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Simultaneous Measurement of Intracellular pH and K^+ or NO_3^- in Barley Root Cells Using Triple-Barreled, Ion-Selective Microelectrodes

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The manufacture and use of triple-barreled microelectrodes, which are capable of simultaneous in vivo measurement of intracellular pH and the activities of K^+ or NO_3^- and cell membrane potential (E_m), are described. Scanning electron micrographs showed that the three tips were aligned and that the overall tip diameter was approximately 0.8 μm. When filled with 100 mM KCl, all three barrels simultaneously reported identical transmembrane potentials, showing that all three tips were located in the same subcellular compartment. Intracellular estimates of E_m in barley (*Hordeum vulgare* L. cv Klaxon) root epidermal cells obtained with these triple-barreled microelectrodes were indistinguishable from those obtained using single- or double-barreled microelectrodes. Measurements made with triple-barreled K^+ and pH-selective microelectrodes in root cells of 7-d-old barley plants grown in a nutrient solution containing 0.5 mM K^+ yielded cytosolic and vacuolar populations having mean K^+ activity values of 71.3 and 68.7 mM, respectively. The associated mean pH values (±SE) were 7.26 ± 0.06 (cytosol) and 5.18 ± 0.08 (vacuole). Analysis of whole-tissue digests confirmed the microelectrode measurements. Measurements made using triple-barreled pH- and nitrate-selective microelectrodes confirmed earlier double-barreled measurements of pH and nitrate in barley root epidermal cells growing in 10 mM nitrate.

The measurement of intracellular K^+ and NO_3^- levels is of obvious interest because of the important nutritional roles of each ion. For example, K^+ is the major inorganic cation in plants and is involved in turgor generation and cell expansion (MacRobbie, 1977), enzyme activation (Evans and Wildes, 1971), and protein synthesis (Wyn Jones et al., 1979). Measurements of cytosolic and vacuolar K^+ levels can provide information relevant to these functions and can also help to elucidate mechanisms of K^+ transport across the plasma membrane and tonoplast (Glass and Fernando, 1992; Maathuis and Sanders, 1993). Nitrate is the major nitrogen source for most plants and is mostly assimilated by the plant, but under replete conditions it is stored in the vacuole at high concentrations, suggesting a major role as an osmoticum (Miller and Smith, 1992).

Intracellular K^+ can be estimated in several ways. X-ray microanalysis has been frequently used for direct measurement of K^+ in suitably prepared tissue (Pitman et al., 1981; Huang and van Steveninck, 1989) and in picoliter (vacuolar) sap samples withdrawn from cells (Malone et al., 1989). However, x-ray signals are difficult to quantify (Leigh and Tomos, 1993), the detection limit is relatively high (20–25 mM; Stelzer et al., 1988), and cytosolic measurements are very difficult to achieve in fully vacuolated tissues (van Steveninck and van Steveninck, 1991).

Compartmentational (efflux) analysis, employing $^{42}K^+$ or $^{86}Rb^+$ as a tracer, has been widely used to estimate K^+ fluxes across the tonoplast and plasma membrane and K^+ levels in the cytoplasm and vacuole (Walker and Pitman, 1976). However, efflux analysis cannot be used to determine cytosolic and vacuolar K^+ levels in "low-salt" roots (Jeschke and Stelter, 1976). Behl and Jeschke (1982) reported that $^{86}Rb^+$ is not a suitable tracer for K^+ in barley (*Hordeum vulgare* L.) roots. ^{39}K-NMR can be used to determine whole tissue (vacuolar) K^+ levels, but it can provide only semiquantitative estimates of cytosolic K^+ levels (Lee and Ratcliffe, 1993).

Ion-selective microelectrodes can be used to give in vivo estimates of the intracellular a_K (MacRobbie and Lettau, 1980; Rona et al., 1982; Maathuis and Sanders, 1993), a_{NO3} (Miller and Zhen 1991), and pH (Felle and Bertl, 1986; Reid and Smith, 1988; Miller and Smith, 1992). K^+-, NO_3^--, and pH-selective sensors all have detection limits that are well below the minimum levels occurring in plant cells (Ammann et al., 1987; Chao et al., 1988; Miller and Zhen, 1991). In addition, double-barreled microelectrodes, incorporating an E_m-sensing barrel as well as an ion-selective barrel, enable the acquisition of information about both the electrical and chemical gradients, and thus allow the calculation of the energetics of the transmembrane ion transport (Rona et al., 1982; Miller and Smith, 1992; Maathuis and Sanders, 1993).

One problem with microelectrode measurements of K^+ and NO_3^- is that the precise intracellular location (cytoplasm or vacuole) of the electrode cannot easily be inferred. Users have had to make many impalements to find two populations of measurements that can then be assigned to particular compartments by other techniques such as vacuolar sap sampling (Zhen et al., 1991) or whole-tissue analysis (Miller and Zhen, 1991). Unfortunately, for plants

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Abbreviations: a_K, K^+ activity; a_{NO3}, NO_3^- activity; E_m, cell membrane potential; FP, flame photometry; ICP, inductively coupled plasma spectrometry.

