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Determination of Intracellular K^+ Activity in Rat Kidney Proximal Tubular Cells

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Abstract. The intracellular K^+ activity of rat kidney proximal tubular cells was determined in vivo, using intracellular microelectrodes. In order to minimize damage from the impaling electrodes, separate measurements on separate cells, were performed with single-barrelled KCl-filled non-selective electrodes and single-barrelled, K^+-sensitive microelectrodes, which were filled with a liquid K^+-exchanger resin that has also a small sensitivity to Na^+. Both electrodes had tip diameters of 0.2 μm or below. The proper intracellular localization of the electrodes was ascertained by recording the cell potential response to intermittent luminal perfusions with glucose. The membrane potential measured with the non-selective microelectrodes was -76.3 ± 8.1 mV ($n = 81$) and the potential difference measured with the K^+-sensitive microelectrode was -7.2 ± 5.8 mV ($n = 32$). Based on the activity of K^+ in the extracellular fluid of ~3 mmol/l the intracellular K^+ activity was estimated to be ~82 mmol/l. Assuming equal K^+-activity coefficients to prevail inside and outside the cell, this figure suggests that the intracellular K^+ concentration is ~113 mmol/l which must be considered as a lower estimate, however. The data indicate that the K^+-ion distribution between cytoplasm and extracellular fluid is not in equilibrium with the membrane potential, but that K^+ is actively accumulated inside the cell. This result provides direct evidence for the presence of an active K^+ pump in the tubular cell membranes, which in view of other observations, must be envisaged as a (not necessarily electroneutral) Na^+/K^+-exchange pump which operates in the peritubular cell membrane and is eventually responsible for the major part of the tubular solute and water absorption.

Key words: Ion-selective microelectrode – Renal tubular cell – Cytoplasmic K^+ activity – Active K^+ transport.

Introduction

The electrophysiological technique is a powerful tool for the investigation of ion transport processes across epithelia because it allows to obtain information from the inside of intact, normally functioning cells. This information is particularly valuable if the measurements include determinations of intracellular ionic activities with ion selective microelectrodes. Several problems, however, arise if the cells are very vulnerable, such as the cells of mammalian kidney tubule. Although we have succeeded already ten years ago in obtaining what we consider reliable cell membrane potential measurements in rat kidneys [8], the success rate of such measurements is still rather low, even though we use electrodes with small tip diameter (below 0.2 μm) which have high resistances and produce less damage. The problem is even more severe with ion-selective microelectrodes. Ideally double-barrelled electrodes should be used, which have one selective barrel to record the activity plus the membrane potential and one non-selective barrel to record the membrane potential separately. Such electrodes, which have already been applied by others [12–14] have larger tips and may thus be expected to damage the cells more readily. On the other hand one cannot simply build electrodes with finer and finer tips, because the resistivity of the ion-exchanger material (or other ligand material) which is filled into the tip of the selective channel is usually very high, so that electrodes with extremely fine tips are not likely to function properly. In order to work with the smallest possible tips and the smallest possible tip resistance we have therefore decided to build only single barreled electrodes and to apply the selective and non-selective electrodes separately. After time-consuming pilot experiments we succeeded in building properly functioning K^+-exchanger microelectrodes with tips of ≤ 0.2 μm, which are smaller than usually described in the literature thus far [15, 19, 28, 32]. The problem of recognizing the proper intracellular position of the

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single-barrelled electrode and judging the validity of the recorded potentials was solved by recording the potential response to intermittent luminal perfusions with glucose containing Ringer's solutions, which are known to produce a rapid initial shift of the membrane potential, but affect the intracellular K^+ activity only slowly. In the present paper we report a study with K^+-selective microelectrodes. Part of the data was already published in abstract form [3].

Methods

The experiments were performed on rat kidney proximal tubular cells using in vivo and situ micropuncture techniques. The animals, male Wistar rats of 230 g body weight, were fed on an Altromin standard diet up to 12 h prior to the experiment and had free access to water until the anaesthesia (Inactin, 50 mg/kg body weight) was begun. Although standard micropuncture techniques were used which have been described elsewhere [27], the following details are worth mentioning: 1. Instead of HCO_3^--Ringers fluid the kidney surface was covered with mineral oil during the experiment. 2. Rather than under free-flow conditions, all cell potential measurements were performed on internally perfused proximal tubules, using the high speed retrograde perfusion technique described in ref. [6]. By switching the luminal perfusate from a bicarbonate Ringers solution to a glucose-bicarbonate Ringers solution, this technique allowed us to identify the proper intracellular localization of the K^+-selective microelectrode tips from the magnitude of the recorded initial potential response to glucose [5] which had to be greater than 5 mV. The perfusion solutions had the following composition (in mmol/l): Na^+: 143, K^+: 4, Ca^{2+}: 1.25, Mg^{2+}: 1, Cl^-: 118.5, HCO_3^-: 30, SO_4^{2-}: 1, PO_4: 1, D-glucose: 5. 3. No i.v. infusions were given throughout the experiment.

