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THE TRANSPORT OF INORGANIC IONS AND *p*-AMINOHIPPURATE IN ISOLATED CELLS OF THE RENAL CORTEX OF THE RABBIT

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SUMMARY

The transport of Na and K ions and of *p*-aminohippurate (PAH) was studied in isolated kidney cells of the rabbit.

1. During aerobic incubation at 25°, with 10 mM acetate as substrate there is active extrusion of Na^+ from the cells and reaccumulation of K^+. K^+ accumulation from the medium into the cells is linearly dependent on the K^+ concentration of the medium.

2. Anaerobic incubation inhibits Na^+ extrusion and water and K^+ accumulation with a concomitant swelling of the cells. After oxygen is reintroduced, the cells again extrude Na^+, reaccumulate K^+ and the cell volume decreases.

3. 10^{-4} M dinitrophenol has the same effect as anaerobiosis on the distribution of cations and water in isolated kidney-cortex cells.

4. PAH was found to accumulate actively in isolated kidney cells. Cells that maintain their K^+ content close to values found in fresh tissue during preparation in an optimal medium show the highest ability to accumulate PAH. At high K^+ concentrations in the medium which increase the K^+ cell content above normal, PAH accumulation is decreased.

5. Increased Ca^{2+} concentration (7.5 mM) in the incubating medium decreases PAH accumulation by isolated kidney cells.

6. The inhibitory effect of Li^+ (20 mequiv/l) on PAH accumulation is partly reduced by increased Ca^{2+} concentrations in the medium; Ca^{2+} also changes the distribution of lithium between the cells and the medium.

INTRODUCTION

It has been demonstrated previously by BOSÁČKOVÁ[1] that isolated cells of the renal cortex can be obtained by a gentle trypsinization procedure and that such cells show some metabolic activities not fundamentally different from those of kidney-cortex slices.

The results presented in this communication show that during aerobic incubation isolated kidney cells extrude sodium against a concentration gradient and accumulate potassium and also PAH.

Abbreviations: PAH, *p*-aminohippurate.

MATERIALS AND METHODS

Cells from the renal cortex of the rabbit were isolated by trypsinization in the saline of ROBINSON[2] containing 5 mequiv K^+ per l, as described previously by BOSÁČKOVÁ[1] (standard procedure) or using a modified saline without K^+ or with 20 mequiv K^+ per l by an equivalent replacement of Na^+ by K^+. Since the trypsinization procedure is carried out by a series of short treatments of the tissue with a trypsin solution, the cells already isolated were maintained at either 0°, when practically all metabolic processes are inhibited, or at 25°, at which temperature the cells respire and transport mechanisms are operative. It was found previously that the respiration of isolated kidney cells is stabilized by glucose (5.6 mM final concentration) which was therefore added to the trypsinizing medium.

The isolated cells were incubated in the saline of ROBINSON (standard procedure). Where the effect of K^+ on K^+ and PAH accumulation was studied, K^+ was substituted for Na^+. For the examination of the effects of Ca^{2+} and Li^+ on PAH transport an equivalent exchange of these cations of Na^+ was made. In addition to its inorganic components the incubating saline contained 5.6 mM glucose, 10 mM sodium acetate and for the study of PAH transport, 0.148 mM PAH.

The volume of the cells was ascertained by using special plexi-glass test tubes. The bore of their upper part was 16 mm in diameter, that of the lower part 3 mm and graduated. The cell suspension was transferred to the test tubes at the start of the experiments or immediately after their termination and centrifuged for 5 min. The main part of the supernatant was discarded and then centrifugation was continued for 20 min at 2000 × g to obtain the final cell volume. Preliminary centrifugation ensured a rapid separation of cells from the main part of the medium and thus prevented possible ion shifts during longer lasting centrifugation. The cell volume was read (about 0.03–0.09 ml), the remaining supernatant was discarded and the test tube dried with cotton wool down to the border of the cellular column. The combined supernatants of each tube were analysed for the Na^+, K^+ or Li^+ content by flame photometry. For the estimation of intracellular cations the cells were suspended in a known amount of distilled water (2–3 ml). After 16 hours the cell debris was removed by centrifugation and cations were determined in the supernatant. Cation contents were expressed in mequiv/l of cells after correcting for the amount of cations present in the extracellular space. Two simultaneously performed analyses of cell volume and cations did not differ by more than 3–5 %.