growing under some nutritional conditions concentrations of the ions of interest in the two intracellular compartments may be indistinguishable, and this can be a major limitation for interpretation. For some ions like Ca^{2+} and H^+, there is a constant activity difference of several orders of magnitude between the cytoplasm and vacuole (Miller and Sanders, 1987; Kurkdjian and Guern, 1989; Miller and Smith, 1992). Here we show that this difference in pH between the cytoplasm and vacuole can be utilized to identify compartments by using triple-barreled microelectrodes, incorporating, in addition to a barrel selective for the ion of primary interest (e.g. K^+ or NO_3^-), a pH-selective barrel, so that measurements can be designated as cytosolic (pH 7.0–7.6) or vacuolar (pH 4.0–6.0) (Kurkdjian and Guern, 1989). There are only a few reports describing the extracellular (Dufau et al., 1982; Coles and Orkand, 1985) and intracellular (Fujimoto and Honda, 1980; Harvey and Kernan, 1984) use of triple-barreled, ion-selective microelectrodes in animal tissues, and no reports of their use in plant tissues. We describe the manufacture of triple-barreled pH- and K^+- or NO_3^--selective microelectrodes and their usage for estimation of intracellular pH, a_K, and a_{NO3} in barley root cells.

MATERIALS AND METHODS

Plant Material

Seeds of barley (*Hordeum vulgare* L. cv Klaxon) were germinated and grown for 5 d over 0.2 mM $CaSO_4$. They were then transferred to a nutrient solution containing 4.5 mM $CaCl_2$, 1.8 mM $Ca(NO_3)_2$, 0.2 mM FeNaEDTA, 2.2 mM $MgSO_4$, 1.1 mM NaH_2PO_4, plus micronutrients (Hoagland and Arnold, 1950). The pH was adjusted to 6.0 with 2 N NaOH. Four plants were grown in a 0.6-L pot, with a constant temperature (day/night) of 20°C, RH of 75%, and a PPFD of 280 to 300 μmol m^{-2} s^{-1} (16-h day). Nutrient solution was replaced daily. Plants were used when 7 d old. For K^+ selective microelectrode measurements, the final K^+ concentration in the nutrient solution was 0.5 mM. However, for the measurements of E_m only, no K^+ was added to the nutrient solution, but ICP showed that the solution contained 2.0 μM K^+, probably as contamination from other chemicals. For nitrate-selective microelectrode measurements, the seedlings were transferred for 24 h to a modified Hoagland solution containing 10 mM nitrate (Zhen et al., 1991).

Development of Electrodes

The K^+ sensor used in the ion-selective microelectrodes consisted of 90% (w/w) Fluka potassium ionophore II cocktail B (Fluka 90038) (Ammann et al., 1987) plus 10% polyvinylchloride to prevent the sensor from being displaced by cell turgor (Miller and Sanders, 1987). This mixture was dissolved in 5 volumes of tetrahydrofuran. The pH sensor was that of Miller and Smith (1992), modified from Chao et al. (1988). The nitrate-selective sensor was prepared as described previously (Miller and Zhen, 1991). All chemicals were of analytical grade and, except for tetrahydrofuran (Sigma), were purchased from Fluka.

Single-barreled electrodes were made using filamented borosilicate glass (o.d. 1.0 mm, i.d. 0.58 mm). Double-barreled microelectrodes were prepared by a modification of the method of Miller and Zhen (1991) using pre-fused filamented borosilicate glass. One barrel of this glass was wider and was used as the ion-selective sensor barrel (o.d. 1.0 mm, i.d. 0.58 mm). The other, narrower barrel (o.d. 0.75 mm, i.d. 0.35 mm) was used as the reference (E_m measuring) barrel. Single- and double-barreled glass was obtained from Hilgenberg Glass (Malsfeld, Germany). Triple-barreled microelectrodes were fabricated from three pieces of the single-barreled glass glued together with Loctite Super Glue (Loctite Holdings, Ltd., Welwyn Garden City, Hertfordshire, UK). The blunt ends of each of the three barrels were broken back to differing lengths: the longest barrel was always filled with the pH sensor; the intermediate-length barrel was filled with the primary ion sensor (NO_3^- or K^+); and the shortest barrel was the reference (E_m sensing) barrel.

Electrodes were pulled as described by Miller and Zhen (1991) to produce overall tip diameters of approximately 0.8 μm, as estimated from scanning electron micrographs. When backfilled with 100 mM KCl, barrel resistances were 50 to 70 MΩ. After pulling triple-barreled microelectrodes were strengthened by applying Super Epoxy resin (Plastic Padding, Ltd., Woburn Green, Buckinghamshire, UK) behind the tip's shoulder. Pulled electrodes were placed under a heating lamp at 140°C for 1 h. Ion-selective barrels were then silanized by the addition of one or two drops of 1% (v/v) dimethyldichlorosilane, in chloroform, to the blunt end of the barrel. After 1 h at 140°C, the heating lamp was removed and the appropriate ion-selective sensor was added. The electrodes were left at room temperature in silica-dried air for at least 48 h to allow excess tetrahydrofuran to evaporate. An exception to this procedure was for triple-barreled microelectrodes. In this case only pH sensor was added immediately after silanization; the other ion-selective sensor was added 48 to 72 h later. We found that when K^+ or NO_3^- sensor was added immediately after pH sensor, triple-barreled microelectrodes were generally unresponsive to any ion. This was not investigated but may have been due to the mixing of the two ion-selective sensor cocktails at the tip.

