$ channel type; cells which were more fully analysed all had type *n* channels. The frequency of occurrence of type *l* channels was estimated by considering only those cells studied *after* our discovery of type *l* channels. Of these, only cells in which the classification suggested by voltage dependence (1) and kinetics of recovery from inactivation (5) was corroborated by tail current kinetics (3) or TEA sensitivity (7) are included

(numbers refer to properties listed at the beginning of this section). Of forty-two cells classified, nine had type *l* channels (21 %). The fraction of classified cells with type *l* channels from a given preparation ranged from none out of six to four out of four, suggesting that type *l* channels do not occur randomly, although cells with type *l* channels were seen in five different preparations, four of which also had cells with type *n* channels.

TABLE 2. Properties of cells with type *l* or type *n* $K^+$ channels

| | Type *l* | Type *n* |
|---|---|---|
| Number | 9 | 33 |
| $g_{K,max}$ (nS) | 1·5 ± 1·3 | 3·1 ± 3·2 |
| Diameter (μm) | 10·7 ± 3·0 | 11·9 ± 2·3 |
| Input capacity (pF) | 6·2 ± 3·8 | 13·3* ± 5·1 |
| $C_m$ (μF/cm²) | 1·7 ± 0·7 | 2·7* ± 0·7 |

* Significantly different from type *l* ($P < 0·005$).

Means ± S.D. are given. The specific capacitance, $C_m$, was calculated assuming each cell was a smooth sphere. The dominant $K^+$ channel type was classified in each cell by criteria described in the text. Of the type *l* cells, eight of nine were identified (as type II cells) by phosphine fluorescence; nine of thirty-three type *n* cells were identified with phosphine.

Several properties of the cells with type *l* or type *n* $K^+$ channels are compared in Table 2. There is no significant difference in the mean cell diameter or $g_{K,max}$ of the groups. The $g_{K,max}$ values are biased toward higher values because cells with small currents were hard to classify; the mean $g_{K,max}$ of 101 other cells (all studied in the absence of phosphine) was 1·6 ± 1·5 nS (± S.D.). Table 2 shows that the input capacity was significantly lower in cells with type *l* channels. This difference remains when the apparent specific capacitance is calculated for each cell, suggesting that cells with type *l* channels have smoother surface membranes. Sorted according to time in culture, eight of twenty-three cells studied within 2 days after isolation had type *l* channels, compared with only one of nineteen cells studied on or later than day 3. This result calls into question the difference found in specific capacitance, since there is a small increase in specific capacitance with time in culture (Fig. 1). However, when only those cells studied on days 0 or 1 are included, the specific capacitance is still lower in the cells with type *l* channels, 1·7 ± 0·8 μF/cm² (mean ± S.D., $n = 7$), than in cells with type *n* channels, 3·0 ± 0·7 μF/cm² ($n = 8$, $P < 0·005$). In summary, these data support the idea that cells with type *l* $K^+$ channels may represent a subtype of type II cells that differs from type II cells with type *n* channels at least with respect to one other feature, surface membrane morphology.

*Changes after achieving whole-cell configuration*

Several changes take place in the properties of ion channels in a variety of cells within about 10 min after achieving whole-cell configuration, including shifts of the voltage dependence of ion channels to more negative potentials (Fenwick, Marty & Neher, 1982; Marty & Neher, 1983; Fernandez, Fox & Krasne, 1984; Cahalan, Chandy, DeCoursey & Gupta, 1985). The whole-cell $g_K$–$V$ relation was determined at different times in the same type II cell and fitted to eqn (1). The first run was started on average 5 min after whole-cell configuration was achieved (range 3–10 min), the