Where experiments with kidney-cortex slices were carried out, the cation content of tissue was determined after drying the latter at 95° overnight and an extraction of the dry solids by 5 ml 0.2 N H_2SO_4 for 5 days was made. Cation content in the slices was expressed in mequiv/kg of intracellular water after correction for the amount of cations present in the extracellular space (25 % of the wet weight of the slices according to ROBINSON[3] and WHITTAM[4]).

The extracellular space in the pellet of cells after centrifugation was determined after incubation of the cells in a saline containing 0.64 % (w/v) of inulin; the pellet of cells was suspended in distilled water as presented above for cation estimation, and an aliquot was taken for the colorimetric determination of inulin by the method of Cole (*cf.* BACON AND BELL[5]) using a Hilger photometer with an IF-500 filter. The inulin space was expressed in ml H_2O per 100 ml of the cell column.

PAH was determined in isolated cells and kidney slices after elution in a suitable volume of distilled water overnight using the method of BRUN[6]. After deproteinization *p*-dimethylaminobenzaldehyde reagent (recrystallized from acid solution by the method of ADAMS *et al.*[7]) was added to an aliquot of the supernatant and the color measured with the Hilger photometer using IF-450 filter. The average error for parallel PAH determinations in kidney cells was ± 3.1 %. The values of PAH were corrected for a blank tissue sample containing an average of 4.45 μg PAH per 100 mg dry substance (GROSS *et al.*[8]) and also for the amount of PAH contained in the inulin space. The amount of accumulated PAH was expressed by the ratio S/M, where S is the apparent PAH concentration in the cells and M in the medium.

The nitrogen content of the isolated kidney cells was determined using the method of COLE AND PARKS[9]. Incinerated samples were distilled in a Markham apparatus. The released ammonia was collected into a 2 % H_3BO_3 solution and titrated with 0.14 N H_2SO_4 using the indicator of MA AND ZUAZAGA[10]. In order to determine the amount of N released by the cells during incubation we determined: a, the total amount of N in a given volume of cell suspension; b, the amount of N in the supernatant after centrifugation of the cells to their final volume at the start of the experiment; c, the amount of N in the supernatant after centrifugation following incubation.

Using these data the amount of N in the sedimented cells before incubation could be determined together with the amount of N released from the cells after incubation. The latter was expressed as percent of the N content of the cells before incubation.

EXPERIMENTAL

The effect of the K^+ concentration in the trypsinizing and incubating media on the Na^+ and K^+ content of isolated kidney cells

Since it could be assumed that during isolation of the cells, lasting 1.5 h, changes in the distribution of inorganic ions might occur, Na^+ and K^+ contents of isolated cells and fresh tissue were compared.

The cation content of isolated cells being expressed per net cell volume, it was necessary to obtain comparable values for fresh tissue (expressed in mequiv Na^+ and K^+ per kg fresh kidney cortex, see AEBI[11], BERNDT[12]) by computing the apparent intracellular cations concentration. This was done by correcting the known tissue values for the extracellular space (25 % wet weight), assuming the cations concentration equals the known plasma data. Preliminary results showed that, as compared with the computed values for the intracellular concentrations in fresh tissue, the concentration of K^+ in the isolated cells decreased and that of Na^+ increased (during the standard preparation procedure without K^+ in the medium and the cells maintained at 0°). Cells prepared in a medium containing 20 mequiv K^+ per l and maintained at 25° did not differ in their ion content from fresh tissue. This is in agreement with the finding of TAGGART *et al.*[13], that a medium with 20 mequiv K^+ per l at 25° is optimal for maintaining normal K^+ concentrations in kidney slices for 2 h. Isolated kidney cells prepared in a medium without K^+, having lost some of their K^+, accumulate this ion from the medium during 30 min of aerobic incubation. Fig. 1 shows that at a K^+ concentration of about 40 mequiv/l in the medium values of Na^+ and K^+ in the isolated cells attain those found for cells prepared in the optimal medium

containing 20 mequiv K^+ per l. The amount of K^+ in the cells increases linearly with increasing K^+ concentration in the medium (Fig. 2). Similar results were obtained by CORT AND KREINZELLER[14] who studied the accumulation of K^+ by kidney slices after leaching in isotonic NaCl at 0° for 2.5 h.

