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THE INTERACTION OF K^+, OUABAIN AND Na^+ ON THE CATION TRANSPORT AND RESPIRATION OF RENAL CORTICAL CELLS OF HAMSTERS AND GROUND SQUIRRELS

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SUMMARY

1. The interactions of K^+ and ouabain on kidney cell respiration and Na^+ extrusion were studied. The influence of intracellular Na^+ on kidney cell respiration was compared with its effect on K^+ transport.

2. Omission of K^+ from the medium in which kidney slices are incubated causes a reduction in Na^+ extrusion and respiration comparable to that caused by maximal ouabain inhibition.

3. The effect of ouabain in K^+-free medium on respiration, Na^+ extrusion and tissue K^+ concentration is greatly enhanced over its effect in the presence of K^+. Na^+ extrusion is virtually blocked; tissue K^+ falls to extraordinarily low concentrations. In hamster kidneys which are normally insensitive to ouabain the sensitivity to low concentrations of ouabain is increased in the absence of K^+.

4. At 5° the omission of K^+ from the medium has a slight effect on respiration in slices of kidneys of ground squirrels but not in those of hamsters. The combined effects of K^+ removal and ouabain on Na^+ extrusion at 5° are similar to those at 38°.

5. Slices of hamster kidney loaded with Na^+ and incubated with Na^+ respire more rapidly at 5° than slices leached of Na^+ and incubated in Na^+-free medium.

6. Na^+-loaded slices in Na^+-free medium also have a high O_2 consumption relative to slices with no Na^+, showing that it is intracellular Na^+ that stimulates respiration. Slices loaded with 30 mM Na^+ and incubated in Na^+-free medium at 5° respired at a rate midway between that of Na^+-rich and Na^+-depleted slices. This finding is in accord with the fact that the rate of K^+ transport from slices loaded with 30 mM Na^+ is also half-maximal.

7. The findings with regard to K^+, ouabain and Na^+ effects on respiration and transport are discussed in terms of four recently suggested hypotheses. It is concluded that on the basis of available evidence the results may be best explained by the existence of long diffusion pathways between the site of the cation pump and the external medium.

8. It is further concluded that while tissue respiration is governed partly and specifically by the rate of total Na^+ transport, it is difficult to obtain a truly basal, non-transport related metabolism. This may also be because of the existence of local pools of cation in the vicinity of the pump activation sites.

INTRODUCTION

Studies of the interaction of cation transport and metabolism in renal cortical slices have shown that (i) removal of Na^+ from the medium inhibits a fraction of cellular respiration and the uptake of K^+ from the medium, and (ii) ouabain in the medium inhibits an equivalent fraction of respiration and also inhibits K^+ uptake[1]. These findings are consistent with the view that cation transport by kidney cells stimulates their respiration, possibly by influencing the cellular concentration of ADP[1,2]. Curiously, however, the hypothesis seems not to have been further tested in this tissue by comparing the effects of removal of K^+ in the medium on metabolism and transport, as has been tried in other tissues. The question of the effect of K^+ in the medium is of particular interest with regard to kidney slices because of the observation that only a part of Na^+ extrusion from kidney cells depends on K^+ in the medium[3,4]. To what then does respiratory regulation correspond: linked Na^+–K^+ transport or total Na^+ transport?

With regard to the effect of Na^+ (point i above) it may also be noted that removal of Na^+ from the medium does not unequivocally demonstrate an effect of intracellular Na^+ on the respiration of kidney cells.

The initial purpose of the present study therefore was to fill these two gaps in our information about the metabolic aspects of cation transport in kidney slices: the effects of external K^+ and the effects of intracellular Na^+. In studying the additional influence of temperature and cardiac glycosides on the primary effects of Na^+ and K^+, results have been obtained which cast further light upon the nature of the K^+-independent, ouabain-insensitive fraction of Na^+ extrusion reported by WHITTEMBURY[3], KLEINZELLER AND KNOTKOVA[5] and WILLIS[4].

METHODS

The methods employed were the same as those described in the preceding paper[6]. The experimental animals were Syrian (golden) hamsters and thirteen-lined ground squirrels. Three incubation media were used: (a) "Standard Na^+ medium": NaCl, 140 mM; $CaCl_2$, 3 mM; $MgSO_4$, 1.5 mM; potassium phosphate buffer, 2.1 mM (providing a K^+ concn. of 3.7 mM); and glucose 10 mM. (b) "Na^+-free, choline medium": Same as (a) except that choline chloride was used to replace NaCl. (c) "Na^+-free, Tris medium": Same as (a) except that Tris buffered to pH 7.4 was used to replace NaCl. (d) "K^+-free medium": Same as (a) except that sodium phosphate replaced potassium phosphate buffer.

There were three "leaching media", one "Na^+-rich", the others "Na^+-free" which were the same as (a), (b) and (c) respectively except that they contained no glucose and were gassed with N_2.

RESULTS

The effects of K^+

Leached slices incubated at 38°

The omission of K^+ from medium in which slices of kidney cortex from ground squirrels were incubated caused a reduction in tissue respiration from 16.1 μl O_2/mg

TABLE I

EFFECT OF K^+ IN MEDIUM ON RESPIRATION, K^+ UPTAKE AND Na^+ EXTRUSION OF GROUND SQUIRREL KIDNEY CORTEX

Slices were leached for 12 min at 38° in Na^+ medium then incubated for 1 h in media with O_2 at 38°. Means are shown with S.E. for those with 6 or more cases. Na^+ values based on limited sample of 4 to 6 pairs of slices.

