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OUABAIN INHIBITION OF ION TRANSPORT AND RESPIRATION IN RENAL CORTICAL SLICES OF GROUND SQUIRRELS AND HAMSTERS

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SUMMARY

1. The inhibitory effects of ouabain on oxygen consumption, K^+ uptake and Na^+ extrusion by renal cortical slices of ground squirrels and hamsters were determined.

2. The maximum inhibition of K^+ uptake at 38° in slices of kidney cortex of ground squirrel which had been leached of K^+ occurred in 25 μM ouabain. Inhibition was actually less in higher concentrations of ouabain.

3. The reduction of respiration at 38° due to ouabain was parallel to the inhibition of K^+ uptake. The uninhibited respiration was 18.0 μl O_2/mg dry wt./h and the minimal O_2 uptake was 11.9 in 25 μM ouabain. Extrusion of Na^+ was inhibited by ouabain in a manner parallel to that of K^+ uptake, but in 25 μM ouabain about half the normal extrusion of Na^+ occurred.

4. At 5° the sensitivity of ground squirrel kidneys to ouabain was less, so that 25 μM ouabain caused only slight inhibition and 3125 μM was required for maximal inhibition of K^+ uptake, of respiration and of Na^+ extrusion. There was greater total inhibition of Na^+ extrusion by ouabain at the lower temperature than at 38°.

5. The reduced sensitivity at low temperature was apparently due to reduced attachment or access of inhibitor to its active site. When slices were pretreated in low concentration of ouabain (25 μM) at 38° they were completely inhibited at 5° with or without additional inhibitor.

6. The inhibition due to ouabain at 38° cannot be readily reversed by washing.

7. Hamster kidney cortex was relatively insensitive to ouabain. In 25 μM ouabain at 38° there was negligible inhibition of K^+ uptake and a concentration of 3125 μM was required for a maximal effect.

INTRODUCTION

An early indication that part of respiration is reciprocally coupled to ion transport was provided by the finding that in kidney slices decrease in O_2 consumption is proportional to inhibition of K^+ uptake by ouabain[1]. On the assumption that this respiratory control is a reflection of (Na^+-K^+)-activated ATPase activity[1,2], the ouabain-sensitive respiration of several cell types has become a useful tool in the study of various aspects of ion transport. These include coupling of Na^+ and K^+

transport, directionality of Na^+ and K^+ effects of these ions on the ATPase and the mode of ouabain inhibition[3,4].

Despite their large transport-coupled respiration, however, renal cortical cells have not been greatly exploited for the study of these problems. Part of the reason for this is that kidney cells are so permeable to cations that rapid equilibration of ion or tracer concentrations occurs before metabolism and rates of ion movement can conveniently be measured. This impediment to such use of kidney slices may perhaps have been diminished by the recent discovery of active ion transport at low temperatures in kidney slices of certain cold resistant species such as rabbits, hamsters and ground squirrels when passive movements are slow[5].

As a first step in the investigation of the questions outlined above, it is necessary to ascertain that the reciprocal coupling between ion transport persists at low temperature. Since the influence of ouabain on cation transport and metabolism is a prime criterion of this coupling, the present study undertook to determine the effect of temperature on these inhibitory influences in kidney cortex of ground squirrels and hamsters.

METHODS

The handling of kidneys, the incubation procedure and the analytical methods have been described in detail elsewhere[1,5]. Kidneys of ground squirrels (*Citellus tridecemlineatus*) or hamsters (*Mesocricetus auratus*) were used. Slices of the kidney cortex were prepared free-hand and then leached for 12 min in a preincubation medium described below. Slices were then incubated at either 38 or 5° in 4 ml of incubation medium within Warburg manometer flasks. (In a few instances slices were incubated in 3 ml of medium in flasks of a Scholander microvolumetric respirometer.) The incubation medium contained NaCl, 140 mM; $CaCl_2$, 3 mM; $MgSO_4$, 1.5 mM; potassium phosphate buffer, 2.1 mM (providing a K^+ concentration of 3.7 mM) and glucose, 10 mM. The ouabain (Sigma) was added as concentrated stock solution (5 or 25 mM) to each of the flasks before the medium to provide appropriate concentrations between 0.2 and 3125 μM. At 38° the high rate of O_2 consumption of kidney slices necessitated the use of 100% O_2 in the gas phase. At 5° the Q_{O_2} (μl O_2/mg dry wt. per h) is extremely low and air was an adequate source of O_2. The leaching media in which the slices were preincubated was the same as the incubation medium except that it lacked K^+ (the buffer having been added as sodium phosphate) and glucose, and the gas phase was N_2. The volume of this medium (approx. 50 ml) was large compared with that of the slices.