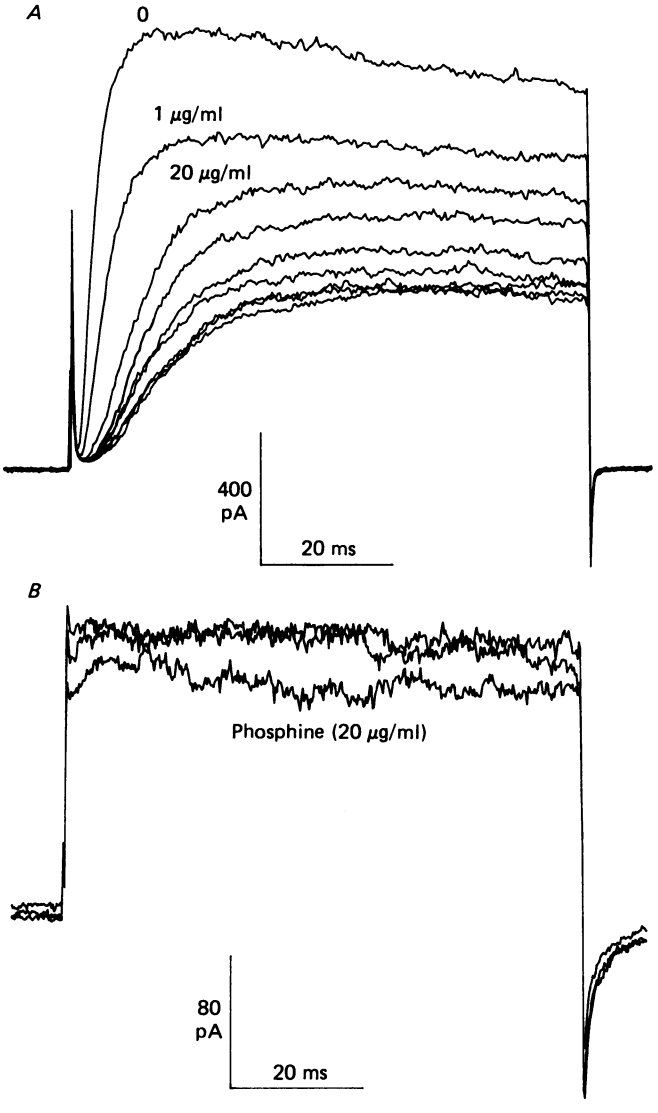


Fig. 6. Reversible effects of phosphine on type $n$ ($A$) and type $l$ ($B$) $K^+$ currents. $A$, this cell with type $n$ $K^+$ currents was pulsed to +20 mV every 30 s in the presence of the indicated concentrations of phosphine. The first seven pulses after addition of 20 μg phosphine/ml are shown to illustrate the gradual onset of the phosphine effect. Note the slowing of $K^+$ channel activation. $B$, this cell with type $l$ currents was pulsed to +40 mV every 30 s. The larger currents were recorded in Ringer solution before and after washout, the smaller current in the presence of 20 μg phosphine/ml.

second run was started 18 min later (range 5–39 min). The fitted mid-point $V_n$ shifted to more negative potentials in twelve of fifteen cells, but the average shift was only −1·8 mV. The slope factor $k_n$ did not change consistently, with the later value on average 1·1 times the first. For the second run $g_{K,max}$ averaged 0·99 times that of the first. In nine of fifteen cells $g_{K,max}$ decreased, probably reflecting 'run-down' often

seen in experiments lasting 1–2 h. In a few cells both the input capacity and $g_{K,max}$ increased gradually but substantially throughout the course of the experiment, as though the membrane area were increasing. This phenomenon was not observed in most cells, and its significance is unclear. In summary, the time-dependent shift in the voltage dependence of $K^+$ channels found in other cells appears to occur only to a small extent, if at all, in rat type II cells.

'Leak' currents in type II cells, recorded at potentials at which no $K^+$ channels were open, varied approximately linearly with potential. The leak conductance was variable, but in 'tight' cells it typically corresponded to an input resistance of 5–20 GΩ. The leak conductance in a given cell varied with time, often increasing rapidly after whole-cell configuration was established, and subsequently decreasing slowly. The leak conductance in some cells increased by an order of magnitude or more from its initial value.

*Effects of phosphine on potassium channels*

Several experiments were done on cells identified as type II cells with the fluorescent dye phosphine (see Methods). Cells were selected which contained distinct, brightly fluorescing inclusion bodies, presumably lamellar bodies. When whole-cell currents in identified cells were studied, it became apparent that the $K^+$ current amplitude increased when the phosphine was washed out of the bath. This phenomenon was studied systematically with the concentration of phosphine used for type II cell identification (1 μg/ml) and also with higher concentrations in order to make the effects more distinct.