After the ion-selective membrane had set in the electrode tip, a 30-G needle attached to a 1-mL syringe was used to backfill each barrel. The backfilling solution for the K^+-selective barrel of K^+ electrodes was 100 mM KCl, whereas the reference barrel was backfilled with 200 mM NaCl. For NO_3^--selective barrels, the backfilling solution contained 100 mM KCl and 100 mM KNO_3, and the reference barrel was backfilled with 100 mM KCl. For pH electrodes, the H^+-selective barrel was backfilled with 0.5 mM KCl plus 100 mM Mes-NaOH, pH 6.0.

Characterization and Calibration of Ion-Selective Microelectrodes

Selectivity coefficients (Guilbault et al., 1976) for the K^+ and pH sensors used in this study were determined using single-barreled microelectrodes by the fixed interference

Table I. *Composition of K^+-selective microelectrode calibration solutions*

Ionic strength and a_K were calculated using the SOLCON program.

$-\log[a_K]$	a_K	[KCl]	[$MgCl_2$]	Ionic Strength
	mM	mM	mM	mM
0.9	124	165	0	165
2.0	10.5	14.0	45.0	149
3.0	1.10	1.40	49.0	148
4.0	0.11	0.14	49.9	150

method (Guilbault et al., 1976), and were found to be very similar to those given by Ammann et al. (1987) and Chao et al. (1988), respectively. No interference with the K^+ response was observed from physiological concentrations of amino acids, anions, pH, protein, or reducing sugars (data not shown). The ionic strength of the K^+-calibration solutions ([K^+] 0.1–125 mM) was maintained at 148 to 165 mM by addition of $MgCl_2$ (Table I). Ion activities were calculated using the SOLCON program written by Drs. D.C.S. White (University of York, UK) and Y.E. Goldman (University of Pennsylvania). The nitrate-selective barrel was calibrated as described previously (Miller and Zhen 1991). All pH calibration solutions contained 120 mM KCl, 10 mM NaH_2PO_4, and 20 mM buffer (Reid and Smith, 1988): either Mes-H_2SO_4 (pH 4.0), Mes-NaOH (pH 6.0), Mops-NaOH (pH 7.0), or *N*-tris[hydroxymethyl]methyl-3-amino-propanesulfonic acid-NaOH (pH 8.5).

Calibration and intracellular measurements were made using a high-input impedance differential electrometer (model FD223; World Precision Instruments, Sarasota, FL). The electrometer output passed via an A/D converter (Labmaster DMA/PGH; Scientific Solutions, Solon, OH), at a sampling frequency of 10 Hz, to an Opus PC V microcomputer. VISER software, developed by I.R. Jennings (University of York, UK), was used to analyze the data. Calibration curves were fitted using a modified Nicolsky-Eisenman equation (Miller and Zhen, 1991) of the type: $E = E_0 + S \log(a_X + C)$, where E is the measured output of the ion-selective electrode, E_0 is the constant electrode reference potential, S is the slope, a_X is activity of H^+ or K^+, and C is a term that subsumes the concentrations of interfering ions and the selectivity coefficients of the electrode for those ions. For NO_3^- the equation takes the form: $E = E_0 - S \log(a_{NO3} + C)$.

Characterization of Microelectrode Tips

Scanning electron microscope photographs of the tips were taken to determine their orientation and diameter. The tips were first splutter-coated with Au using a Semcoat