Respiration was measured for 1 h at 38° and for 2 h at 5°. Slices were then dried at 105° overnight, digested in concentrated HNO_3 and analyzed for K^+ and Na^+ by flame photometry. Values for tissue K^+ and Na^+ concentration are in terms of tissue dry weight throughout (*e.g.* μequiv/g dry wt.).

RESULTS

Ground squirrels

Effects of ouabain at 38°

After 12 min of leaching, slices of kidney cortex with an average K^+ content of 102 μequiv/g dry wt. were placed in Warburg manometer flasks containing various

concentrations of ouabain and were incubated for 1 h at 38°. The uptake of K^+ during this interval in uninhibited slices was 179 μequiv/g dry wt., giving a final tissue K^+ concentration of 277 μequiv./g dry wt. (Fig. 1). Slices incubated with 1 μM ouabain exhibited a significantly lower ($P < 0.01$) final concentration of 184 μequiv/g dry wt., which represents an uptake (86 μequiv/g dry wt.) about half that observed in the uninhibited control slices. Maximum inhibition of K^+ uptake was obtained with 25 μM ouabain, in which the final K^+ content of slices was only 116 ± 7 μequiv/g dry wt. (S.E., 8 cases), a concentration whose difference from the initial starting value was not significant ($P > 0.05$). Curiously, in higher concentrations of ouabain there was a tendency for less inhibition of K^+ uptake. The tissue K^+ concentration after incubation in 625 μM ouabain was 132 μequiv/g dry wt. and in 3125 μM ouabain it was 148 μequiv/g dry wt, both of which values were above that obtained in 25 μM ouabain.

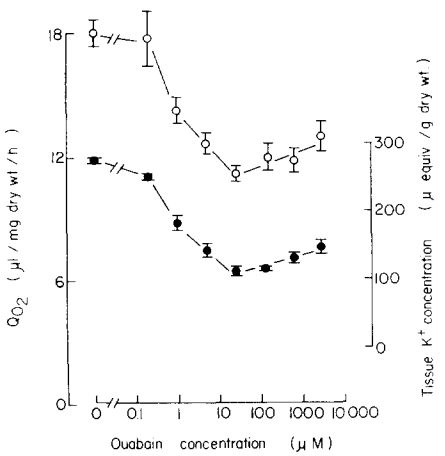

Fig. 1. Effect of ouabain on K^+ uptake and respiration in renal cortical slices of ground squirrels. Slices were leached 12 min in medium without O_2, glucose or K^+ and then incubated one hour at 38° in Na^+ medium with K^+, glucose, O_2, and various concentrations of ouabain. Tissue K^+ concn. of slices after leaching and before incubation was 102 ± 7 μequiv/g dry wt. (S.E., 11 cases). ○, O_2 consumption; ●, tissue K^+ content after incubation. Means of 6–10 cases are shown (except for 3125 μM ouabain for which $n = 4$). Standard errors are indicated.

The rates of O_2 consumption of the same slices were inhibited by ouabain in a manner parallel to that of K^+ uptake (Fig. 1). The respiratory rate of control, uninhibited slices was 18.0 μl O_2/mg dry wt. per h, whereas that of slices incubated with 1 μM ouabain was 14.2. The Q_{O_2} in 25 μM ouabain of 11.9 was the minimum observed. As with K^+ uptake, there was a slight reversal of inhibition at very high ouabain concentrations so that in 3125 μM ouabain the Q_{O_2} was 12.9.