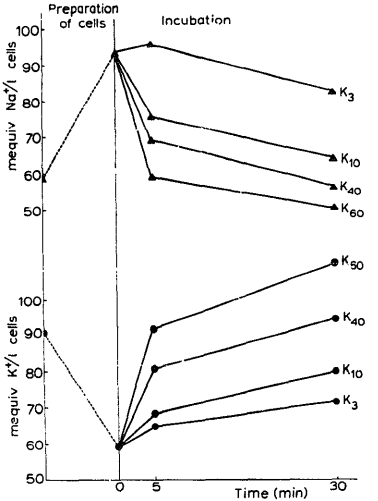

Fig. 1. Na^+ and K^+ contents in isolated kidney cells incubated at 25° in saline of varying K^+ concentrations. Cells prepared in medium without K^+ and maintained at 0°. Mean values for Na^+ and K^+ content in isolated cells prepared in medium containing 20 mequiv K^+ per l are given on the ordinate for comparison.

In order to determine whether active transport of Na^+ and K^+ occurs and whether the above results were not due to diffusion only, depending on the K^+ concentration of the medium isolated cells were first incubated anaerobically for 20 min in Robinson's medium with 20 mequiv K^+ per l and then the medium was aerated with oxygen making it aerobic. Fig. 3 shows that isolated cells are capable of accumulating K^+ and extruding Na^+ to values found before incubation if they are again placed under aerobic conditions. Since the K^+ content in the medium did not change appreciably during the experiment the above results may be taken as evidence that under aerobic conditions the cations are transported against their respective concentration gradients.

The effect of anaerobic conditions and dinitrophenol on Na^+ and K^+ transport and on the volume of isolated kidney cells

When isolated kidney cells, prepared in normal Robinson's medium are incubated at 25° anaerobically or in the presence of 10^{-4} M dinitrophenol there is a fall in the K^+ and a rise in the Na^+ content of the cells as compared to values found on aerobic

incubation, (Table I) as is the case for kidney slices (MUDGE[15], WHITTAM AND DAVIES[16]). The slight increase in the inulin space may be due to damage of a portion of the cells as compared with aerobic condition. This is also indicated by the increased loss of nitrogen from the cells during anaerobic incubation and in the presence of dinitrophenol.

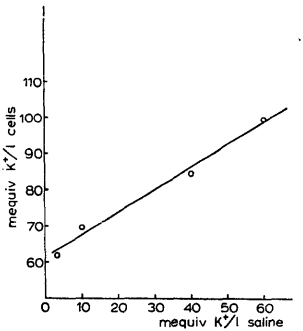

Fig. 2. The dependence of K^+ content of isolated kidney cells, on the K^+ concentration in the incubating medium. Cells prepared in a trypsinizing medium without K^+ and then incubated aerobically 30 min at 25° in media of varying K^+ concentrations. Individual points on the curve are the means from 4 experiments.

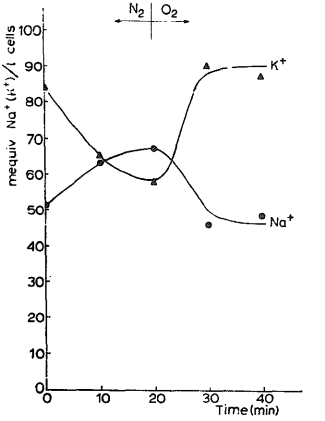

Fig. 3. The effect of anaerobic and aerobic condition on the cation distribution in isolated kidney-cortex cells. Trypsinization and incubation in a medium with 20 mequiv K^+/l. The cells incubated at 25° first anaerobically for 20 min, then aerobically for a further 20 min.