Figure 6*A* illustrates type *n* $K^+$ currents during identical depolarizing voltage pulses in a cell bathed in Ringer solution (record labelled 0). Addition of phosphine to the bath at 1 μg/ml reduced the current amplitude; addition at 20 μg/ml further reduced the current. At this higher concentration the currents were reduced progressively during the first several pulses (given with 30 s intervals). After a few minutes, a new steady state was attained, with no further change in current amplitude. In four experiments the time constant of the attenuation of $K^+$ currents by phosphine, fitted by a single exponential, ranged from 50 to 210 s. The reduction of $K^+$ current amplitude by 1 μg phosphine/ml varied substantially from cell to cell, averaging 16% in eight cells. The mean reduction by 20 μg phosphine/ml was 36% in four cells; 100 μg/ml reduced the $K^+$ current by 83% in two cells. The reduction of $K^+$ current by phosphine was at least partially reversible immediately upon wash-out. Recovery after long exposures was sometimes incomplete; however, 'run-down' probably contributes to this effect. Wash-out of phosphine in cells exposed only for a few minutes was usually accompanied by complete recovery of the $K^+$ currents.

Examination of the currents in Fig. 6*A* suggests that in addition to reducing the amplitude of type *n* $K^+$ currents, phosphine also changes the kinetics of $K^+$ currents, slowing the rising phase, as quantified by measuring the time-to-half-peak $K^+$ current (data not shown). This effect could result from a shift in the voltage dependence of $K^+$ channel activation to more positive potentials. In two cells, 20 μg phosphine/ml shifted the $g_K$–$V$ curve by +16 mV. The effects of 1 μg phosphine/ml were small and varied from cell to cell, with $V_n$ (eqn (1)) shifted by $+3 \pm 7$ mV (mean ± S.D., $n = 7$). In one cell, the voltage dependence of the rate of $K^+$ channel