The changes in Na^+ concentration of slices were the converse of K^+ (Table IA). The tissue Na^+ concentration following leaching (which results in loading with Na^+) was 582 μequiv/g dry wt. and during subsequent uninhibited incubation this fell to 271 μequiv/g dry wt., a net loss of 311 μequiv/g dry wt. Slices incubated with 1 μM ouabain showed a higher content of Na^+ (330 μequiv/g dry wt.) and, therefore, less extrusion (252 μequiv/g dry wt.), while those in 25 μM ouabain lost only 143 μequiv/g dry wt. to give a final tissue Na^+ concentration of 439. At concentrations

TABLE I

DOSE-RESPONSE TO OUABAIN OF TISSUE Na^+ CONCENTRATION OF SLICES OF KIDNEY CORTEX OF GROUND SQUIRRELS

Slices were leached 12 min in O_2-free, K^+-free, glucose-free Krebs medium then incubated for 1 h at 38° or 2 h at 5° in medium containing glucose, 4 mM K^+ and various concentrations of ouabain. The data in rows A and B refer to the experiments described in Figs. 1 and 2 respectively. Means ± S.E. are indicated.

Conditions	*Number of cases*	*Tissue Na^+ concn. (μequiv/g dry wt.)*							
		After leaching	*After incubation*						
	Ouabain concn. (μM):	*0*	*0*	*0.2*	*1*	*5*	*25*	*125*	*625*	*3125*
A. 38°	8–13	582 ±12	271 ±12	289 ±9	330 ±11	407 ±21	439 ±14	434 ±16	394 ±12	357 ±14
B. 5°	6	565 ±22	382 ±24	—	—	—	378 ±25	490 ±42	512 ±22	522 ±14

above 25 μM ouabain inhibition was less and in 3125 μM ouabain the final tissue Na^+ concentration was 357 μequiv/g dry wt., nearly as low as that of slices in 1 μM.

Effects of ouabain at 5°

In the set of slices which were to be incubated at 5° the tissue K^+ concentration after leaching was 80 μequiv/g dry wt. Uptake during 2 h incubation was 140 μequiv/g dry wt. to a final concentration of 220 μequiv/g dry wt. (Fig. 2). This uptake was only slightly reduced by 25 μM ouabain in which the final tissue K^+ concentration was 203 μequiv/g dry wt. An ouabain concentration of 125 μM reduced the final tissue K^+ concentration to 136 μequiv/g dry wt., an uptake of 56 μequiv/g dry wt., and 3125 μM ouabain prevented uptake of K^+ entirely. The Q_{O_2} of slices at 5° was only 0.8 ± 0.1 (S.E., 8 cases) and with Warburg manometers small changes were difficult to measure. Nevertheless the respiratory rate in 125 μM ouabain was 0.6 ±

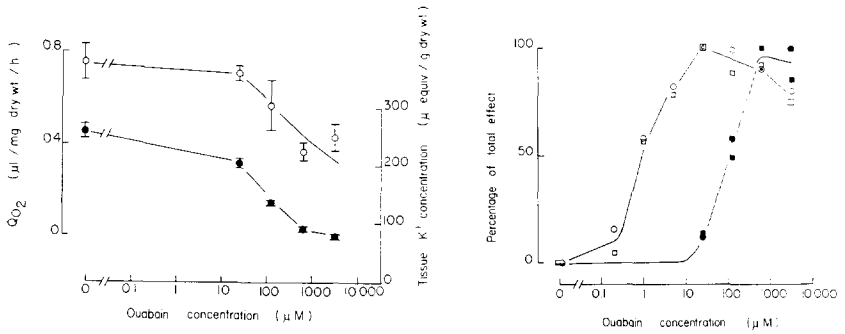

Fig. 2. Effect of ouabain at 5° in renal cortical slices of ground squirrel. Procedure same as described in Fig. 1 except slices were incubated at 5° in air for 2 h. ○, O_2 consumption; ●, final tissue K^+ content. Tissue K^+ concn. (μequiv/g dry wt.) after leaching, before incubation was 80 ± 5, 12 cases. Means ± S.E. for 6–8 pairs of slices are shown.

Fig. 3. Comparison of relative effects of ouabain at 38° and 5°. ○, the relative inhibition by ouabain on tissue K^+ content at 38°; □, tissue respiration at 38°. ● and ■, the inhibitory effects of ouabain at 5°. Values are computed from the results shown in Figs. 1 and 2.

0.1 (7) and in 625 and 3125 μM it was 0.4 $\pm$ 0.04 (6) and 0.4 $\pm$ 0.06 respectively (Fig. 2).