To measure the cell membrane potential Ling-Gerard type glass microelectrodes of ≤ 0.2 µm tip diameter were used, which were filled with 2.7 molar KCl solution. The resistance of these electrodes ranged between 60 and 150 MΩ and the tip potential was less than 5 mV. The electrodes were connected to a Keithley 604 electrometer and all measurements were recorded on a Servogor 512 chart recorder. All measurements with the K^+-selective microelectrodes, the production of which is described in detail below, were performed separately, using a WP F 223 electrometer (obtained from Science trading, Frankfurt/M., FRG) which features high input shunt resistance ($\sim 10^{15}$ Ω) and compensation of leakage current. These measurements were also recorded on a chart recorder. The resistance of the selective microelectrode was repeatedly tested during the calibration steps and during the in vivo measurements. It was calculated from the potential change recorded in response to connecting at 10^{11} Ω resistor between the electrometer input and ground.

Production of K^+-selective microelectrodes: With a tip size of 0.2 µm or below, which one must use to obtain reliable cell potential records, the production of liquid ion exchanger electrodes turned out to be difficult. The commonly used techniques for the siliconization of the electrode tips [15, 19, 28] failed, because the pressure required to suck in and expel the siliconizing solution was impractically great. We have therefore developed the following technique, which is partially adopted from Zeuthen et al. [32]. After cleaning with detergent, filament capillaries of borosilicate glass (K. Hilgenberg, 3509 Malsfeld, FRG) of 1.5 mm o.d. and 0.85 mm i.d. are boiled for 2 h in distilled water and then dried for 15 min in an oven at 200° C. They are then pulled to a tip length of 24 mm and silanized inside, by exposing the inside of the shank for 35–40 s to dichlor-dimethyl-silane vapour. The capillaries are then baked for 30 min at 130° C and filled with the exchanger (Corning No. 477317) by injecting a drop of the resin with a Hamilton syringe from the shank. They are then allowed to stand for 1–4 h until the tips are filled, whereafter the shaft is filled with the reference solution (in the present experiments 0.16 molar KCl solution) using again a Hamilton syringe. The yield of this technique is ~80%.

The electrode properties were tested in mixtures of isotonic NaCl-KCl solutions, by inserting the electrode into a WP EH 1 holder and measuring the potential difference against a broken tip microelectrode which had been filled with saturated KCl solution and was connected to ground via a saturated calomel half cell. Measurements were also performed in which the reference electrode was connected to the test solution under flowing boundary conditions, but the results were identical.

Theoretical Consideration

The principle of electrometric activity measurements consists of determining in a sample of unknown composition the potential difference ($\Delta\varphi_i$*[1]) between an electrode that is selective for the ion i and a non-selective electrode such as a calomel electrode with saturated KCl solution forming the liquid junction between the calomel electrode and the sample fluid. The activity of the ion i in the test fluid (a_i*) is then read from a calibration curve of $\Delta\varphi_i$* versus log a_i* obtained with samples of known composition, which in the ideal case obeys the Nernst equation:

$$\Delta\varphi_i^* = \frac{RT}{z_i F} \ln a_i^* + C \tag{1}$$

provided the junction potential between the test fluid and the saturated KCl solution of the reference electrode is negligible. In Eq. (1) R, T, z_i and F have their usual meaning and C is a constant summarizing all constant phase boundary potentials in the measuring chain. For the sake of simplicity the polarity of the potential $\Delta\varphi_i$* in this equation and in Eqs. (6), (7) and (9) below (which all describe electrode test experiments) is taken as the polarity indicated by the selective electrode if the reference electrode is connected to ground.

Although the principle of this approach is retained in the present experiments the technical realization differs in that we measure separately: 1. with a non-selective (KCl-filled) microelectrode the potential change (membrane potential, $\Delta\varphi$, with $\Delta\varphi = \varphi' - \varphi''$) which is observed, when the non-selective microelectrode is advanced from the extracellular space (compartment ″) into the intracellular space (compartment ′), and 2. with a selective microelectrode the potential change $\Delta\varphi_i$ (with $\Delta\varphi_i = \varphi_i' - \varphi_i''$) which is observed while advancing it from the extracellular space into the intracellular space, both measurements being taken with reference to a constant indifferent reference electrode, which is connected to the extracellular fluid compartment via the deskinned rat's tail. Since the potential difference $\Delta\varphi_i$ recorded with the selective electrode equals the electrochemical potential difference $\Delta\eta_i$ of the ion i that exists across the phase boundary between compartments ′ and ″, divided by the charge we obtain:

$$\Delta\varphi_i = \frac{\Delta\eta_i}{z_i F} = \frac{RT}{z_i F} \ln \frac{a_i'}{a_i''} + \Delta\varphi \tag{2}$$

which can be rearranged to

$$\Delta\varphi_i - \Delta\varphi = \frac{RT}{z_i F} \ln \frac{a_i'}{a_i''}$$

and from which the unknown intracellular activity a_i' is calculated as

$$a_i' = a_i'' \, e^{\frac{z_i F}{RT}\left(\Delta\varphi_i - \Delta\varphi\right)} \tag{3}$$