Conditions	*Number of samples*	Q_{O_2} *(µl O_2/mg dry wt. per h)*	*Tissue K^+ concn. (µequiv/g dry wt.)*	*Tissue Na^+ concn. (µequiv/g dry wt.)*
(a) Leached 12 min in Na^+ medium	26	—	87 ± 5	581 ± 10
(b) Incubated 1 h in standard Na^+ medium	25	16.1 ± 0.3	205 ± 8	296
(c) K^+-free medium	15	12.7 ± 0.3	55 ± 8	450 ± 15
(d) K^+-free medium + 25 µM ouabain	6	9.1 ± 0.3	34 ± 8	540 ± 21
(e) K^+-free medium + 625 µM ouabain	5	8.6	—	—
(f) (e) and (d) combined	11	8.9 ± 0.3	—	—

dry wt. per h (Q_{O_2}) to 12.7 (Table I). The tissue Na^+ concentration in slices after leaching was 581 µequiv/g dry wt. and in those which were then incubated with K^+ this value fell to 296 µequiv/g dry wt. while in K^+-free medium it only fell to 450 µequiv/g dry wt. (Table I). Thus, the tissue Na^+ content and the Q_{O_2} of slices incubated in K^+-free medium were similar to those of slices incubated with an optimally effective concentration of 25 µM ouabain (439 ± 14 and 11.9 ± 0.4 µequiv/g dry wt. respectively[6]). The tissue K^+ concentration of slices in K^+-free medium fell during incubation (Table I).

In kidney slices of hamsters the Q_{O_2} in K^+-free medium was 13.7 compared with that of 16.7 in slices incubated with K^+ (Fig. 1). The difference, though statistical-

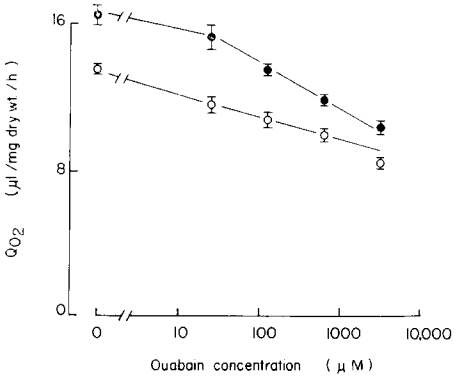

Fig. 1. Effect of ouabain on respiration of kidney cortex slices of hamsters. Slices were leached 12 min in Na^+-rich leaching medium with no K^+, no O_2, and no glucose. They were transferred to Na^+-rich media either with 4 mM K^+ (●) or without K^+ (○) and incubated for 1 h with O_2 and glucose at 38°.

TABLE II

EFFECT OF OUABAIN ON TISSUE Na^+ CONCENTRATION OF HAMSTER KIDNEY CORTEX INCUBATED IN K^+-FREE MEDIUM AT 38°

Slices treated as in Table I. Means ± S.E. are indicated.

Procedure	*Number of samples*	*Ouabain concn. (μM):*	*Tissue Na^+ concn. (μequiv/g dry wt.)*				
			0	*25*	*125*	*625*	*3125*
(a) Leached	17		651 ± 19				
(b) Incubated 1 h in K^+-free media	6–13		507 ± 11	534 ± 13	529 ± 14	565 ± 8	619 ± 10
Net extrusion (a − b)			144	117	122	86	32
(c) Leached*	17		609 ± 14				
(d) Incubated* 1 h in standard medium with 4 mM K^+	6–13		332 ± 6	329 ± 7	360 ± 6	438 ± 10	469 ± 16
Net extrusion (c − d)			277	280	249	171	140

* Same experiments are reported in ref. 6.

ly significant ($P < 0.01$), is not as great as that caused by 625 or 3125 μM ouabain which resulted in a Q_{O_2} of 12.0 and 10.5 respectively (Fig. 1 and ref. 6). As reported previously[4] omission of K^+ from the medium causes a decrease in extrusion of Na^+ from hamster kidney slices. In the present study the loss of Na^+ from slices in medium containing K^+ was 277 μequiv/g dry wt. and in K^+-free medium it was 144 μequiv/g dry wt. (Table II). The latter figure corresponds to the Na^+ extrusion observed in slices incubated with 3125 μM ouabain in a K^+-rich medium (137 μequiv/g dry wt.). Thus, neither ouabain nor K^+-free incubation acting alone reduces Na^+ extrusion by more than half.

The effect of ouabain in K^+-free medium at 38°

Since removal of K^+ appeared to be equivalent to full ouabain inhibition of Na^+ transport and, in ground squirrels, of respiration, it was of interest to see whether the two effects would be additive. That is, would ouabain cause any further effect in K^+-free medium? Slices of kidney cortex of ground squirrel were, therefore, incubated in K^+-free medium at 38° with 25 μM ouabain, and with the results that the Q_{O_2} was reduced to a new low value of 9.1 (Table I) while the transport of Na^+ was almost totally blocked (Table I). The latter result had not previously been achieved at 38° with any concentration of ouabain in media containing K^+ (ref. 6). In addition, the tissue K^+ concentration also fell below that of the leached value to 34 μequiv/g dry wt. In no previous circumstances had such a profound further decrease occurred (*i.e.* under anaerobic incubation, ouabain inhibition in K^+ medium, or incubation in K^+-free medium).