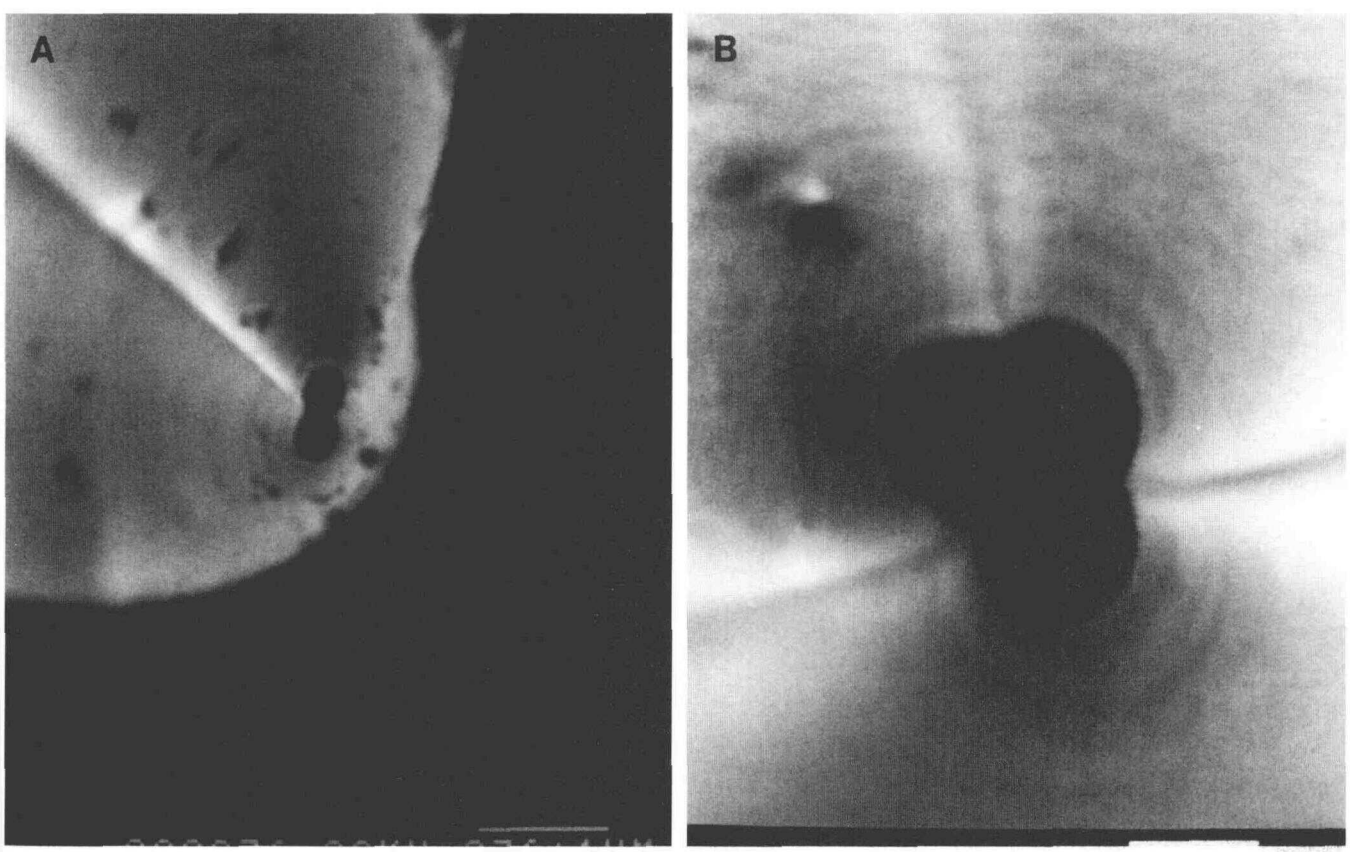

Figure 1. Scanning electron micrographs showing double- (A) and triple-barreled (B) microelectrode tips. A, Scale bar = 0.76 μm; B, scale bar = 0.2 μm.

Table II. *Resistances of single-, double-, and triple-barreled microelectrodes filled with 100 mM KCl*

Values are mean ± SE with the number of measurements shown in parentheses. For double-barreled electrodes, number 1 would become the ion-selective barrel, whereas for triple-barreled electrodes, 1 would be the pH barrel and 2 either the K^+ or NO_3^- barrel.

Electrode Type	Barrel Measured	Resistance
		MΩ
Single-barreled		53.1 ± 2.1 (8)
Double-barreled	1	65.4 ± 8.9 (10)
	2	69.9 ± 7.4 (10)
Triple-barreled	1	60.5 ± 1.7 (10)
	2	53.4 ± 2.6 (10)
	3	55.7 ± 2.4 (10)

Splutter Coater MkII (EMScope, Ltd., Ashford, Kent, UK) and then viewed under a Hitachi S-450 scanning electron microscope (Nissie-Sanyo Instruments, Ltd., Wokingham, Berkshire, UK). An additional estimate of relative tip pore diameter was provided by measuring tip electrical resistance using the resistance test facility of World Precision Instruments, Inc. (New Haven, CT) "Ωmega-tip."

Intracellular Recordings

For intracellular impalements, a root, still attached to a 7-d-old barley plant, was clamped in a plexiglass chamber (volume 2.0 mL) and perfused with nutrient solution (flow rate = 5.0 mL/min). Impalements with microelectrodes were always made in mature epidermal cells 1 to 2 cm from the root tip. Microelectrodes were calibrated separately for pH and K^+ or NO_3^- both before and after an intracellular measurement, and the combined data were used to produce a calibration curve from which the intracellular a_K, a_{NO3}, and pH were calculated (using VISER software). Results from electrodes that failed to recalibrate were discarded. Before ion-selective microelectrodes were calibrated, they were preimpaled once or twice for 5 to 10 s each time into a barley root to eliminate any tips that were unable to withstand cell turgor. We also found that the electrode performance can sometimes improve, by extending the range of the calibration curve at lower ion activities, after such brief impalements. Results are expressed as mean values ± SE (number of observations), except for intracellular a_K and a_{NO3} values, which are expressed as the means plus 95% confidence limits, following conversion from the corresponding $-\log[a_K]$ or $-\log[a_{NO3}]$ values (Fry et al., 1990).

Determination of Whole-Tissue K^+ Levels

For 7-d-old barley plants the fresh weights of the whole roots were recorded and sap was extracted by a freeze-thaw technique (Tomos et al., 1984) and analyzed for K^+ by FP. Alternatively, roots were acid digested with perchloric acid and analyzed for K^+ by ICP.

RESULTS

Figure 1 shows scanning electron micrographs of the tips of both double- and triple-barreled microelectrodes. These clearly show that the tip diameters of both types of electrode are less than 1 μm, with a slightly larger tip in the triple barrel. The internal cross-walls are not visible on these micrographs, but they could be seen on the scanning electron microscope display screen. The electrical resistance of each barrel of the triple-barrel tips was similar to that of both individual barrels of double- and single-barreled tips (Table II). Tip resistance of electrodes, which are all filled with 100 mM KCl, must be determined largely by the actual bore diameter of the microelectrode glass at the tip.