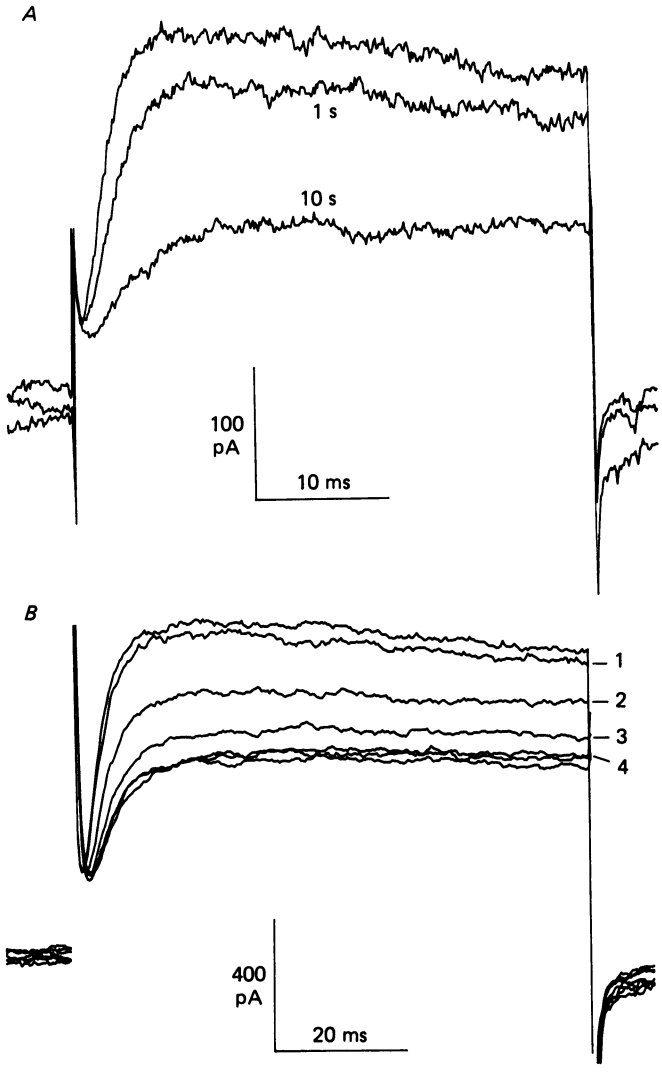


Fig. 7. Photodestruction of type $n$ $K^+$ channels in the presence ($A$) or absence of phosphine ($B$). $A$, reduction of type $n$ $K^+$ current by phosphine fluorescence. The three superimposed currents were all recorded during pulses to +20 mV in the presence of 1 $\mu$g phosphine/ml. The largest current was recorded before the cell had been flashed. The record labelled 1 s was taken after exposure to high-intensity blue light for 1 s. During this flash, discrete, brightly fluorescent inclusion bodies were visible. After another 1 s flash (not shown), a current–voltage run (as in Fig. 2) was recorded to evaluate the properties of the remaining $K^+$ channels. The cell was then flashed for 10 s, further reducing the $K^+$ current amplitude (record labelled 10 s). $B$, effect of exposure to blue light (see Methods) on a cell never exposed to phosphine. Potassium current–voltage relations measured 4 and 15 min after achieving whole-cell configuration were superimposable; voltage dependence and tail current kinetics of $K^+$ currents were distinctly type $n$. Starting after about 20 min equilibration the cell was pulsed briefly to +20 mV every 30 s. Superimposed here are the last control current before the shutter for the mercury lamp was opened (largest current), the next four pulses during which the shutter was open continuously (numbered 1–4), and two more control pulses after the shutter was closed. The average $K^+$ current, after leak

deactivation (tail current time constant) was not detectably affected by 20 $\mu$g phosphine/ml. In summary, addition of phosphine to the bathing solution decreases $g_{K,max}$ and shifts $K^+$ channel activation to more positive potentials. Both effects are reversed upon wash-out of phosphine, at least for short exposures.

Type *l* $K^+$ channels are also affected by phosphine. The type *l* $K^+$ current in the cell illustrated in Fig. 6*B* was reduced reversibly by about 20% by phosphine at 20 $\mu$g/ml, with no detectable effect at 1 $\mu$g/ml.

*Flash experiments.* Several experiments were done to determine the effects of inducing phosphine fluorescence in type II cells. Fig. 7*A* shows the effect on type *n* $K^+$ currents of flashing a cell in the presence of 1 $\mu$g phosphine/ml. Activation of the dye for about 1 s reduced the $K^+$ current amplitude by 19%. A second 1 s flash reduced the remaining $K^+$ current amplitude by 12% (not shown), while a 10 s flash reduced the currents further by 44%. The voltage dependence and kinetics of the $K^+$ currents remaining after a flash were not detectably altered. Although a reduction of $K^+$ current amplitude was seen consistently in cells with type *n* channels, the magnitude of this effect was variable. In three cells flashed for 1 s in the presence of 1 $\mu$g phosphine/ml, the average reduction of $K^+$ current was 30%. The fractional reduction consistently decreased with repeated or prolonged flashes; the average reduction of $K^+$ current after 12–15 s cumulative exposure was $< 50\%$ (three cells). This kind of result would be expected if phosphine lost its effect on $K^+$ channels as it became photobleached. When repeated or prolonged flashes were applied, the input resistance of the cell eventually decreased, suggesting membrane damage. For all of these reasons, it is clearly desirable to minimize the duration of fluorescence. In practice, since phosphine bleached within a few seconds, exposure of cells to the blue activating light for the purpose of identifying type II cells was usually brief (1–2 s). If intact type II cells had the same sensitivity to fluorescence-induced $K^+$ current reduction, then our usual identification procedure would reduce the measured value of $g_{K,max}$ by about 30%.

Flashing type II cells in the absence of phosphine also reduced $K^+$ currents, but only very slowly. Fig. 7*B* shows that exposure to the blue excitatory light for 2 min reduced the $K^+$ current by $< 50\%$. Effects of light alone during cell identification would therefore be negligible.