1 Throughout this paper all electrode test experiments are marked by asterisks

if the activity coefficient, γ_i'', and the concentration, c_i'', of the extracellular fluid are known, with

$$a_i = \gamma_i c_i \,. \tag{4}$$

In the special case that $\Delta\varphi_i$ and hence $\Delta\eta_i$ are zero the relation holds

$$\Delta\varphi = -\frac{RT}{z_i F}\ln\frac{a_i'}{a_i''} \equiv E_i \,. \tag{5}$$

E_i as defined in Eq. (5) is usually named equilibrium potential. Irrespective of the actual value of $\Delta\varphi_i$ the equilibrium potential E_i is commonly used to describe the relationship between the intra- and extracellular activities of i.

In practice, however, activity measurements are more complicated than suggested by Eq. (3), because most of the presently available electrodes do not only respond to a single ion i, but respond also to one or more interfering ions j; this means these electrodes do not measure $\Delta\varphi_i$ as defined in Eq. (2), but indicate a potential, which we may call $\Delta\tilde{\varphi}_i$ that reflects the activities of two or more different ions, with i being the ion to which the electrode is most sensitive. In the presence of one interfering ion j, with $z_i = z_j = +1$, such electrodes are generally thought to obey the equation

$$\Delta\tilde{\varphi}_i^* = \frac{RT}{z_i F}\ln\left(a_i^* + k_{i,j}^{\text{pot}}\, a_j^*\right) + C \tag{6}$$

which was originally derived for glass electrodes by Nicolsky [20] but was recently recommended for general use [11]. It is equivalent to the Goldman equation if the empirical selectivity coefficient $k_{i,j}^{\text{pot}}$ is interpreted as relative permeability of the ions i and j across the selective barrier, which determines the electrode properties. Although Eq. (6) was widely used in the past it was also frequently noticed that the empirical selectivity coefficients were not constant but varied with concentration [15–17, 26, 29], which suggests that Eq. (6) does not describe the interference behaviour properly. Among various alternative treatments the one of Sandblom, Eisenman and Walker [23] is particularly noteworthy, because it considers the case of a liquid ion exchange membrane under measuring conditions which are identical to those chosen in the present paper. For isomolar mixtures of two univalent salts with common anion the authors arrive at

$$\Delta\varphi_i^* = \frac{RT}{F}\left\{(1-\tau)\ln\left(a_i^* + K_1\, a_j^*\right) + \tau\ln\left(a_i^* + K_2\, a_j^*\right)\right\} + C \tag{7}$$

with $0 \le \tau \le 1$

where τ, K_1 and K_2 are coefficients, which are composed of molecular properties (such as mobilities and binding constants) of the ions i and j and of the mobile site of the exchanger in the loaded and unloaded configuration (for details see ref. [23]). To our knowledge the validity of this equation for liquid ion exchanger microelectrodes was not yet demonstrated.

In case that Eq. (6) can be applied, the determination of a_i' from measurements of $\Delta\varphi$ and $\Delta\tilde{\varphi}_i$, the latter being the potential change observed with a non-ideally selective electrode upon passing from the extra- into the intracellular space, leads to

$$a_i' = \left(a_i'' + k_{i,j}^{\text{pot}}\, a_j''\right) e^{\frac{z_i F}{RT}(\Delta\tilde{\varphi}_i - \Delta\varphi)} - k_{i,j}^{\text{pot}}\, a_j' \tag{8}$$

and a more complicated relation holds in case of Eq. (7). This means that in order to calculate a_K' we must know the activities of i and j in the extracellular space (a_i'' and a_j'') and the activity of j in the intracellular space (a_j') as well as the selectivity coefficient $k_{i,j}^{\text{pot}}$. Although the extracellular activities a_i'' and a_j'' are usually known, this is rarely true for the intracellular activity of the interfering ion j, so that an independent estimate of a_j' must be obtained. In case neither Eq. (6) nor Eq. (7) are found to be appropriate, one can prepare a calibration curve and determine a_i' graphically. In that case one of the test solutions must have the composition of the extracellular fluid, and the other solutions must be prepared on the basis of a reasonable assumption of a_j'. When determining intracellular K^+ concentrations with Na^+ being the interfering cation, one reasonable assumption would be $c_{Na}' + c_K' = c_{Na}'' + c_K''$ which we use below. As demonstrated below the influence of a_j' on the value of a_i' is fortunately very small.