Kidney slices of hamsters responded in a similar manner to the combination of ouabain and K^+-free incubation. In Fig. 1 the dose-response of the respiration of the slices to ouabain in K^+-free medium is compared with that of slices in medium with K^+ (same data as in ref. 6).

Because the sensitivity of hamster tissue to ouabain is so low[6], it was of interest to discover whether in the absence of K^+ the kidney cells would become more sensitive to low concentrations of the glycoside. In Fig. 1 it may be seen that the curves tend to converge slightly. If one sets the inhibition in 3125 μM ouabain as 100%, then in medium with K^+, 25 μM ouabain causes an effect only 19% of the total and the difference in respiration from that of uninhibited slices is not significant statistically ($P > 0.05$). Further, in medium with K^+, 125 μM ouabain causes 48% inhibition. By contrast in K^+-free medium a concentration of only 25 μM ouabain causes a reduction in respiration which is highly significant ($P < 0.01$) and represents 48% of the total. Thus, removal of K^+ exerts not only an added effect to that of ouabain, but also causes a slight increase in the relative sensitivity to ouabain.

As in the ground squirrel kidney, Na^+ transport in kidney cortex of hamsters is almost entirely inhibited by high ouabain concentration in the absence of K^+; the tissue Na^+ concentration of slices incubated in 3125 μM ouabain (619 $\pm$ 10 μequiv/g dry wt.) is not significantly different from the initial leached value (651 $\pm$ 19 μequiv/g dry wt.). Also, as in ground squirrel, ouabain in the K^+-free medium causes the tissue K^+ content to fall to the unusually low value of 36 μequiv/g dry wt., a value never previously observed with any adverse set of conditions.

Unleached slices at 38°

The previous experiments showed that if Na^+-loaded, K^+-deficient slices were deprived of K^+ in the medium, thereby preventing K^+ uptake and part of Na^+ extrusion, the respiration of the slices remained unstimulated. This finding then raises the question of whether the metabolism of K^+-rich slices with relatively low Na^+ content would be so affected by K^+-free treatment. Interest in this question is enhanced by the consideration that the metabolic effects of low K^+ in the earlier experiments may have been minimized by the anaerobic leaching process which might have tended to raise the cellular concentration of ADP, and therefore the rate of respiration toward a maximum.

Slices of hamster kidney were therefore made which were transferred directly (*i.e.* without leaching) to incubation flasks containing medium with and without K^+ (Table III). The Q_{O_2} of the four pairs of such slices in K^+-free medium was found to

TABLE III

EFFECT OF OMISSION OF K^+ ON Q_{O_2} OF SLICES OF KIDNEY CORTEX OF HAMSTER WITH HIGH K^+ CONTENT

Slices were transferred directly to incubation flasks (*i.e.* without leaching) and were incubated 1 h at 38°.

Expt. No.	Q_{O_2} ($\mu l\ O_2$/mg dry wt. per h)		*Final tissue K^+ concn.** (μequiv/g dry wt.)		*Final medium K^+ concn.* (mM)	
	4 mM K^+	*0*	*4 mM K^+*	*0*	*4 mM K^+*	*0*
285	18.8	18.5	274	208	4.5	1.3
286	16.2	18.9	378	256	4.7	1.0
287	16.4	17.7	260	188	4.6	1.3
288	16.9	17.7	253	169	4.2	1.1
Mean	17.1	18.2	279	205	4.5	1.2

* Concentration of K^+ of two slices in same series: 331 μequiv/g dry wt.

be no different from that of a like number of slices incubated in medium with K^+ (Table III). It was of interest to note that while the loss of K^+ during incubation from the slices incubated without K^+ was about twice as great as that of slices with K^+ in the medium, the final tissue concentration (205 μequiv/g dry wt.) was well above that commonly observed in leached slices (about 120 μequiv/g dry wt.). This loss of K^+ in the slices incubated in the initially "K^+-free" medium raised the concentration in the medium to 1.2 mM by the end of the experiment.

Effect of K^+ at 5°

Ground squirrels. When slices of kidney cortex from ground squirrel were incubated at 5° without K^+, their Q_{O_2} (0.7 ± 0.04) was midway between that of slices incubated with K^+ (0.8 ± 0.04) and that of slices incubated with K^+ and ouabain (0.6 ± 0.04) and it was not statistically different from either (Table IV).

TABLE IV

EFFECT OF K^+-FREE INCUBATION ON RESPIRATION AND TISSUE Na^+ CONCENTRATION OF GROUND SQUIRREL KIDNEY SLICES AT 5°

Slices were leached at 38° in Na^+ with no glucose, then incubated 2 h at 5°. Means ± S.E. are indicated. Number of slices is 14 for all cases.