## DISCUSSION

Rat alveolar type II epithelial cells maintained in primary culture can be studied in the whole-cell configuration using the gigohm-seal technique. The cell membrane of cultured type II cells has a large apparent specific capacitance, suggesting substantial irregularity. Type II cells *in vivo* have microvilli on their apical surface. Type II cells under the conditions employed had primarily $K^+$-selective channels. Although most cells had $K^+$ channels, the channel density was low. A typical type

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subtraction, immediately after the 2 min exposure to blue light was 387 pA, 49% of the control value. The voltage dependence of $K^+$ channel activation after the flash was indistinguishable from that before exposure to light. The cell was cultured for 2 days, was 13 $\mu$m in diameter, and had many inclusion bodies when viewed under Nomarski optics.

II cell has a $g_{K,max}$ of about 1·6 nS. Dividing by the single type $n$ $K^+$ channel conductance of about 12 pS (Jacobs *et al.* 1986) gives an estimate of 130 $K^+$ channels/cell. This represents a density of about 0·2 channels/$\mu m^2$ apparent area (9 channels/pF), corrected to $< 0{\cdot}1$ $\mu m^{-2}$ if the specific capacitance is 1 $\mu F/cm^2$.

*Two types of potassium channels*

Two distinct types of $K^+$ channels were present in rat type II cells. Both types of $K^+$ currents activate with a sigmoid time course upon depolarization of the cell membrane, inactivate with maintained depolarization, and are blockable by externally applied TEA. Compared with type $n$ currents, type $l$ currents activate at more positive potentials, deactivate more rapidly upon repolarization, and are 100-fold more sensitive to block by TEA. Most type II cells had type $n$ channels, which resemble delayed rectifier $K^+$ channels found in a wide variety of cells including skeletal muscle (Adrian, Chandler & Hodgkin, 1970), snail neurones (Aldrich, Getting & Thompson, 1979), lymphocytes (Cahalan *et al.* 1985) and macrophages (Gallin & Sheehy, 1985). A small number of type II cells had type $l$ $K^+$ channels. To our knowledge type $l$ $K^+$ channels have been described only in mouse lymphocytes, in which they occur in small numbers in some normal T lymphocytes (DeCoursey, Chandy, Gupta & Cahalan, 1987*b*), and in large numbers in a subset of thymocytes from normal mice (R. S. Lewis & M. D. Cahalan, personal communication) and in the abnormal T lymphocytes from mutant mice, homozygous for the *lpr* gene locus, with genetically induced lymphoproliferation (Chandy, DeCoursey, Fischbach, Talal, Cahalan & Gupta, 1986). It is tempting to speculate that cells with type $l$ $K^+$ channels might represent a specific subset of type II cells. The lower apparent specific capacitance of the cells with type $l$ $K^+$ channels (Table 2) supports the idea that these channels are present in a distinct subset of type II cells. The possibility that the cells with type $l$ channels were a contaminating cell type rather than type II cells can be ruled out because most of these cells were identified with phosphine. It seems unlikely that type $n$ channels are converted into type $l$ channels either by phosphine, or by activation of phosphine to fluoresce, because (a) many cells identified with phosphine had type $n$ currents, (b) no detectable change in the $g_K$–$V$ relation resulted from flashing cells with type $n$ channels, and (c) no change in the kinetics of tail currents were detected in cells studied without prior exposure to phosphine, either upon addition of phosphine to the bath or upon flashing the cell in the presence of phosphine.

*Changes after achieving whole-cell configuration*

Several changes take place in the properties of ion channels in a variety of cell types after whole-cell configuration is achieved. The voltage dependence of several types of ion channels in a variety of cells shifts by about 10–20 mV to more negative potentials (Fenwick *et al.* 1982; Marty & Neher, 1983; Fernandez *et al.* 1984; Cahalan *et al.* 1985; Fukushima & Hagiwara, 1985; Gallin & Sheehy, 1985). It has been proposed that this shift is due to dissipation of a Donnan potential resulting from the presence of slowly diffusing large anions in the cytoplasm (Marty & Neher, 1983; Fernandez *et al.* 1984). However, no such shift is seen in the voltage dependence of inward rectifier $K^+$ channels in macrophages in spite of the presence of a shift in these

cells for delayed rectifier channels, a result which appears to rule out a Donnan potential mechanism (Gallin & Sheehy, 1985). The shift in the voltage dependence of delayed rectifier $K^+$ channels in type II cells averaged only $-2$ mV, much less pronounced than in other cells.