Table 1. Properties of K^+-selective microelectrodes (at 22° C)

Resin	Corning No. 477317
Tip diameter	$<0.2\,\mu m$
Resistance	1.5, S.D. $\pm 0.3 \cdot 10^{10}\,\Omega$ ($n = 5$)
Slope (pure sol.)	58.5, S.D. $\pm$ 1.6 mV/decade ($n = 4$)
(mixed sol.)	53.0, S.D. $\pm$ 1.4 mV/decade ($n = 5$)
$1/k_{K,Na}^{\text{pot}}$	~15–50 (conc. dependent)
Rise time	<0.5 s
Yield of stable electrodes	~80% ($\pm$2 mV/1 h)

Results

A. Electrode Properties

The electrode properties are summarized in Table 1. Because of the smaller tip diameter the resistance is approximately one order of magnitude higher than that reported for Corning No. 477317 exchanger microelectrodes from other laboratories [15, 29]. The sensitivity of the electrode, as indicated by its "slope" in pure KCl solutions is excellent (58.5 $\pm$ 1.6 mV/decade, $n = 4$). It was calculated from measurements in 160 and 80 mmolar KCl solutions using $\gamma_K = 0.7345$ and $\gamma_K = 0.784$ respectively for the activity coefficients, which are interpolated from the data of Table 8.9 in ref. [22]. The selectivity coefficients calculated from the Nicolsky equation are strongly concentration-dependent. These calculations are based on the following form of the Nicolsky equation:

$$\Delta\tilde{\varphi}_K^* = \frac{RT}{F}\left\{\ln\left(a_K^* + k_{K,Na}^{\text{pot}}\, a_{Na}^*\right) - \ln a_K^{**}\right\} + C' \tag{9}$$

in which a_K^{**} is the K^+-activity of the reference solution (0.16 molar KCl) which was filled into the shank of the ion selective electrode and C' summarizes all constant phase boundary potentials, which do not disappear, if the 0.16 molar reference solution of the electrode shank is also used as test solution so that $a_K^* = a_K^{**}$ and $a_{Na}^* = 0$. Furthermore, the calculations assumed that γ_K and γ_{Na} were constant in isotonic mixtures of KCl and NaCl solutions and identical to the γ_K of pure 0.16 molar KCl. The concentration dependence of the selectivity coefficients is more readily

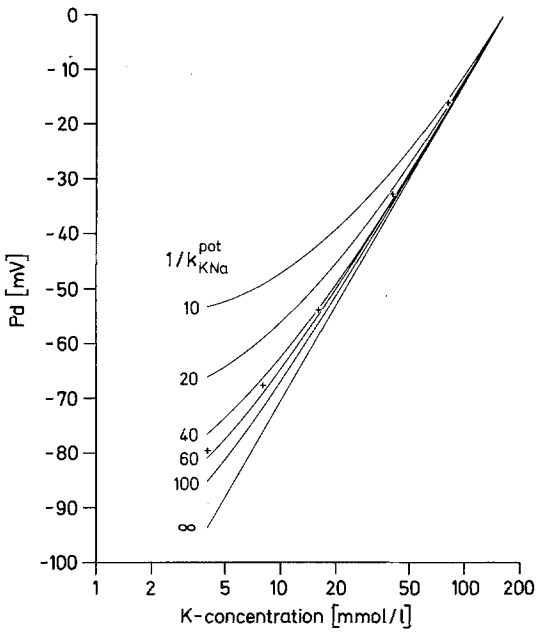


Fig. 1. Electrode calibration curve in mixed isomolar NaCl-KCl solutions. Abscissa: K^+ concentration of the test solutions in mmoles/l, ordinate: potential difference ($\Delta\tilde{\varphi}_K^*$) between microelectrode and grounded saturated calomel half cell in mV. The curves represent isoselectivity lines calculated from Eq. (9) for the different selectivity coefficients $k_{K,Na}^{pot}$ which are indicated by their reciprocals at the lower end of each curve. The data points (+) represent mean values from repeated measurements with 5 different electrodes. The standard deviation of the measurements was 3.3, 0.5, 0.5, 0.5, 0.4 (in order of increasing K^+ concentration). Note that the data points fall on increasingly higher curves as the K^+ concentration increases, indicating a decrease in K^+ selectivity

seen in the calibration curve of Fig. 1 in which the data points fall on progressively higher isoselectivity lines as the K^+ concentration of the test solution decreases.

To test whether the simplifying assumption of constant γ_K might be responsible for the observed concentration dependence of the $k_{K,Na}^{pot}$ we have tried to obtain estimates of the activity coefficient, γ_K, as a function of solution composition from the literature. This was done by assuming γ_+, to equal γ_{salt} and calculating the γ-values from Harned's rule:

$$\log \gamma_i = \log \gamma_{i(0)} - \alpha_i c_j - \beta_i c_j^{\,2} \qquad (10)$$

where $\gamma_{i(0)}$ is the activity coefficient of i in pure solution of i. Estimates of α and β were obtained by extrapolating data of Robinson [21]. They are: $\alpha_{KCl} = -0.020$, $\beta_{KCl} = 0$, $\alpha_{NaCl} = 0.028$ and $\beta_{NaCl} = -0.005$. The resulting γ-values are listed in Table 2. Using these values to calculate the selectivity coefficients from the Nicolsky-equation, however, did not change the result. The concentration dependence of the selectivity coefficients is still apparent (compare columns A and B in Table 3).