E_m recordings made using single-, double-, and triple-barreled microelectrodes, without ion sensors and with all barrels backfilled with salt solutions, were similar (Table III; Fig. 2). Figure 2 also shows that all three barrels of a single, triple-barreled tip report the same E_m value, indicating that the three individual tips were residing in the same subcellular compartment and providing more evidence that the three apertures are adjacent to each other. At the onset of the impalement the three barrels reported a small potential difference of approximately −50 mV, which may be due to the Donnan potential of the cell wall (Lew, 1991); we do not know why channel B reported a smaller value compared to the other two barrels (see Fig. 2). However, in all measurements using multi-barreled electrodes backfilled with salt solution only, we found that there was never more than a 2-mV difference between the voltage reported by each of the barrels. The similarity of E_m for all types of electrodes implies that the cell impalement and resulting membrane seal are not very different between single-, double-, and triple-barreled electrodes. The results in Table III also show that there is no effect on E_m when the backfilling salt solution is changed from KCl to NaCl. These measurements were all made using barley roots that had been grown on nutrient solution containing only 2 μM K^+, in order to obtain E_m values that were as large as possible.

We also compared E_m, pH, and a_K measurements made using triple-barreled K^+-selective microelectrodes in which the reference barrel contained either 200 mM NaCl or KCl, because of concerns raised about diffusion of KCl from reference barrels containing high KCl concentrations that may significantly alter measured values of E_m and a_K (Blatt and Slayman, 1983; Blatt, 1987). We could not measure any drift in cytosolic a_k during recordings with either type of electrode, so there was no evidence of significant diffusion of K^+ from the KCl-filled electrode tip (data not shown).

Table III. *Measurements of E_m in barley root epidermal cells made using single-, double-, or triple-barreled microelectrodes backfilled with 100 mM KCl or 200 mM NaCl in all barrels*

Values are means ± SE with the numbers of measurements in parentheses. Measurements were made in nutrient solution containing only 2 μM K^+.

Number of Barrels	Backfill Solution	E_m
		mV
1	NaCl	−156.1 ± 5.2 (15)
1	KCl	−151.5 ± 6.3 (12)
2	KCl	−147.6 ± 6.1 (12)
3	KCl	−149.9 ± 5.4 (12)

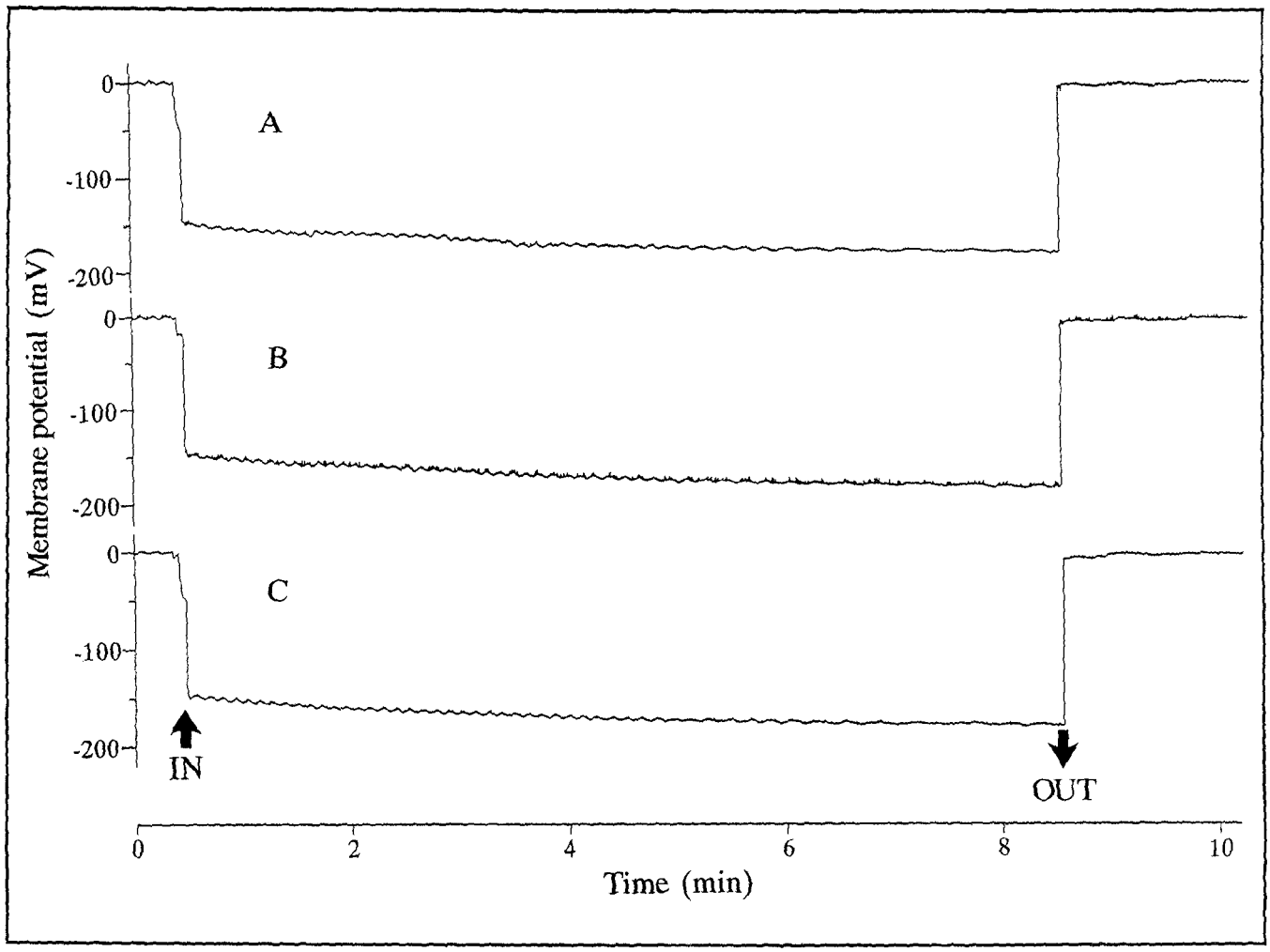

Figure 2. Simultaneous recordings of E_m of a barley root epidermal cell from all three barrels (A, B, and C) of a triple-barreled microelectrode. All barrels were backfilled with 100 mM KCl. The microelectrode tip was inserted into and removed from the cell at the points shown. The E_m values at time = 8 min were −177.5 mV (A), −178.0 mV (B), and −178.0 mV (C). Measurements were made in nutrient solution containing only 2 μM K^+.