*Effects of phosphine on potassium channels*

In the light of the effects of phosphine on $K^+$ currents in type II cells, its usefulness as a means of prospectively identifying individual type II cells for study must be questioned. Addition of phosphine to the bath reduced $g_{K,max}$ and shifted the voltage dependence of $K^+$ channel activation to more positive potentials. Since these effects were reversible, distortion of one's results in principle could be avoided simply by washing out the phosphine after identification of a cell. However, identification requires activation of the dye to fluoresce, which was found to reduce $K^+$ current amplitude irreversibly. Flashing a cell for about 1 s in the presence of 1 $\mu$g phosphine/ml reduced $g_{K,max}$ by about 30%. No evidence was found to suggest that the properties of the $K^+$ channels remaining after flashing a cell had been altered, so one could correct for this effect by scaling the $g_{K,max}$ by a factor of about 1·4. A preferable procedure would be to identify cells only *after* recording, by adding phosphine to the bath and flashing the cell. This approach was found to have several drawbacks. Type II cells are often 'blown away' from the pipette when solutions are changed, precluding their identification. Secondly, identification of type II cells with phosphine is not clear-cut for every cell. Selecting a positively stained cell from a field is straightforward, because some cells contain a large number of distinct brightly fluorescing bodies. On the other hand, other cells are hard to classify with certainty, so that data collected from one given cell would often be equivocal. Finally, fluorescent dyes are concentrated in lamellar bodies by an ATP-dependent hydrogen ion gradient (Chander, Johnson, Reicherter & Fisher, 1986), which would dissipate in a dialysed cell. An alternative approach is simply to assume that most cells selected for study on the basis of size and appearance are likely to be type II cells. Any conclusions reached should be confirmed on positively identified cells. Thus, it is significant that type *l* $K^+$ channels were present in cells identified with phosphine, since their presence in only a small fraction of cells might otherwise suggest that they exist only in a contaminating cell type.

The irreversible reduction of $K^+$ currents in type II cells resulting from excitation of phosphine is reminiscent of the photodynamic damage associated with excitation of a variety of membrane potential-sensing dyes in the presence of oxygen (Ross, Salzberg, Cohen, Grinvald, Davila, Waggoner & Wang, 1977). That phosphine in the absence of light reversibly alters the voltage dependence and kinetics of $K^+$ channel gating, suggests that the dye may bind to a site on or very near $K^+$ channels.

*Other channels*

Schneider, Cook, Gage & Young (1985) reported an anion-selective channel in a cell line derived from mouse alveolar type II cells, with a large unitary conductance (350–400 pS). We have noticed a variety of high-conductance non-selective channel-like currents in rat type II cells, especially at positive potentials. In a small number of experiments in which the extracellular ionic strength was reduced, these currents

reversed at more negative potentials, indicating cation selectivity. Although there may be species differences, our experimental conditions were sufficiently different from those used by Schneider *et al.* (1985) to preclude direct comparison of our results.

In summary, rat alveolar type II epithelial cells in primary culture studied with tight-seal techniques have typically about 130 $K^+$ selective channels resembling delayed rectifier channels in other cells. A small fraction of type II cells have a distinctly different type of $K^+$ channel. Additional types of channels were observed in some cells which under the conditions employed did not contribute as obviously to the whole-cell membrane conductance.

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## REFERENCES

ADRIAN, R. H., CHANDLER, W. K. & HODGKIN, A. L. (1970). Voltage clamp experiments in striated muscle fibres. *Journal of Physiology* **208**, 607–644.

ALDRICH, R. W., GETTING, P. A. & THOMPSON, S. H. (1979). Inactivation of delayed outward current in molluscan neurone somata. *Journal of Physiology* **291**, 507–530.