As an alternative to the Nicolsky-equation we have tried to fit Eq. (7) to the data, assuming different values of τ and using a least-square fit program to select best estimates of K_1 and K_2. Although the quality of the fit improved when increasing τ stepwise from 0 to 0.5 [2] the theoretical curve never reached the data points in the upper K^+-concentration range (40 and 80 mmolar KCl) and did not even approach the average measured potential difference closer than by one standard deviation.

In further experiments we have tested the temperature dependence of the calibration curve (Fig. 2). When going from 22 – 37° C the slope increased in the upper concentration range by 5.7% as expected from the

2 Increasing τ above 0.5 does not change the fit because K_1 and K_2 are then interchanging

Table 2. Activities of KCl and NaCl in mixed isotonic solutions at 25° C (extrapolated from ref. [21])

Sol.	i	c_i (mmol/l)	γ_i –	a_i (mmol/l)
1	KCl:	160	0.7345	117.50
	NaCl:	–	0.7410	–
2	KCl:	80	0.7372	59.00
	NaCl:	80	0.7447	59.60
3	KCl:	40	0.7386	29.50
	NaCl:	120	0.7466	89.60
4	KCl:	16	0.7393	11.80
	NaCl:	144	0.7477	107.70
5	KCl:	8	0.7396	5.90
	NaCl:	152	0.7481	113.70
6	KCl:	4	0.7398	2.96
	NaCl:	156	0.7483	116.70
7	KCl:	–	0.7400	–
	NaCl:	160	0.7485	119.80

Table 3. Selectivity coefficients as calculated from the Nicolsky equation

Composition of test solutions (mmol/l)		$1/k_{K,Na}^{pot}$		
		22° C	22° C	37° C
KCl	NaCl	A	B	C
80	80	15	14	15
40	120	30	29	24
16	144	47	45	32
8	152	50	49	32
4	156	54	53	38

The selectivity coefficients of column A were calculated from Eq. (9) using the activities from Table 2. The data of columns B and C were calculated from Eq. (9) assuming that the activity coefficients γ_K and γ_{Na} are equal and independent of concentration

Nernst-factor (5.1 %) but there was also an increase of curvature. As a result the selectivity coefficients calculated from the Nicolsky-equation for low K^+ concentrations fell (compare columns B and C of Table 3). The close coincidence of both curves in the upper and lower concentration range suggests that calibration curves obtained at room temperature may be used for intracellular K^+ determinations at body temperature without introducing greater errors.

B. Tubular Cell Punctures

a) Determination of $\Delta\varphi$. Figure 3 shows the frequency distribution of the peritubular membrane potential from 156 punctures obeying different stability criteria. In agreement with previous results we find that the more stable values (drift < 1 mV for ≥ 5 min) show a bell-shaped distribution, while the distribution of less stable data (< 1 mV for $\geqq 2$ min) is skewed. The average potential difference of the former group is also higher (-76.3 S.D. ± 8.1 mV, $n = 81$) than that of the latter (73.6 ± 9 mV, $n = 156$). Under the reservations discussed below we conclude from these observations that -76.3 mV is the best estimate of the average tubular cell membrane potential which we can obtain. The fact that it is slightly higher than previously reported [8, 24] may be due to the measuring conditions. In the present study all tubules were luminally perfused with artificial HCO_3^- Ringers solution which did not contain any organic solutes such as glucose or amino acids, while the previous measurements were performed under free flow.

b) Determination of $\Delta\tilde{\varphi}_K$. Figure 4 shows a trace record of the potential difference measured with a K^+-selective microelectrode. Although the absolute potential value is small, it can be seen that it responds properly to luminal perfusion with glucose-containing

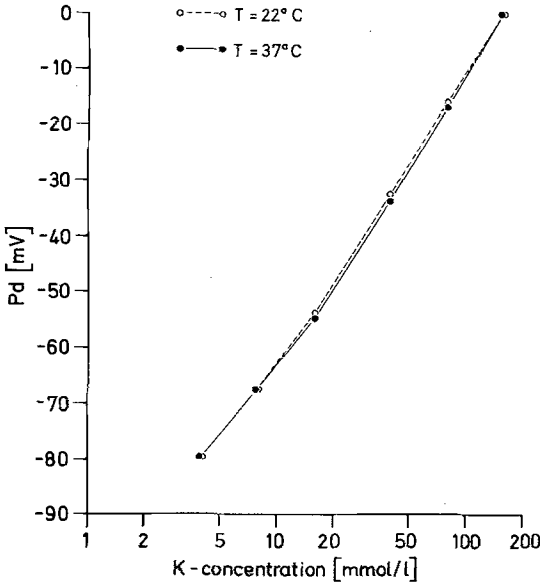


Fig. 2. Effect of temperature on calibration curve. Details as in Fig. 1. Open symbols: 22° C, closed symbols: 37° C. The same 5 electrodes were used in both series of experiments