	Conditions			
K^+ in medium (4 mM):	+	+	o	o
Ouabain in medium (625 mM):	o	+	o	+
Q_{O_2} (μl O_2/mg dry wt. per h)	0.8 ± 0.04	0.6 ± 0.04	0.7 ± 0.04	0.6 ± 0.04
Tissue Na^+ concn.* (μequiv/g dry wt.)	400 ± 18	572 ± 19	534 ± 19	612 ± 17

* Initial concentration of leached slices = 586 ± 12.

The Q_{O_2} of slices incubated without K^+ but with 625 μM ouabain was the same (0.6 ± 0.04) as those treated with ouabain in the presence of K^+. Omission of K^+ from the medium caused a retention of Na^+ (534 ± 19 μequiv/g dry wt.) about equal to that caused by ouabain (572 ± 19 μequiv/g dry wt.), and both procedures represent a greater relative inhibition of Na^+ extrusion than at 38°. The combination of K^+-free incubation and ouabain inhibition, however, again led to complete blocking of Na^+ extrusion (612 ± 17 μequiv/g dry wt. compared with an initial tissue Na^+ concentration of 586 ± 12 μequiv/g dry wt.).

Hamsters. The Q_{O_2} of slices of kidney cortex of hamsters which were incubated at 5° in K^+-free medium (0.9 ± 0.05) was identical with that of slices incubated with K^+ (Table VI). Ouabain in high concentrations of 625 and 3125 μM caused the same decrease in Q_{O_2} to 0.7 as observed in slices incubated with K^+ and ouabain. At 5°, as at 38°, the extrusion of Na^+ in slices treated both with 3125 μM ouabain and with K^+-free incubation was diminished further than in slices treated with either ouabain or omission of K^+ alone.

Effects of Na^+

"Vectorial" effect on metabolism; preliminary considerations

WHITTAM AND WILLIS[1] observed that decreasing the Na^+ concentration in the medium by replacement with choline caused a progressive decrease in Q_{O_2} of rabbit

kidney slices. Since this effect was parallel with reduction of final tissue Na^+ concentration and of final tissue K^+ concentration the not unreasonable conclusion was drawn that the effect of decreasing Na^+ in the medium was secondary to the lowering of intracellular Na^+ concentration which in turn diminished the rate of transport of cations. Obviously, since changes in the cell and medium were roughly proportional, the alternative possibility that the primary effect of Na^+ was external to the cell membrane could not be rigorously excluded. Subsequent findings[8] have shown that extracellular Na^+ does have effects on events within the cell, so that this alternative explanation is at least conceivable. WILLIS[4] has found that at low temperature, hamster kidney slices will accumulate K^+ in a Na^+-free medium if the slices have been preloaded with Na^+ and that such slices will extrude Na^+ into a Na^+-free medium by a mechanism which is sensitive to K^+ or to metabolic inhibitors in the medium. A natural sequel to these two previous studies, therefore, was to determine the effects of Na^+ on metabolism of Na^+-loaded slices in Na^+-free medium at low temperature in the slices of hamster. Before doing this, however, it is necessary first to consider whether the stimulatory effects on respiration of Na^+ in the medium at 38° are apparent and similar to those of rabbit. Another previous study[7] showed that replacing Na^+ in the medium with choline caused a lowering of respiration from 16.2 ± 0.5 to 10.2 ± 0.3. The former figure is in good agreement with that of 16.7 observed in control slices in this study (Fig. 1), and the value of 10.2 ± 0.3 for Na^+-free incubation is close to that of 10.5 ± 0.4 observed for slices inhibited with 3125 μM ouabain (Fig. 1). Table V shows that in four sets of slices the addition of ouabain to Na^+-free medium does not lower the respiration appreciably below that of slices in Na^+-free medium with no ouabain.

TABLE V

EFFECT OF Na^+-FREE INCUBATION ON RESPIRATION OF SLICES OF KIDNEY CORTEX OF HAMSTERS

Slices were leached 12 min in media without O_2 or glucose then incubated 1 h at 38° with O_2 and glucose. Means are shown with S.E. for those with more than 6 cases or ranges for those with less.

Conditions of incubation	*Number of samples*	Q_{O_2} (*μl O_2/mg dry wt. per h*)
Standard Na^+ medium*	18	16.2 ± 0.5
Na^+-free, choline medium*	15	10.2 ± 0.3
Na^+-free, choline medium	4	9.5 (7.9–10.3)
Na^+-free choline medium + 625 μM ouabain	4	9.0 (7.9–10.3)

* Data from ref. 7.

Effect of internal Na^+

The rate of respiration of Na^+-rich slices previously leached at 38° in Na^+ and then incubated at 5° in Na^+ medium was found to be 0.9 ± 0.05 compared with that of 0.5 ± 0.04 found in Na^+-depleted slices leached in and incubated with Na^+-free choline media (Table VI). Slices which were leached in Na^+ at 38° and then incubated in Na^+-free, choline medium at 5° exhibited a Q_{O_2} of 0.8 ± 0.06 a value which was not significantly different ($P > 0.05$) from that of Na^+-rich slices incubated in Na^+ but which was significantly above that of Na^+-depleted slices leached in and incubated in Na^+-free, choline medium ($P < 0.01$).