CAHALAN, M. D., CHANDY, K. G., DECOURSEY, T. E. & GUPTA, S. (1985). A voltage-gated potassium channel in human T lymphocytes. *Journal of Physiology* **358**, 197–237.

CASTRANOVA, V., JONES, G. S. & MILES, P. R. (1983). Transmembrane potential of isolated rat alveolar type II cells. *Journal of Applied Physiology: Respiratory and Environmental Exercise Physiology* **54**, 1511–1517.

CHANDER, A., JOHNSON, R. G., REICHERTER & FISHER, A. B. (1986). Lung lamellar bodies maintain an acidic internal pH. *Journal of Biological Chemistry* **261**, 6126–6131.

CHANDY, K. G., DECOURSEY, T. E., FISCHBACH, M., TALAL, N., CAHALAN, M. D. & GUPTA, S. (1986). Altered $K^+$ channel expression in abnormal T lymphocytes from mice with the *lpr* gene mutation. *Science* **213**, 1197–1200.

DECOURSEY, T. E., CHANDY, K. G., GUPTA, S. & CAHALAN, M. D. (1987*a*). Two types of potassium channels in murine T lymphocytes. *Journal of General Physiology* **89**, 379–404.

DECOURSEY, T. E., CHANDY, K. G., GUPTA, S. & CAHALAN, M. D. (1987*b*). Mitogen induction of ion channels in murine T lymphocytes. *Journal of General Physiology* **89**, 405–420.

DECOURSEY, T. E. & JACOBS, E. R. (1987). Two types of potassium channels in rat type II alveolar epithelial cells: differential sensitivity to the fluorescent dye phosphine 3R. *Biophysical Journal* **51**, 368a.

DIGLIO, C. A. & KIKKAWA, Y. (1977). The type II epithelial cells of the lung. IV. Adaptation and behavior of isolated type II cells in culture. *Laboratory Investigation* **37**, 622–631.

DULHUNTY, A. F. & FRANZINI-ARMSTRONG, C. (1977). The passive electrical properties of frog skeletal muscle fibres at different sarcomere lengths. *Journal of Physiology* **266**, 687–711.

FENWICK, E. M., MARTY, A. & NEHER, E. (1982). Sodium and calcium channels in bovine chromaffin cells. *Journal of Physiology* **331**, 599–635.

FERNANDEZ, J. M., FOX, A. P. & KRASNE, S. (1984). Membrane patches and whole-cell membranes: a comparison of electrical properties in rat clonal pituitary ($GH_3$) cells. *Journal of Physiology* **356**, 565–585.

FISHER, A. B., FURIA, L. & BERMAN, H. (1980). Metabolism of rat granular pneumocytes isolated in primary culture. *Journal of Applied Physiology: Respiratory and Environmental Exercise Physiology* **49**, 743–750.

FUKUSHIMA, Y. & HAGIWARA, S. (1985). Currents carried by monovalent cations through calcium channels in mouse neoplastic B lymphocytes. *Journal of Physiology* **358**, 255–284.

GALLIN, E. K. & SHEEHY, P. A. (1985). Differential expression of inward and outward potassium currents in the macrophage-like cell line J774. *Journal of Physiology* **369**, 475–499.

GALLO, R. L., FINKELSTEIN, J. N. & NOTTER, R. H. (1984). Characterization of the plasma and mitochondrial membrane potentials of alveolar type II cells by the use of ionic probes. *Biochimica et biophysica acta* **771**, 217–227.

GOLDMAN, D. E. (1943). Potential, impedance, and rectification in membranes. *Journal of General Physiology* **27**, 37–60.

GOODMAN, B. E. & CRANDALL, E. D. (1982). Dome formation in primary cultured monolayers of alveolar epithelial cells. *American Journal of Physiology* **243**, C96–100.

HODGKIN, A. L. & HUXLEY, A. F. (1952). A quantitative description of membrane current and its application to conduction and excitation in nerve. *Journal of Physiology* **117**, 500–544.

HODGKIN, A. L. & KATZ, B. (1949). The effect of sodium ions on the electrical activity of the giant axon of the squid. *Journal of Physiology* **108**, 37–77.