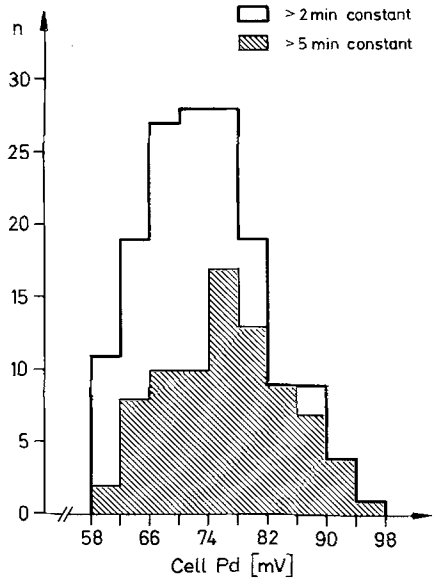
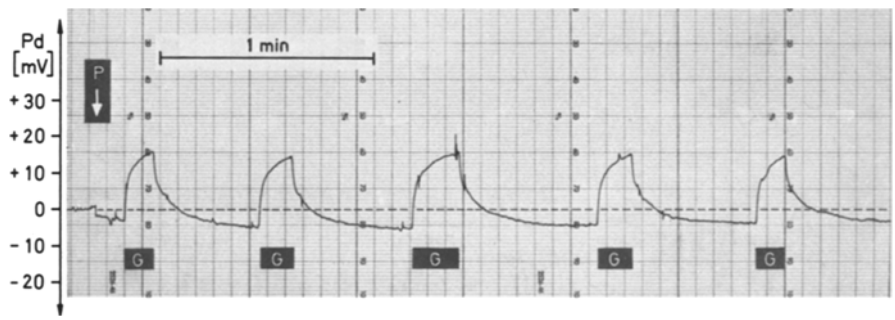


Fig. 3. Frequency distribution of measurements of the membrane potential $\Delta\varphi$ obeying different stability criteria. Abscissa: membrane potential ($\Delta\varphi$) in mV, ordinate: number of observations in interval of 4 mV

Fig. 4
Trace record of a cell puncture with a K^+-selective electrode. Abscissa: time (bar indicating 1 min), ordinate: potential difference $\Delta\tilde{\varphi}_K$ in mV. At mark P the electrode was advanced into a cell and during the marks G was the luminal perfusion switched to glucose containing Ringer's solution. Note magnitude and reproducibility of the response to glucose as well as long term stability of the small value of $\Delta\tilde{\varphi}_K$



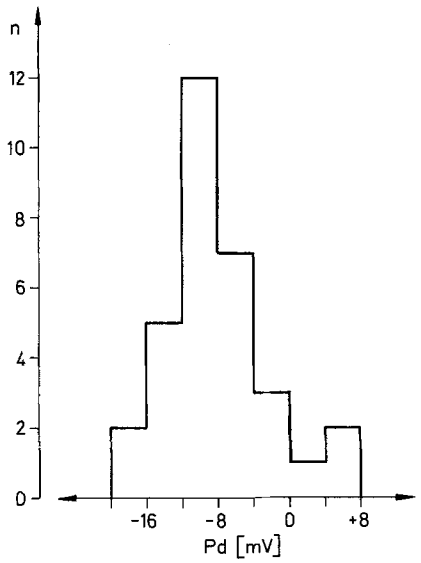


Fig. 5. Frequency distribution of 32 measurements of $\Delta\tilde{\varphi}_K$. Details as in Fig. 3

Ringers solution [5] and that the response to glucose is also stable with time indicating the absence of leaks or cell membrane damage. Figure 5 gives the histogram of all $\Delta\tilde{\varphi}_k$ measurements in which the potential difference and the response to glucose were stable for at least 2 min. The average $\Delta\tilde{\varphi}_K$ was -7.2 S.D. $+$ 5.8 mV ($n = 32$). Applying a more stringent stability criterion does not significantly change this result, possibly because of the low absolute value and the limited number of observations. $\Delta\tilde{\varphi}_K$ measurements with a stability of $\geqq 5$ min yield an average of -8.8 ± 7.4 mV ($n = 5$).

C. The Intracellular K^+ Activity and Other Derived Values

Two methods were used to derive the average intracellular K^+ activity. In the first method the average $\Delta\varphi$ of -76.3 mV was subtracted from the value of $\Delta\tilde{\varphi}_K$ which had been observed in a given experiment and a_K' was read from the calibration curve of the particular electrode which had been used. Averaging all individual a_K' values thus derived, yields a mean of 82.6, S.D. ± 23.6, range 48 to 145 mmol/l. This range, however is most likely without biological significance because it may well be that the more negative $\Delta\tilde{\varphi}_K$ values were associated with cell membrane potentials above average and the reverse, which would have been noticed if $\Delta\tilde{\varphi}_K$ and $\Delta\varphi$ had been measured within the same cell. In the second method all electrode calibration curves ($\Delta\tilde{\varphi}_K^*$ versus a_K^*) were averaged and the intracellular activity was directly read from this curve using the mean values of $\Delta\tilde{\varphi}_K$ and $\Delta\varphi$ described above. This method yields the same value of 82 mmol/l for the intracellular K^+ activity which was found with method 1.