Figure 3 shows a typical recording from a triple-barreled K^+ and pH electrode. This impalement shows that the microelectrode tip was located in the cytosol when the measured pH was 7.22, whereas the corresponding value of a_K was 59 mM.

Figure 4 shows a frequency histogram plot of a_K measurements obtained using triple-barreled microelectrodes. Clearly, these data points do not separate into two populations. However, when the a_K values measured with triple-barreled electrodes were plotted against the pH values obtained (Fig. 5), the data points separated into two populations, one around pH 5 and the other above pH 7, indicating that the electrodes can distinguish measurements from two compartments that probably represent the vacuole and the cytosol.

The microelectrode measurements in Table IV show that there were no significant differences in cytosolic and vacuolar a_K and pH between cortical and epidermal cells of roots growing in 0.5 mM K^+ nutrient solution. This lack of difference between the two different cell types justifies combining the values to give the lower two rows of Table IV and Figures 4 and 5. Whole-root tissue K^+ analysis by acid digest and ICP gave a tissue concentration of 98 mM, whereas FP analysis of whole-root sap samples gave a tissue concentration of 120 ± 5 (4) mM. These values for whole-root K^+ concentration estimated directly do differ slightly from triple-barreled K^+-selective microelectrode measurements of vacuolar a_K (see Table IV).

Measurements were also made with triple-barreled nitrate- and pH-selective microelectrodes, and these confirm earlier measurements made using double-barreled nitrate and pH-selective microelectrodes (Miller and Smith, 1992). When comparing values for cytosolic and vacuolar nitrate and pH in Table V, there were no significant differences between results obtained using double- and triple-barreled electrodes. Overall, the above results give confidence that the triple-barreled electrodes yield measurements that are indistinguishable from those made with single- or double-barreled electrodes, and that all three barrels of the electrode report from the same compartment.