HODGKIN, A. L. & NAKAJIMA, S. (1972). Analysis of the membrane capacity in frog muscle. *Journal of Physiology* **221**, 121–136.

JACOBS, E. R., CLAYPOOL, W. D., SILVER, M. R., BONE, R. C. & DECOURSEY, T. E. (1986). Voltage-gated potassium channels are present in rat pulmonary type II epithelial cells. *American Review of Respiratory Disease* **133**, A89.

JACOBS, E. R., SILVER, M. R., BONE, R. C. & DECOURSEY, T. E. (1987). Effects of phosphine 3R and quinacrine on potassium channels in pulmonary alveolar epithelial cells. *American Review of Respiratory Disease* **135**, A139.

KIKKAWA, Y. & YONEDA, K. (1974). The type II epithelial cell of the lung. 1. Method of isolation. *Laboratory Investigation* **30**, 76–84.

MARTY, A. & NEHER, E. (1983). Tight-seal whole-cell recording. In *Single-Channel Recording*, ed. SAKMANN, B. & NEHER, E., pp. 107–122. New York: Plenum Press.

MASON, R. J., DOBBS, L. G., GREENLEAF, R. D. & WILLIAMS, M. C. (1977*a*). Alveolar type II cells. *Federation Proceedings* **36**, 2697–2702.

MASON, R., WILLIAMS, M. C. & CLEMENTS, J. A. (1975). Isolation and identification of type 2 alveolar epithelial cells. *Chest* **67**, 36–37s.

MASON, R. J., WILLIAMS, M. C., GREENLEAF, R. D. & CLEMENTS, J. A. (1977*b*). Isolation and properties of type II alveolar epithelial cells from rat lung. *American Review of Respiratory Disease* **115**, 1015–1026.

MESSMER, T. O., ARMOUR, R. & HOLLEY, R. W. (1982). Factors influencing the growth of alveolar type II epithelial cells isolated from rat lungs. *Experimental Cell Research* **142**, 417–426.

RAE, J. L. & LEVIS, R. A. (1984). Patch voltage clamp of lens epithelial cells: theory and practice. *Molecular Physiology* **6**, 115–162.

RANNELS, S. R. & RANNELS, D. E. (1986). Type II pneumocytes in culture: correlation of cell shape and function. *American Review of Respiratory Disease* **133**, A292.

ROONEY, S. A. (1985). The surfactant system and lung phospholipid biochemistry. *American Review of Respiratory Disease* **131**, 439–460.

ROSS, W. N., SALZBERG, B. M., COHEN, L. B., GRINVALD, A., DAVILA, H. V., WAGGONER, A. S. & WANG, C. H. (1977). Changes in absorption, fluorescence, dichroism, and birefringence in stained giant axons: optical measurement of membrane potential. *Journal of Membrane Biology* **33**, 141–183.

SCHNEIDER, G. T., COOK, D. I., GAGE, P. W. & YOUNG, J. A. (1985). Voltage sensitive, high-conductance chloride channels in the luminal membrane of cultured pulmonary alveolar (type II) cells. *Pflügers Archiv* **404**, 354–357.

SIMON, R. H., MCCOY, J. P., CHU, A. E., DEHART, P. D. & GOLDSTEIN, I. J. (1986). Binding of *Griffonia simplicifolia* I lectin to rat pulmonary alveolar macrophages and its use in purifying type II alveolar epithelial cells. *Biochimica et biophysica acta* **885**, 34–42.

SMITH, F. B., KIKKAWA, Y., DIGLIO, C. A. & DALEN, R. C. (1980). The type II epithelial cells of the lung. VI. Incorporation of $^3$H-choline and $^3$H-palmitate into lipids of cultured type II cells. *Laboratory Investigation* **42**, 296–301.

WEIBEL, E. R., GEHR, P., HAIES, D., GIL, J. & BACHOFEN, M. (1976). The cell population of the normal lung. In *Lung Cells in Disease*, ed. BOUHUYS, A., pp. 3–16. Amsterdam: Elsevier/North Holland Biomedical Press.