It is known that the water content of kidney slices increases during anaerobic incubation and in the presence of metabolic inhibitors (MUDGE[15], LEAF[17]). Hence it was of interest whether similar changes could be found in isolated cells. Fig. 4 shows that, during anaerobic incubation the cell volume increases by an average of 14.5 %, dinitrophenol increases the volume by 18 %. Very similar changes in water content of kidney slices were found by LEAF[17]. On return to aerobic conditions cell volume returned to control values as is the case for kidney slices (ROBINSON[18]).

Accumulation of PAH by isolated kidney cells and the effect of K^+ concentration thereon

Table II shows that isolated kidney cells are able to accumulate aerobically PAH against a concentration gradient. Addition of 10 mM acetate increases PAH accumulation 70 %, while anaerobically no PAH is accumulated. Experiments using two types of cell preparations are given in Table II. In one, cells were prepared in a trypsinizing medium without K^+, in the other type 20 mequiv K^+ per l were present. Isolated cells prepared in the latter medium accumulated PAH to a greater extent; the average S/M ratio 8.65 was about 27 % lower than the value 11.0 for kidney slices (CROSS *et*

al.[8]). *S/M* values were much smaller for cells prepared without K^+ and incubated in the medium with 10 mequiv K^+ per l. It is of interest to note that only a medium which ensured apparently normal intracellular K^+ values was optimal for PAH accumulation. Respiratory activity of both types of cell preparations did not differ significantly.

TABLE I

Na^+ AND K^+ CONTENTS IN ISOLATED KIDNEY CELLS, INULIN SPACE AND PERCENT OF RELEASED N UNDER DIFFERENT INCUBATING CONDITIONS

Cells were isolated in the standard saline of Robinson. Incubation for 30 min at 25° in the saline of Robinson containing 10 mequiv K^+ per l. Number of experiments 8.

Condition of incubation	*Na+*	*K+*	*Inulin space*	*Loss of N in % of*
	(mequiv/l cells)		*(ml H_2O/100 ml cells)*	*total cell N*
Initial values	83.3 ± 5.75	65.0 ± 2.7	38.8 ± 2.05	0
O_2	76.6 ± 3.99	76.0 ± 2.99	37.6 ± 2.0	5.9
O_2, 10^{-4} M dinitrophenol	95.0 ± 4.20	41.0 ± 2.80	42.2 ± 1.95	13.8
N_2	105.8 ± 3.51	39.4 ± 2.76	42.7 ± 1.83	10.9

TABLE II

THE EFFECT OF IONIC COMPOSITION OF THE TRYPSINIZING MEDIUM ON PAH TRANSPORT IN ISOLATED KIDNEY CELLS

Trypsinization and incubation in Robinson's medium in which Na^+ was substituted for K^+ equivalently. Incubation at 25° for 60 min.

Trypsinizing medium (mequiv K^+/l)	*Incubating condition*	*Incubating medium*		*PAH transport (S/M)*
		mequiv K^+/l	*mM Na acetate*	
0	O_2	10	—	2.59 ± 0.44
	O_2	10	10	4.47 ± 0.38
	N_2	10	10	1.02
20	O_2	20	—	5.27 ± 0.32
	O_2	20	10	8.65 ± 0.91
	N_2	20	10	0.95

The dependence of PAH accumulation on the apparent intracellular K^+ concentration was further studied after incubation of isolated cells in media with different K^+ concentrations. The results are shown in Fig. 5. It will be seen that cells which have lost part of their K^+ are maximally capable of accumulating PAH at K^+ concentration of 50 mequiv/l. Even though cell preparations under such conditions accumulate K^+ to values close to those computed for fresh-tissue cells, they are less efficient in accumulating PAH than cells prepared in such a way that they did not lose K^+ during trypsinization (see Section 1 of EXPERIMENTAL). These latter cell preparations accumulate PAH even at very low K^+ concentrations in the incubating saline to about the same extent as when incubated with 20 mequiv K^+ per l. The K^+ content of these cells after being incubated in a medium with only 2.5 mequiv K^+ per l differs only slightly from those values computed for fresh tissue cells. A high

K^+ concentration in the medium leading to increased cellular K^+ content above normal values decreases PAH accumulation.