Table 4. Results of K^+-electrode studies on rat kidney proximal tubular cells

Membrane p.d.	$\Delta\varphi$	$=-$	76.3, S.D. $\pm$ 8.1 [mV] ($n = 81$)
K^+ electrode p.d.	$\Delta\tilde{\varphi}_K$	$=-$	7.2, S.D. $\pm$ 5.8 [mV] ($n = 32$)
Intracell. K^+ activity	a_K'	$=$	82 [mmol/l][a]
Intracell. K^+ conc.	c_K'	$=$	113 [mmol/l][b]
Intracell. K^+ conc. (flame photometry)	c_K'	$\sim$	129 [mmol/l][c]
K^+ activity coeff.	γ_K'	$\sim$	0.64
K^+ equilibrium p.d.	E_K	$=-$	88.7 [mV]

[a] Read from the calibration curve
[b] Lower limit, calculated from a_K' assuming $\gamma_K' = \gamma_K''$
[c] Average of data of Burg and Orloff [1] and Khuri et al. [13]

Table 4 summarizes the results and some further values which were derived from them. Based on a average value of the intracellular K^+ concentration of $\sim$129 mmol/l determined by Burg and Orloff [1] and Khuri et al. [13] using flame photometry, our activity measurements suggest that the activity coefficient of K^+ in the cytoplasm is $\sim$0.64. This estimate, however, does not consider a possible compartmentalization of K^+ in cytoplasmic substructures, which cannot be recognized with the presently available measuring techniques. On the other hand, assuming that intracellular γ_K' equals extracellular γ_K'' we calculate a free K^+ concentration in the cytoplasm of 113 mmol/l. This value must be a lower estimate, however, because the presence of polyvalent anions in the cytoplasm suggests that contrary to the above assumption $\gamma_K' < \gamma_K''$, so that the cytoplasmic K^+ concentration is likely to be higher than 113 mmol/l and hence closer to the value of 129 mmol/l determined with flame photometry. Finally using a_K' and a_K'' we calculate from Eq. (5) that E_K is -86 mV.

Discussion

The value of intracellular K^+ activity of 82 mmol/l which we have reported in the present paper differs largely from the value of 39 mmol/l determined by Khuri et al. [13] previously. We interpret this discrepancy as reflecting primarily technical differences. Khuri and collaborators used double-barrelled electrodes with larger tips, which according to our experience damage the membrane more readily than do fine-tip electrodes, and furthermore, when analyzing the data these authors applied less stringent stability criteria than we did in the present study. Double-barrelled electrodes have of course several advantages, but they must be carefully weighed against their disadvantages.

For example, the fact that they allow – at least in principle – $\Delta\varphi$ and $\Delta\varphi_i$ to be measured simultaneously in the same cell is – despite its general advantage – of little importance in the present context, since we are only interested in obtaining an estimate of the mean intracellular K^+ activity here. Furthermore, the possibility of localizing the intracellular position from the potential of the non-selective channel, although convenient, was not indispensable. We have solved the problem of localization adequately by recording the potential response to intermittent luminal perfusions with glucose.

Regarding the reliability of the reported data we favour our results, particularly because we find that E_K (−88 mV) is more negative than $\Delta\varphi$ (−76 mV). This finding agrees with the hyperpolarization of 5–10 mV seen upon increasing the transference number of the peritubular cell membrane for K^+ (during peritubular surfusion with alkaline solutions [31] or during application of acetazolamide [9]). The value of E_K (−67 mV) which can be calculated from the data of Khuri and collaborators [13], in contrast, would be more positive than the membrane potential, so that increasing τ_K should have caused a depolarization, rather than the hyperpolarization which was observed. Despite all confidence in the general validity of our result we must admit, however, that the precision of intracellular activity measurements is limited. The main sources of error are the following:

1. The tip potential of the *non*-selective electrode. Upon insertion into the cytoplasm the tip potential of a Ling-Gerard type KCl-filled microelectrode may either increase or decrease, the former because the tip may be blocked by cell debris, the latter because the tip potential is sensitive to K^+ and because the intracellular K^+ concentration is high. Although clogging of the tip may be reversible upon withdrawal of the electrode and hence may be overlooked when comparing only the pre- and postpuncture tip potential level in the bath, it is known that bigger tip potential changes are usually associated with increased drift and noise of the potential record so that such records do not meet our strict stability criteria and are automatically excluded from the final average. Small changes, however, may not be recognized so that some uncertainty remains. The uncertainty is kept minimal, however, in our experiments because we carefully boil the capillary glass before pulling which virtually eliminates the occurrence of tip potentials a priori and reduces all ensuing tip potential changes.