TABLE VI

EFFECT OF EXTRACELLULAR K^+ AND OF INTRACELLULAR AND EXTRACELLULAR Na^+ ON RESPIRATION OF HAMSTER KIDNEY CORTEX

Slices were leached in media as indicated with no glucose or O_2 for 12 min at 38° and incubated 2 h at 5° in media indicated. In Na^+-free media, choline replaced Na^+. In K^+-free media sodium phosphate buffer was used.

Expt.	*Procedure*			*Number of samples*	Q_{O_2} ($\mu l\ O_2$/*mg dry wt. per h*)
	Leaching medium	*Incubation medium*			
	Na^+ *concn.* (*mM*)	Na^+ *concn.* (*mM*)	K^+ *concn.* (*mM*)		
A	140	140	4	40	0.9 ± 0.05
B	140	140	0	21	0.9 ± 0.06
C	140	0	4	19	0.8 ± 0.06
D	0	0	4	19	0.5 ± 0.04
E	0	140	4	7	0.7 ± 0.04
F	30	30	4	6	0.8 ± 0.05
G	30	0	4	19	0.7 ± 0.06
Statistical comparisons:					
A *vs.* D		$P < 0.01$			
A *vs.* E		$P < 0.01$*			
E *vs.* D		$P < 0.01$			
A *vs.* F		$P < 0.02$			
D *vs.* F		$P < 0.01$			

* Difference in F is highly significant for two samples. Quasi-"T" test applied as described in ref. 23.

WILLIS[4] found that when slices were leached in a choline medium with 30 mM Na^+ their Na^+ concentration after leaching (in μequiv/ml tissue water) was 38 ± 2. When incubated in Na^+-free medium these low Na^+ slices accumulated K^+ at an initial rate half that of slices loaded with 140 mM Na and incubated in Na^+-free medium. To see how such a low Na^+ concentration would influence metabolism, slices were leached at 38° in a medium with 30 mM Na^+ and 110 mM choline (Table VI). During 1 h at incubation following transfer to Na^+-free, choline medium the uptake of O_2 by these slices was 0.7 ± 0.06, half way between that of slices leached and incubated without Na^+ (0.5) and those leached and incubated with 140 mM Na^+ (0.9). The average Q_{O_2} of seven pairs of slices leached in 30 mM Na^+ at 38° and incubated in 30 mM Na^+ at 5° was 0.8 ± 0.05.

Effect of external Na^+

The converse experiment to showing that alterations in intracellular Na^+ alone cause the full effect previously observed with changes in external Na^+, is to test whether high external Na^+ at low temperature has any effect on respiration of slices depleted of Na^+. Slices which had been leached in Na^+-free choline medium were, therefore, incubated in the standard Na^+-rich (140 mM) medium at 5°. The Q_{O_2} of these slices (0.7 ± 0.08) was significantly below ($P < 0.01$) that of Na^+-rich slices leached in Na^+ and incubated with Na^+, but it was also above that of slices leached in and incubated in Na^+-free media (Table VI). The elevation of metabolism over the basal level could of course have resulted from inward leakage of Na^+ from the large external pool into the relatively small volume of the cells.

Effect of Na^+-free media on ground squirrel kidney cortex

In order to compare the decrease in Q_{O_2} caused by ouabain and by omission of K^+ in the medium with that caused by omission of Na^+ from the medium, slices of kidney cortex from ground squirrel were leached in Na^+-free choline medium and then incubated in Na^+-free choline at 38°. Rather surprisingly the Q_{O_2} of these slices (14.9 ± 0.4, Table VII) was observed to be only slightly less than that of slices incubated in Na^+ (16.7) and it was well above the minimum value obtained with ouabain in media with K^+ (11.9, ref. 6). It was further observed, however, that addition of ouabain to the Na^+-free, choline medium did not further inhibit the respiration of slices (Table VII). It would seem therefore that the previous result occurs not necessarily as a consequence of lack of stimulatory effect of Na^+, but rather as an indication of the presence of a non-specific stimulatory effect of choline on the previously defined[1] "basal" rate of metabolism.

TABLE VII

EFFECT OF Na^+ ON RESPIRATION, K^+ AND Na^+ OF KIDNEY CORTEX SLICES OF GROUND SQUIRRELS

Slices were leached for 12 min in N_2 then incubated for 1 h in media with O_2. Means ± S.E. are indicated.

Conditions	*Number of samples*	Q_{O_2} *(µl O_2/mg dry wt. per h)*	*Tissue K^+ concn. (µequiv/g dry wt.)*	*Tissue Na^+ concn. (µequiv/g dry wt.)*
Leached 12 min in Na^+ medium*	26	—	87 ± 5	581 ± 10
Incubated 1 h in Na^+ medium*	25	16.1 ± 0.3	205 ± 8	296
Leached 12 min in Na^+-free choline medium	10	—	163 ± 7	59 ± 12
Incubated 1 h in Na^+-free, choline medium	14	14.9 ± 0.4	131 ± 3	25 ± 5
Incubated 1 h in Na^+-free, choline medium + 125 µM ouabain	7	13.4 ± 0.2	124 ± 6	27 ± 10
Leached 1 h in Na^+-free Tris medium	18	—	123 ± 4	24 ± 3
Incubated 1 h in Na^+-free Tris medium	19	9.7 ± 0.6	64 ± 4	13 ± 4
Incubated 1 h in Na^+-free Tris + 125 µM ouabain	8	10.2 ± 0.8	72 ± 4	13 ± 4
Leached 12 min in Na^+ + Tris medium**	8	—	105 ± 8	253 ± 18
Incubated 1 h in Na^+ + Tris medium**	8	15.4 ± 0.4	143 ± 6	165 ± 13

* Same data as in Table I.
** Na^+ concn. in 70 mM.