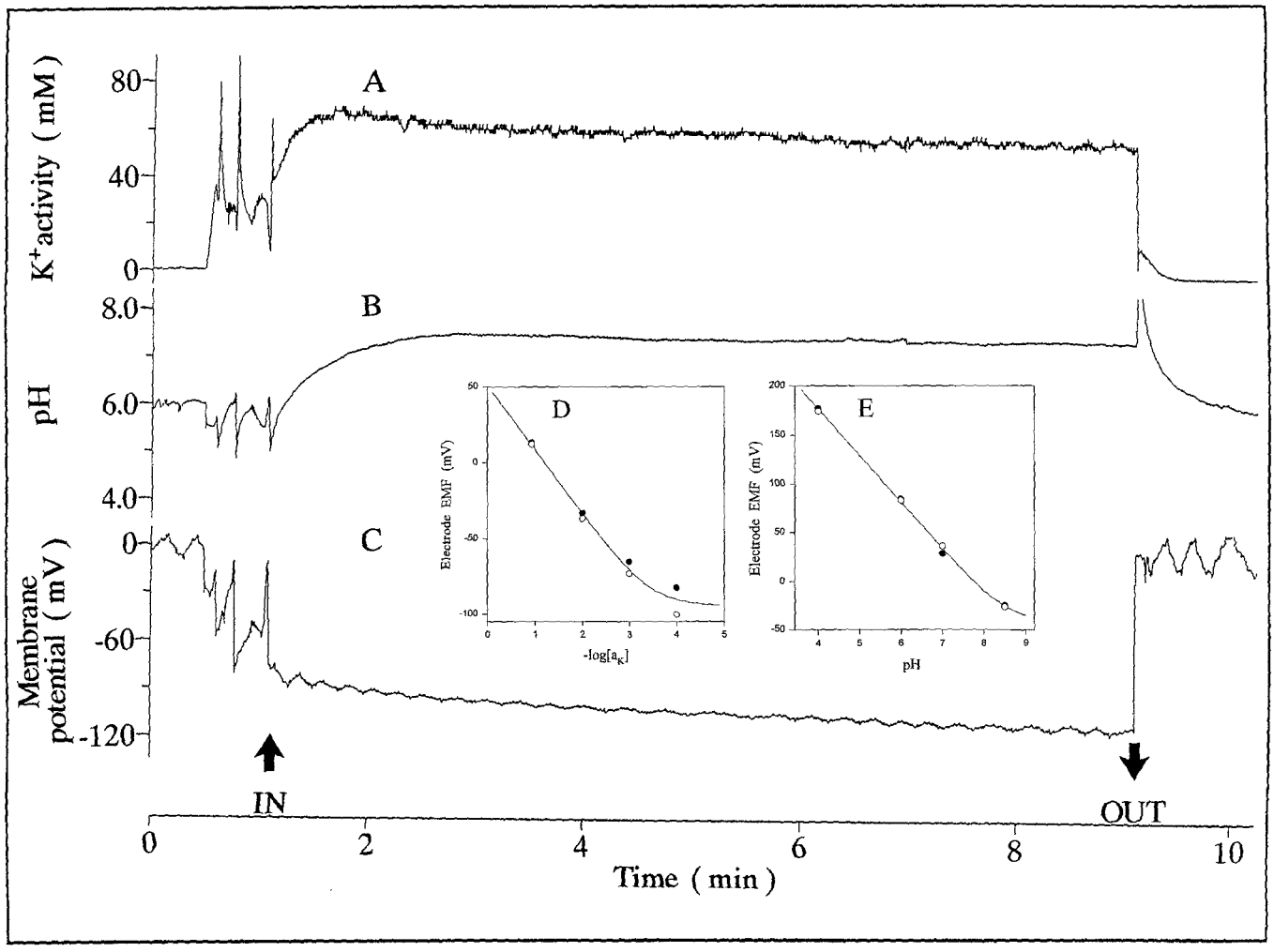

Figure 3. Triple-barreled microelectrode recording in the cytosol of a barley root cortical cell. A, a_K (mM); B, pH; C, E_m (mV). The microelectrode tip was inserted into the cell and removed at the points shown. The E_m-sensing barrel was backfilled with 200 mM NaCl. Insets D and E show the calibration data before (●) and after (○) the impalement for the K^+-selective barrel (D) and the pH-selective barrel (E). Calibration curves were fitted to combine before and after data, using modified Nicolsky-Eisenman equations. Fitted values were: K^+ electrode, E_0 = 51.0 mV; S = 46.4 mV; C = 0.4 mM. pH electrode, E_0 = 367.6 mV; S = 47.8 mV; C = 2.7 nM.

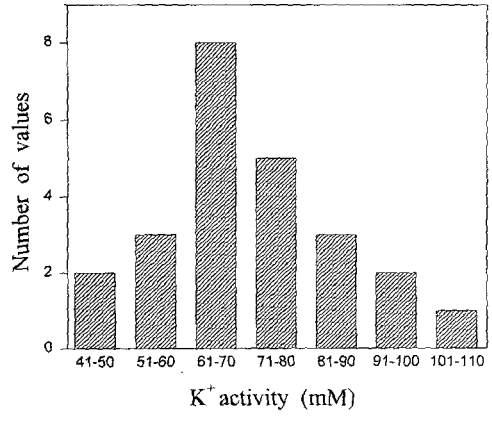

Figure 4. Histogram showing the distribution of triple-barreled K^+-selective microelectrode measurements of intracellular a_K in barley root epidermal and cortical cells growing in nutrient solution containing 0.5 mM K^+.

DISCUSSION

Triple-Barreled, Ion-Selective Microelectrodes as a Physiological Tool

To our knowledge, this paper reports the first intracellular use of triple-barreled, ion-selective microelectrodes in plants. Triple-barreled, ion-selective microelectrodes have been used previously in animal cells (Fujimoto and Honda, 1980; Harvey and Kernan, 1984), and multi-barreled electrodes have been used for voltage-clamp measurements in plant cells (Lew, 1991; Blatt and Armstrong, 1993).

The triple-barreled electrodes give reliable results that report from the same intracellular location. They measure E_m values similar to those obtained with single- and double-barreled electrodes and to those previously reported for barley roots (Mertz and Higinbotham, 1976). For triple-barreled microelectrode measurements it is vital that the tips of all three barrels are oriented such that after impale-

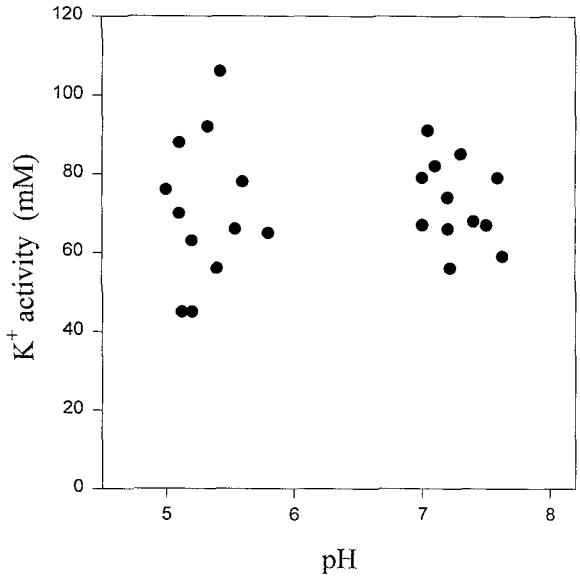

Figure 5. Scatter diagram showing the relationship between intracellular a_K and pH measured with triple-barreled K^+- and pH-selective microelectrodes in barley root epidermal and cortical cells growing in nutrient solution containing 0.5 mM K^+.

ment they are all located in the same subcellular compartment, i.e. cytosol or vacuole. The incorporation of a third barrel, selective for a "marker" ion whose activity is known to vary by several orders of magnitude between cytosol and vacuole, e.g. Ca^{2+} or H^+, allows the compartmental location of the second ion-selective barrel (in this case K^+ or NO_3^-) to be identified as cytosolic (pH 7.0–7.8) or vacuolar (pH 5.0–5.5).