2. The non-ideal behaviour of the selective microelectrodes. As demonstrated in the first part of the results section, we have not been able to describe the behaviour of our K^+-ion exchanger microelectrodes in mixed NaCl-KCl solutions appropriately. The fit obtained by Eq. (7) assuming $\tau = 0.5$, which then equals an equation for liquid ion-exchanger electrodes derived by Morf et al. [18], though superior to that of the Nicolsky-equation, was not satisfactory. Unfortunately we have not found similarly detailed calibration curves in the literature to check the consistency of our observations with those from other laboratories; however, the data of Neher and Lux [19], which cover a smaller concentration range (4–40 mmol/l of K^+ concentration), fit exactly with our data points. Although it is of course dangerous to work with a tool whose operation is not completely understood, the possible error can be kept to a minimum, if the calibrating solutions are made similar to the biological fluids. For that reason we have used isotonic mixtures of NaCl and KCl, assuming that $c_K' + c_{Na}' \approx c_K'' + c_{Na}''$, rather than determining $k^{pot}_{K,Na}$ from measurements in pure KCl solutions and pure NaCl solutions, as suggested by others [10, 17]. The interference from Na^+ and hence the possible error which we might introduce by the foregoing assumption is rather small, however. As far as the potential reading in the extracellular fluid is concerned all errors are eliminated by the fact that we use a calibrating solution the composition of which resembles closely that of the extracellular fluid. With regard to the intracellular potential reading on the other hand, we can say that in the range of intracellular K^+ activity which we observe, the electrode potential is so strongly K^+-dependent that the interference from Na^+ is almost negligible. Indeed, when passing from the calibration solution of $c_K^* = 4$ and $c_{Na}^* = 156$ (both in mmol/l) into a solution of $a_K^* = 82$ mmol/l (which consists of $c_K^* = 113$ mmol/l, $c_{Na}^* = 47$ mmol/l with a_{Na}^* being 39 mmol/l), the Na^+ ions contribute only less than 1 mV to the new electrode potential which is, however, 69 mV higher than the initial potential. This can be readily seen from the calibration curve in Fig. 1, in which the distance between the iso-selectivity lines and the Nernst-slope indicates the contribution of the interfering ions directly. Other ions such as NH_4^+, Ca^{2+} and Mg^{2+} do not contribute to the potential at all, because either their concentration is too small (NH_4^+ and Ca^{2+}) or the exchanger is too little sensitive (Mg^{2+} see ref. [4]).

3. Another point of concern is the magnitude of the activity coefficients of Na^+ and K^+ in mixed solutions, as well as the question of whether γ_K and γ_{Na} can be adequately enough represented by the activity coefficients of the respective salts as we have tried in the present paper. Although we cannot answer the latter question we conclude from the data of Table 2 that the concentration dependence of the γ's in mixed NaCl-KCl solutions is not so strong that it need necessarily be considered. Indeed deriving a_K' from a calibration curve that was prepared under the assumption that γ_K

and γ_{Na} in mixed isotonic solutions are equal and constant yields the same figure of 82 mmol/l as derived above. It is true of course that our attempt to evaluate γ_K and γ_{Na} in mixed solutions by extrapolating the data from measurements of Robinson [21] beyond the measuring range may be criticized, however, the observation that the condition $\gamma_{(0)j} = \gamma_{(0)i}$ is approximately fulfilled is comforting (see Table 2). It means that the activity coefficient of i, with i being present in vanishing concentrations in a solution of j, is approximately equal to the activity coefficient of j, with j being present in vanishing concentrations in a solution of i, as is normally found.

Accepting then the results from Table 4 as fairly reliable estimates of the intracellular K^+ activity we ask what these data indicate about ion transport in proximal tubular cells of rat kidney. The main conclusion from Table 4 is that $E_K > \Delta\varphi$ or in other words that $\Delta\eta_K \neq 0$ and hence that the K^+ distribution across the peritubular cell membrane ist *not* in equilibrium with the membrane potential but that there exists a constant passive driving force for K^+ efflux from the cell into the interstitium (and into the lumen), which must be counteracted by an active K^+-uptake mechanism to maintain the intracellular K^+ concentration constant. This result demonstrates clearly the existence of an active K^+-uptake pump in the tubular cell membranes, after such a pump has been variously negated [13] and postulated [7, 25, 30] in the past, the latter based on more or less indirect evidence, such as the finding of a Na^+/K^+-stimulated ATPase in basolateral cell membranes [25], flux studies on kidney slices in the presence and absence of ouabain [30] and electrical potential measurements in response to sudden applications of ouabain [7]. Taking these and other recent findings into account [2] we conclude therefore that a coupled Na^+/K^+ pump operates in the peritubular cell membrane, which works similarly as in erythrocytes and other cells, and which is obviously responsible for the greater part of the tubular salt and fluid absorption.

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