As a further attempt to determine a "basal" respiration in Na^+-free medium, slices of kidney cortex of ground squirrels were leached and then incubated at 38° in Na^+-free Tris medium (Medium c). The Q_{O_2} in this medium (Table VII) was 9.7 and with 125 µM ouabain it was 10.2, a difference which was not statistically significant. In order to estimate whether Tris might have a depressant effect upon metabolism, slices were leached and incubated in a medium made up half of Na^+-free Tris and half of the standard Na^+ medium. The Na^+ concentration of this mixture was about 70 mM, which, if Tris had no generally deleterious effect, should give nearly maximal stimulation of respiration. The rate of O_2 consumption in this solution

(Table I) was in fact 15.4, a value not significantly lower than the rate of 16.1 observed in control slices (Table VII).

DISCUSSION

A discussion of the interactions of Na^+,K^+, ouabain and respiration can be very confusing. Table VIII shows that among these four factors, considered both as dependent and independent variables, there are about twenty-one possible combinations of interaction, without even separating the effects of intracellular and extracellular Na^+ and K^+. Of this number about twenty interactions have actually been observed, thirteen of these in kidney cortex. Of this thirteen, nine interactions are directly relevant to the present discussion. These are: (1) The activation of K^+ uptake by Na^+ within the cell[1,4]. (2) K^+ activation of about one half of Na^+ extrusion[3,4].

TABLE VIII

POSSIBLE INTERACTIONS BETWEEN Na^+, K^+, OUABAIN AND RESPIRATION

+ = activates or is required; I = inhibits.

Independent variables	*Dependent variables*					
	Na^+ extrusion	*K^+ uptake*	*Ouabain inhibition*			*Respiration*
			Na^+ extrusion	*K^+ uptake*	*Respiration*	
Na^+	+	+	(+)*	(+)*	(+)*	+
K^+	+	+	I	?	I	+
Ouabain	I	I	—	—	—	I
Respiration	+	+	(+)*	(+)*	(+)*	—

* *cf.* ref. 9.

(3) Ouabain inhibition of net K^+ uptake[1,6]. (4) Ouabain inhibition of about half of Na^+ extrusion in media with K^+ (refs. 5 and 6). (5) Excess inhibition of Na^+ extrusion by ouabain in K^+-free medium (ref. 10; Tables I and II). (6) Ouabain inhibition of a fraction of respiration[1,6]. (7) Increased inhibition of respiration by ouabain in media without K^+ (Table I, Fig. 1). (8) Inhibition of part of respiration by omission of Na^+ from the medium[1] and, more importantly, from the cell (Table VI). (9) Partial inhibition of respiration by removal of K^+ from the medium (Table I, Fig. 1).

Five of these findings are difficult to explain on the basis of an unmodified hypothesis of a simple Na^+–K^+ exchange pump. These are the persistence of Na^+ transport both in the absence of K^+ in the medium (2) and also in the presence of ouabain with K^+ in the medium (4), the inhibition of only a part of the ouabain-sensitive fraction of respiration by removal of K^+ from the medium (9), and the increased inhibitory effect of ouabain on Na^+ extrusion and respiration in the absence of K^+ (5 and 7). In order to account for such discrepant results two modifications of the simple hypothesis have been proposed explicitly for kidney cortex and two others may be adapted from the studies of other tissues. These proposed modifications are illustrated in Fig. 2 and may be summarized as follows: (A) Two parallel Na^+ pumps, one requiring K^+ and exchanging Na^+ for K^+, the other a non-coupled (electrogenic)

Na^+ pump[11]. (B) Contraction of leached and swollen kidney cells which causes extrusion of cellular fluid including Na^+, K^+ and Cl^- (ref. 5). (C) A linked Na^+--K^+ pump in which Na^+ may exchange for Na^+ when external concentration of K^+ is decreased (see ref. 12). If normally two Na^+ exchange for one K^+, then in the absence

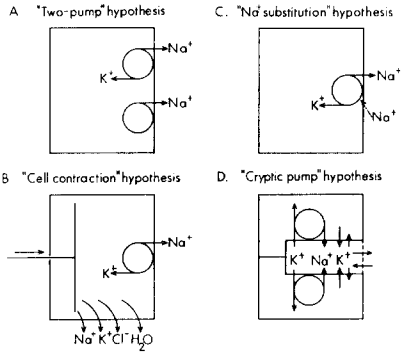

Fig. 2. Four proposed modifications of a simple Na^+–K^+ exchange mechanism in kidney slices. (A) two-pump hypothesis[11]; (B) cell-contraction hypothesis[5]; (C) Na^+-substitution hypothesis (based on ref. 12); (D) cryptic-pump hypothesis (based on refs. 13, 14). For description see text.

of K^+ net Na^+ extrusion would be reduced by about half. (D) A coupled Na^+--K^+ pump which operates in contact with a restricted extracellular space between tubular cells or within infoldings of the cell membrane (see refs. 13 and 14). Because of the long diffusion pathway between this "cryptic pump" and the outside medium the K^+ concentration in the vicinity of the pump could very well be different from that in the medium. In particular, the K^+ concentration of this space in slices incubated with K^+-free medium would tend to be above zero. Swelling of cells under conditions adverse for transport would tend to close the channels (*cf.* ref. 14) making transport sites less accessible to inhibitors such as ouabain.