The effect of Ca^{2+} and Li^+ on PAH accumulation

Studies on the mechanism of action of Ca^{2+} on electrolyte metabolism in muscle, nerve and kidney tissue (SHANES[19], FRANKENHAUSER *et al.*[20], KLEINZELLER *et al.*[21]) led to the conclusion that Ca^{2+} stabilizes the membrane potential and decreases the permeability of the cell membrane for Na^+ and K^+. Hence the question arose whether

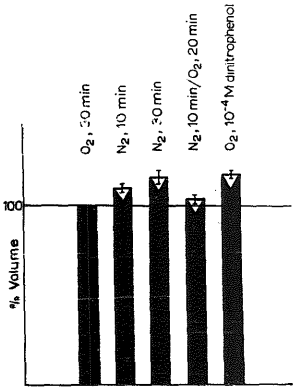

Fig. 4. The effect of anaerobiosis and 10^{-4} M dinitrophenol on the volume of isolated kidney cells incubated for 30 min at 25°. Volume changes are expressed in % of cell volume found aerobically. The effect of dinitrophenol, anaerobic conditions is statistically significantly different for $P < 0.05$.

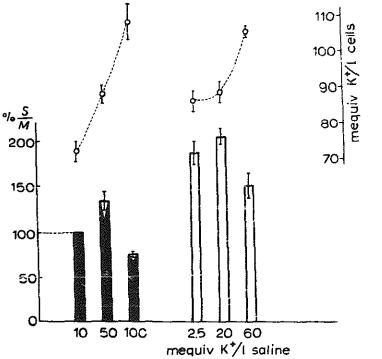

Fig. 5. Dependence of PAH accumulation in isolated kidney cells on the K^+ concentration in the incubating medium. Incubation at 25° for 60 min in media with varying K^+ concentration. Values for PAH accumulation (S/M) expressed in % of the average S/M value = 8.3 found for cells prepared and incubated in saline with 20 mequiv K^+/l. Black columns, % S/M for cells prepared in medium without K^+; white columns, % S/M for cells prepared in medium with 20 mequiv K^+/l. Curves show changes in the K^+ content in mequiv/l cells.

Ca^{2+} also affects PAH accumulation in kidney cells; for comparison also data for kidney slices are included. Fig. 6 shows that in both isolated cells and slices an increase in the Ca^{2+} concentration to 7.5 mM decreases PAH accumulation. This effect is statistically significant in both cases.

Such results might be explained either by effect of Ca^{2+} on the characteristics of the cell membrane, or by the effect on the distribution of water and cations within the cell, assuming the PAH accumulation to be affected by the intracellular cation concentration. This second alternative could be tested by studying the effect of Ca^{2+} on the distribution of some ion affecting PAH accumulation. Since it is known that Li^+ considerably decreases PAH accumulation (TAGGART *et al.*[13]), the effect of Ca^{2+}

on the inhibitory action of this ion was followed together with the distribution of Li^+ as dependent on the Ca^{2+} concentration in the medium. Fig. 6 shows that the inhibitory effect of Li^+ is much more apparent in both isolated cells and kidney slices in a normal medium than in a medium containing thrice the normal amount of Ca^{2+}

TABLE III

THE EFFECT OF Ca^{2+} IN THE INCUBATING MEDIUM ON Li^+ ACCUMULATION INTO KIDNEY SLICES

Robinson's saline containing 20 mequiv Li^+/l equivalently exchanged for Na^+. Incubation for 60 min at 25°. Values for each experiment are average from 4 parallel determinations.

Ca^{2+} concentration in the incubating medium (mM)	*mequiv Li^+/kg intracellular water*			
	Expt. 1	*Expt. 2*	*Expt. 3*	*Expt. 4*
0	22.80	18.92	19.90	21.10
2.5	14.22	13.88	14.46	14.05
7.5	10.92	9.40	11.00	9.80

(7.5 mM). In the control experiment Li^+ decreases PAH accumulation by 46 % on the average in both cells and slices while at 7.5 mM Ca^{2+} this decrease is 30 % for cells and 35 % for slices of the control value without Li^+. In the experiment using kidney slices, in agreement with the above, Ca^{2+} decreases the entry of Li^+ into the the intracellular space (Table III). These experiments indicated that PAH accumulation might be indirectly affected by Ca^{2+} through a change in the distribution of Li^+ in the cells and the medium.