The Na^+ concentration of slices after leaching and before incubation was 565 μequiv/g dry wt., and in uninhibited slices this fell to 382 μequiv/g dry wt. after 2 h incubation at 5° (Table IB). In 125 μM ouabain it fell to 490 μequiv/g dry wt. and in 3125 μM to 522 μequiv/g dry wt.

The close correspondence between inhibition of K^+ uptake and inhibition of O_2 consumption by ouabain is illustrated in Fig. 3 in which the results previously described are expressed as per cent of total effect of ouabain. The parallel between K^+ uptake and Q_{O_2} is apparent not only at 38° but also at 5°. At the lower temperature, however, the sensitivity of both is greatly reduced so that half-maximal inhibition is at 125 μM ouabain instead of 1 μM, and total inhibition is at about 625 μM ouabain instead of at 25 μM, which at 5° causes almost no inhibition.

Effect of pre-exposure to ouabain at high temperature

Since the previous results indicated a greatly decreased sensitivity of K^+ transport to ouabain at 5°, the question arose of whether this consisted of diminished affinity of the drug for its site of action or a diminished effect on the mechanism itself. To test these two possibilities, slices of ground squirrel kidney cortex were leached in a medium containing 25 μM ouabain. They were then incubated at 5° with and without 25 μM ouabain and their final tissue K^+ concentration was compared with that of slices treated in the same way except for the presence of ouabain in the leaching medium (Table II).

TABLE II

EFFECT OF PRE-EXPOSURE TO OUABAIN AT 38° BEFORE INCUBATION AT 5°

Slices were leached with (or without) 25 μM ouabain at 38° then incubated with and without ouabain at 5°. Number of cases is 6–9. Means $\pm$ S.E. are shown.

	Tissue K^+ concn. (μequiv/g dry wt.)				
	After leaching		*After incubation*		
Ouabain (μM):	*0*	*25*	*0*	*25*	*625*
A	72 $\pm$ 2	—	192 $\pm$ 4	163 $\pm$ 4	82 $\pm$ 2
B	—	87 $\pm$ 3	101 $\pm$ 4	92 $\pm$ 3	74 $\pm$ 2

After leaching in a medium with 25 μM ouabain, the K^+ content of slices was slightly, but significantly ($P < 0.01$), higher than when leached without ouabain. When these slices were incubated with 25 μM ouabain there was virtually no K^+ uptake (Table II). Even when incubated in ouabain-free medium they showed much less uptake than did those slices leached in ouabain-free medium and then incubated in ouabain-free medium.

Effect of pre-exposure to ouabain at low temperature

Since it appeared that pre-exposure of slices to ouabain at high temperature influenced its effectiveness at low temperature, it was of interest to see if the converse were also true. That is, would pre-exposure to ouabain at low temperature have any effect on K^+ uptake during subsequent incubation at high temperature? Slices were

leached without ouabain then incubated for 1 h at 5° with 25 μM ouabain; they were then transferred to flasks containing media prewarmed to 38° and were incubated at that temperature for a second hour with or without 25 μM ouabain. The results showed (Table III) that although, at 5° slices reaccumulated K^+ in 25 μM ouabain to a concentration of 161 μequiv/g dry wt. (from an initial concentration of 75 μequiv/g dry wt.), they lost most of this reaccumulated K^+ (to a concentration of 111 μequiv/g dry wt.) during a second hour of incubation at 38° even in a medium to which no ouabain had been added. In the 38° medium containing 25 μM ouabain, the loss of K^+ from the reincubated slices was still more profound (to a final tissue K^+ concentration of 66 μequiv/g dry wt.).

TABLE III

EFFECT OF PRE-EXPOSURE TO OUABAIN AT 5° FOLLOWED BY INCUBATION AT 38°

Slices of ground squirrel kidney cortex were leached without ouabain, incubated for 1 h at 5° with 25 μM then transferred (with or without washing in ouabain free medium) to 38° and incubated 1 h. Means ± S.E. are shown.