TABLE IX

SUMMARY OF THE EFFECTIVENESS OF FOUR MODELS TO ACCOUNT FOR FIVE ANOMALOUS RESULTS

For details see text and Fig. 2. x = model accounts for finding; xx = model does not account for finding; ( ) = uncertainty about prediction of model. Numerals before findings refer to list in text.

Anomalous finding	*Model*			
	A *Two pump*	*B* *Cell contraction*	*C* *Na^+ substitution*	*D* *Cryptic pump*
(2) K^+ activates 1/2 Na^+ extrusion	x	x	x	x
(4) Ouabain inhibits 1/2 Na^+ extrusion (in presence of K^+)	x	x	xx	x
(5) Ouabain inhibits all Na^+ extrusion in absence of K^+	xx	xx	(xx)	x
(7) Ouabain exerts added inhibition on O_2 consumption in absence of K^+	xx	xx	(x)	x
(9) K^+ inhibits 1/2 Na^+ sensitive O_2 uptake (in presence of Na^+)	x	xx	xx	x

The adequacy for each of these proposals to account for the discrepant observations outlined above are summarized in Table IX. Three models account equally well for the excess Na^+ extrusion in ouabain or the absence of K^+. The "two pump" and "cell contraction" hypotheses were proposed specifically for this purpose. The "cryptic-pump" hypothesis would suggest that removing K^+ from the medium does not remove all K^+ from the relevant activating site on the membrane, hence Na^+ transport and elevated metabolism would continue at a reduced rate. The partial effect of ouabain in K^+ medium would be a result of a high K_0 at the site of attachment resulting in an interference with the binding of ouabain[15].

The "Na^+ substitution" model accounts for Na^+ extrusion in the absence of K^+, but it cannot explain the partial effect of ouabain on Na^+ extrusion in K^+ medium without an *ad hoc* amendment (*e.g.* ouabain increases the affinity of the system for extracellular Na^+).

The enhanced effect of ouabain on Na^+ extrusion and respiration in the absence of K^+ are not accounted for by either the "two-pump" or the "cell-contraction" hypothesis. There is no reason why K^+ should necessarily protect a unilateral, non-coupled pump from ouabain. It is even less apparent why a "cellular contraction" should suddenly become sensitive to ouabain in the absence of K^+. Furthermore KLEINZELLER AND KNOTKOVA[5] indicated that this process was apparently not coupled reciprocally to respiration, so that even if the extrusion of Na^+ by squeezing were to become ouabain sensitive, the increased inhibition of respiration would still have to be accounted for.

The same observation can be made with equal force regarding the effect of removing K^+. There is no apparent reason why, if the excess Na^+ extrusion is a result of non-specific contraction, removal of K^+ should have less of an effect on respiration than ouabain inhibition or removal of Na^+.

Although the cryptic-pump hypothesis could account for all the observations noted, the other three suggestions are not unreasonable, and it is altogether possible that more than one of these mechanisms is operative.

Other observations can also be considered but in general these are less conclusive.

Chloride. WILLIS[4] found that the ratio of Na^+ extruded to Cl^- extruded from leached kidney slices of hamster remained the same regardless of whether extrusion was into medium with or without K^+. On the face of it, this observation would seem to diminish the two-pump hypothesis, since one would suppose that in the absence of Na^+–K^+ exchange the Na^+/Cl^- ratio would increase. WHITTEMBURY[16], however, has reported that removal of K^+ from the medium does not alter the kinetics of Cl^- extrusion. This discrepancy awaits resolution.

Tissue water. One might expect that under the "super inhibition" of Na^+ extrusion caused by ouabain in the absence of K^+ (ref. 10, Tables I and II) that there would be no loss of water. This is not the case. KLEINZELLER AND KNOTKOVA[5] and MACKNIGHT[10] found extrusion of water in kidney slices treated with ouabain in K^+-free medium. In the slices incubated in K^+-free media in this study there was less loss of water in those with ouabain than in those without, but there was a net decrease in tissue water from the value of leached slices, in both cases. This persistent loss of water may be ascribed to the increased loss of K^+ reported by MACKNIGHT[10] and in Table I and Fig. 1.

Residual K^+. It is usually not possible to remove all K^+ from kidney cortex slices by leaching and metabolic inhibition. The observation that ouabain in K^+-free medium causes a drastic, almost total loss (ref. 10, Table I) tends to confirm the view of BURG AND ORLOFF[17] that this residual retention is due to transport. At the same time, the observation may suggest that under other conditions, closure of the extracellular channels leads to reduction in the loss of K^+.