For the work described here, the compartmental location of the tip is easily distinguished by the pH measurement, because large pH gradients exist across the tonoplast, with the cytosol maintained at relatively constant values, whereas the vacuole has much higher proton concentrations. This ability to distinguish the cytosol and the vacuole should be particularly useful when plants are grown under nutrient regimes that produce similar cytosolic and vacuolar activities of the "primary" ion. This avoids the need for more indirect methods to demonstrate that a collection of intracellular ion activity measurements can be divided into two (cytosolic and vacuolar) populations (e.g. Miller and Zhen, 1991; Maathuis and Sanders, 1993). The use of triple-barreled microelectrodes extends the range of ion-selective electrode measurements to conditions in which cytosolic and vacuolar concentrations are likely to be similar. For example, nitrate measurements during induction when nitrate is first supplied to tissues, and K^+ measurements in K^+-replete cells.

Triple-barreled microelectrodes should be a powerful tool for the elucidation of transplasma membrane or tonoplast ion-transport mechanisms, since they yield values for both transmembrane potential differences and ion gradients from which the electrochemical gradient for a given ion can be determined. For example, in these barley root cells the K^+-selective microelectrode measurements of the electrochemical gradient for K^+ across the plasma membrane showed that the equilibrium potential for K^+ (E_K) was approximately −125 mV, whereas the measured membrane potential was −103 ± 5.1 (Table IV). This indicates that active uptake is required to maintain the K^+ gradient across the plasma membrane in barley root epidermal cells growing under these conditions. Another advantage of using these triple-barreled electrodes is that they also report the electrochemical gradients of H^+ across both the plasma membrane and the tonoplast, so that the proton motive force can be calculated for proton-coupled transport such as that recently described for K^+-starved wheat roots (Schachtman and Schroeder, 1994).

Whole-root tissue K^+ analysis gave concentrations ranging from 98 mM (ICP) to 120 mM (FP), but the triple-barreled electrode measurements indicate a lower vacuolar K^+ activity of 69 mM (Table IV). The different values obtained using the different methods can be partly explained by the fact that the K^+-selective microelectrodes were calibrated against activity rather than concentration (see "Materials and Methods"). An activity coefficient of 0.75 for K^+ in the cell is not an unreasonable value (Robinson and Stokes, 1968). However, we do not know why the two different types of whole-tissue analysis gave different estimates of tissue K^+ concentration; perhaps there is some water loss during the freeze-thawing process that results in an overestimation.

The proton-nitrate-selective, triple-barreled microelectrodes give measurements that confirm previous work us-

Table IV. *Vacuolar and cytosolic E_m, pH, and a_K of barley root epidermal and cortical cells, measured using triple-barreled microelectrodes; reference barrel backfilled with 200 mM NaCl*

Values of E_m and pH are means ± SE and a_K are means with 95% confidence limits in square brackets. Compartments were identified on the basis of their pH. The number of measurements is given in parentheses. The same data have been used to plot Figures 4 and 5; there are fewer vacuolar measurements of a_K than pH because in some measurements the K^+-selective barrel did not recalibrate after a measurement.

Cell Type	Cell Compartment	Measurements with Microelectrodes		
		E_m	a_K	pH
Epidermal	Cytosol	−110.4 ± 7.4 (7)	73.0 [70.1,76.2] (7)	7.21 ± 0.07 (7)
	Vacuole	−90.3 ± 7.9 (6)	67.6 [64.3,71.1] (6)	5.35 ± 0.13 (6)
Cortical	Cytosol	−94.3 ± 5.4 (5)	68.9 [62.8,75.5] (5)	7.34 ± 0.12 (5)
	Vacuole	−81.1 ± 4.1 (11)	69.7 [59.4,82.0] (6)	5.09 ± 0.10 (11)
Pooled data	Mean cytosol	−103.0 ± 5.1 (12)	71.3 [68.2,74.5] (12)	7.26 ± 0.06 (12)
	Mean vacuole	−84.0 ± 3.8 (17)	68.7 [63.5,74.3] (12)	5.18 ± 0.08 (17)

Table V. *Comparison of double- and triple-barreled electrode measurements of nitrate and pH in barley root epidermal cells*

E_m and pH values are means ± SE, and a_{NO3} are means with 95% confidence limits in brackets; *n* is the number of measurements.

Electrode Type	Cell Compartment	NO_3-Selective Microelectrode			pH-Selective Microelectrode		
		a_{NO3}	*n*	E_m	pH	*n*	E_m
		mM		mM			mM
Double-barreled[a]	Cytosol	4.9 (4.5, 5.5)	19	−73.0 ± 6	7.12 ± 0.06	10	−76.7 ± 4
	Vacuole	39 (36.7, 41.7)	35	−65.0 ± 4	4.93 ± 0.11	22	−67.6 ± 5
Triple-barreled	Cytosol	4.5 (3.8, 5.2)	4	−79.3 ± 6	7.23 ± 0.09	4	−79.3 ± 6
	Vacuole	42 (33.9, 51.3)	6	−71.7 ± 8	5.20 ± 0.20	6	−71.7 ± 8

[a] Data from Miller and Smith, 1992.

ing double-barreled, nitrate-selective microelectrodes (Miller and Smith, 1992). For barley root epidermal cells growing under these conditions, nitrate transport at both the plasma membrane and the tonoplast must be active, confirming findings made using double-barreled, nitrate-selective microelectrodes (Zhen et al., 1991).

In conclusion, we have shown that triple-barreled, ion-selective microelectrodes can be used to measure intracellular K^+ or NO_3^- and pH in barley root cells. Several different methods have been used to check that the electrodes do not damage cells and that all three tips are located in the same intracellular compartment.

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