Our results thus showed that the degree of inhibition of PAH accumulation depends on the concentration of Li^+ within the cells. It was further examined whether Li^+ also affects PAH accumulation at low concentrations, where no considerable

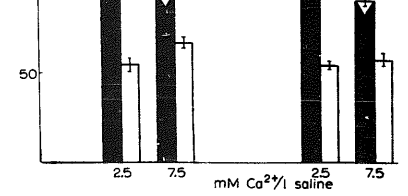

Fig. 6. The effect of Ca^{2+} and Li^+ on PAH accumulation in isolated kidney cells and slices. Incubation at 25° for 60 min. Values for PAH accumulation S/M are expressed as % of the average S/M value obtained for isolated cells and slices incubated in a medium with 2.5 mM Ca^{2+}. Black columns, % S/M for cells and slices incubated without Li^+; white columns, the same with 20 mequiv Li^+/l medium.

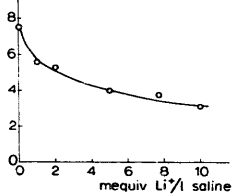

Fig. 7. The effect of Li^+ on PAH transport in leached slices. Slices leached at 0° for 2.5 h in 0.154 M NaCl to which 1/10 the volume of 0.1 M phosphate buffer (pH 7.4) was added. Incubation at 25° for 60 min aerobically in saline of Robinson without K^+.

effect on the activity of key reactions in the cells could be expected. Hence the effect of Li^+ within the range of 0–10 mequiv/l on PAH accumulation was studied in kidney slices. The slices were first leached in isotonic NaCl and then incubated in a medium without K^+, the presence of which might affect the action of Li^+. Fig. 7 shows that with increasing Li^+ concentrations PAH accumulation decreases.

DISCUSSION

A comparison of results obtained with isolated kidney-cortex cells and cortex slices indicates some qualitatively similar phenomena in the transport of solutes.

Both slices (KLEINZELLER[22]) and isolated kidney cells actively excrete sodium and accumulate potassium. The transport of potassium is linearly related to the concentration of this ion in the medium and may be characterized as being essentially passive. In accordance with the results of TAGGART *et al.*[13] on kidney-cortex slices, our results show that the potassium concentration in isolated cells must be maintained at values close to those in fresh tissues for PAH accumulation to be maximal. Isolated kidney cells deprived of potassium during preparation are stimulated to accumulate more PAH by higher potassium concentrations in the incubating medium (potassium is reaccumulated to normal values), but the extent of PAH accumulation is lower than in cells not deprived of potassium.

Several suggestions might serve to explain these findings. At higher concentrations of K^+ in the incubating medium, the respiration of isolated cells decreases (BOSÁČKOVÁ[1]) and then the active transport processes might be affected; external concentrations of potassium also affect the membrane potential (LI AND MCILWAIN[23]); finally it is possible that potassium more firmly "bound" to structural elements was also released during preparation procedure and that during aerobic reaccumulation it did not become localized at the initial sites.

The effect of calcium on PAH accumulation is comprehensible in view of the known mechanism of action of this ion. Since cell-membrane permeability is decreased for some non-electrolytes, cations and anions it may be assumed that permeability for PAH is also decreased. Our experiments did not exclude the possibility of Ca ions affecting PAH accumulation by a change in the distribution of diffusible ions (*e.g.* Li^+) between the cell and its environment.

Inhibition of PAH transport in the presence of Li^+ indicated that this ion affects both processes of active PAH accumulation, as they are considered by FOULKES[24]. Inhibition of PAH accumulation by very low lithium concentrations, where an effect on intracellular enzyme activity can hardly be expected, indicate that membrane mechanisms might be influenced. At higher lithium concentrations in the medium, when potassium accumulation in the cells is decreased (MUDGE[15]) the intracellular concentrating reaction might be affected, which requires the maintainance of normal potassium concentration within the cell. It is also possible that lithium decreases PAH accumulation by imparing some key reactions in the cell. The known inhibition of CoA acetylation by lithium ions[25,26] might cause decreased utilization of acetate for PAH accumulation.

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