Ethacrynic acid. WHITTEMBURY[11] has reported an inhibition of the 'extra' (supposedly non-coupled) Na^+ extrusion by ethacrynic acid. The significance of this is not clear. HOFFMAN AND KREGENOW[18] found that ethacrynic acid inhibited a fraction of Na^+ extrusion in erythrocytes which did not require extracellular K^+ but did depend upon extracellular Na^+. Other evidence suggests, however, that the drug is an inhibitor of (Na^+-K^+)-activated ATPase though less specific than ouabain[19]. WHITTEMBURY's finding therefore may only reflect an additive inhibitory effect of ethacrynic acid on the same mechanism that is also inhibited by ouabain.

Species and temperature effects. Variances in results have been reported by groups working with kidney cortex slices in different species and at various temperatures. Such divergent findings include differential ouabain sensitivity among different species (*e.g.* see ref. 6), differences between species in maximal effectiveness of ouabain on Na^+ extrusion (*cf.* ref. 6 on ground squirrel and ref. 5 on rabbit), and at different temperatures[6], and differences in the effectiveness of external K^+ with temperature (Tables I and IV). Many of these differences might be accounted for merely by the geometry of the tissue, *i.e.* the length and tortuosity of the extracellular diffusion pathways, and by the balance between membrane permeability to K^+, all of which would tend to influence the true extracellular concentrations of K^+, Na^+ and ouabain. For example, the greater effect of ouabain on Na^+ extrusion in kidney slices of ground squirrels at low temperature than at high temperature might be explained either by decreased membrane permeability or more open channels to the outside, leading to lower interspace K^+ concentration.

Q_{O_2}. Finally, to return to the original question posed in INTRODUCTION, it is apparent that the "basal" O_2 consumption (*i.e.* that not linked with transport) is more difficult to estimate than previously supposed[1,7]. It seems fairly clear that ouabain in K^+-free medium exerts a greater inhibitory effect than in media without K^+, in accord with the greater inhibition of Na^+ extrusion under the same condition. (In hamsters the difference between 8.5 in 3125 μM ouabain with 0 mM K^+ is significantly less ($P < 0.01$) than that of 10.5 in 3125 μM ouabain with 4 mM K^+. In ground squirrel kidneys, the values for slices in K^+-free medium of 9.1 in 25 μM ouabain and 8.6 in 625 μM ouabain can be compared with 10.9 and 10.3 for the slices incubated in comparable ouabain concentrations with K^+. In the previous study[6] the Q_{O_2} of maximally inhibited slices, *i.e.* those in 25 μM ouabain was 11.2, but the controls in that series also had a higher rate of respiration. In the present study the conglomerate averages for slices in ouabain were 10.7 ± 0.3 and 8.9 ± 0.3 for K^+ and K^+-free medium, respectively. This difference is highly significant statistically ($P < 0.01$).) What then of the Q_{O_2} of slices in Na^+-free media? In previous studies[1,7] the rate of respiration in Na^+-free medium has been taken to be the same as in ouabain media with K^+. Yet the latter now seems to give a value too high, and one should expect the former, *i.e.* removal of Na^+, to be a very effective means of blocking Na^+ transport. The results of this study are ambivalent on this score. The respiratory rate in Na^+-free

media of 9.7 in kidney slices of ground squirrels is not significantly different ($P > 0.05$) from the respiratory rates of slices in ouabain with K^+ (10.7) or without K^+ (8.9). The same could be said of the hamster kidney slices, in which the Q_{O_2} of 9.5 in Na^+-free medium compares with 10.5 in ouabain *plus* K^+ and with 8.5 in K^+-free ouabain. It should be noted, however, that a larger series of determinations in hamster gave a Q_{O_2} of 10.2 $\pm$ 0.3 in Na^+-free medium[7].

It is conceivable, however, that removal of Na^+ yields a respiratory rate slightly greater than truly "basal". Even slices leached of Na^+ retain some Na^+ (roughly 2 to 10 mM in tissue water, see Table I). If this Na^+ were concentrated near the membrane and extruded into a similarly restricted extracellular compartment, an appreciable stimulation of respiration might result.

To summarize, then, although the stoichiometric relationship between K^+ and Na^+ transport in kidney slices remains obscure, most of the evidence still indicates a close correspondence between Na^+ transport and O_2 consumption. Whenever Na^+ extrusion is decreased, whether by ouabain, by removal of K^+, by the combination of these two, or by removal of Na^+, respiration is diminished in proportion. The one exception to this rule is the result with hamster kidney slices at 5° in which omission of K^+ from the medium does decrease Na^+ extrusion[4] but not respiration (Table VII). Although the small O_2 consumption at 5° is near the limits of determination of the respirometer, the large number of consistent observations prevents the discounting of this observation. Neither can it be supposed that respiration is not coupled to Na^+ extrusion in this species, for a comparatively large decrease is seen in Na^+-free medium. While it is difficult to reconcile this finding with those at 38° in hamster and with the results with ground squirrel kidney cortex, it does suggest again that a fraction of "internal transport" can occur within the slices which is not detected by measurement of net changes.

If, as it seems, respiration is coupled to the entire Na^+ extrusion, rather than merely to the fraction which is inhibited by omission of K^+ or presence of ouabain, then the Na^+/O_2 ratio would be about twice that of the K^+/O_2 ratios previously observed[1,6] and would range between 7 and 16, in better agreement with results obtained in intact tissues[20–22].

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