Conditions	*Number of cases*	*Tissue K^+ concn. (μequiv/g dry wt.)*
A. After leaching	8	75 ± 3
B. After 1 h at 5° + 25 μM ouabain	5	161 ± 3
C. After transfer to 38° without washing	8	111 ± 7
D. After transfer to 38° + 25 μM ouabain without washing	8	66 ± 4
E. After transfer to 38° (ouabain-free medium) with washing before transfer	5	146 ± 6

The somewhat surprising result obtained in slices incubated without ouabain during the second hour could have meant merely that the ouabain in the interstitial spaces of the slices became attached to its site of action before being diluted by fresh

TABLE IV

IRREVERSIBILITY OF OUABAIN INHIBITION AT 38°

Slices of ground squirrel kidney were leached without ouabain then incubated with and without ouabain 1 h at 38° then transferred to a fresh cup of medium with or without ouabain for a second hour. Slices were washed in ouabain-free medium between transfers. The tissue K^+ concentration of slices after leaching was 74 μequiv/g dry wt. and the values beneath represent the means of 3–5 cases.

Conditions				*Tissue K^+ concn. (μequiv/g dry wt.)*
Incubated 1 h at 38°	*Transferred to 2nd flask and incubated another hour*	*Ouabain concn.*		
		1st h	*2nd h*	
+	+	0	0	178
+	+	25	0	57
+	+	25	25	52
+	—	0	—	178

ouabain-free medium. To test this possibility, slices were washed in ouabain-free iced medium after removal from the medium at 5° and before transfer to ouabain-free medium at 38°. With this added procedure most of the inhibition disappeared, although the final tissue K^+ concentration of 146 μequiv/g dry wt. was still lower than one would have expected for a uninhibited slice incubated at this temperature (about 180 μequiv/g dry wt., *cf.* Table IV).

Reversibility of ouabain inhibition

The previous experiments showed that at 5° ouabain inhibition persisted in the absence of ouabain and that at 38° there was residual inhibition even after washing. As a further test of irreversibility and the effects of washing, without the complication of a changing temperature, slices were incubated at 38° for 1 h with 25 μM ouabain, washed in ouabain-free medium, then reincubated in fresh medium for another hour at 38° with and without 25 μM ouabain. The results (Table IV) indicated essentially no difference in K^+ content between slices which were exposed to ouabain during the second hour and those which were not. It appears that ouabain inhibition does persist after the removal of the drug from the medium and is relatively irreversible.

Hamsters

Dose-response to ouabain at 38°

Previous studies have indicated that hamster tissues are less sensitive to ouabain than are those of other mammals[6]. Examination of the effects of high concentrations of ouabain on K^+ and O_2 uptake at 38° in slices of kidney cortex of hamster (Fig. 4, Table V), shows that the dose-response of these activities resembles that of ground squirrel kidneys at 5°: At a concentration of 25 μM, ouabain had a slight but negligible effect, in 625 μM the final tissue K^+ concentration was no greater than the initial, leached value and at 3125 μM the final tissue K^+ concentration was below this level. The decline of O_2 consumption was parallel to that of K^+ uptake except that there was a slightly greater inhibition of respiration at 25 μM ouabain (19%) than of K^+ uptake (7%) (Fig. 4). As in ground squirrels at 38° about half the Na^+ extrusion was inhibitable by ouabain, but the relative inhibition caused by lower concentrations of ouabain was less for Na^+ extrusion than for K^+ uptake (Table V).

Effects of ouabain at 5°

Because the sensitivity to ouabain is so low at 38° it was of interest to determine whether it would drop still further at low temperature or whether the

TABLE V

TISSUE Na^+ CONCENTRATION OF KIDNEY CORTEX SLICES OF HAMSTERS TREATED WITH OUABAIN DURING INCUBATION

For procedure see Table I.

Temp. of incubation	*Number of cases*	*Tissue Na^+ concn. (μequiv/g dry wt.)*					
		After leaching at 38°	*After incubation*				
	Ouabain (μM):	*0*	*0*	*25*	*125*	*625*	*3125*
38°	6–17	609 ± 14	332 ± 6	329 ± 7	360 ± 6	438 ± 10	469 ± 16
5°	4	582	388	—	—	435	474

properties causing low affinity are maximal at 38°. The results of incubation of hamster kidney slices at 5° indicate (Fig. 5) that 625 μM ouabain had relatively little effect on K^+ uptake and that even in 3125 μM ouabain K^+ uptake was about one-third that occurring in uninhibited slices. Again, however, about half the extrusion of Na^+ was inhibited by 3125 μM ouabain (the decrease in tissue Na^+ during 2 h of incubation was 108 μequiv/g dry wt. in ouabain compared with 194 μequiv/g dry wt. in uninhibited slices, Table V).

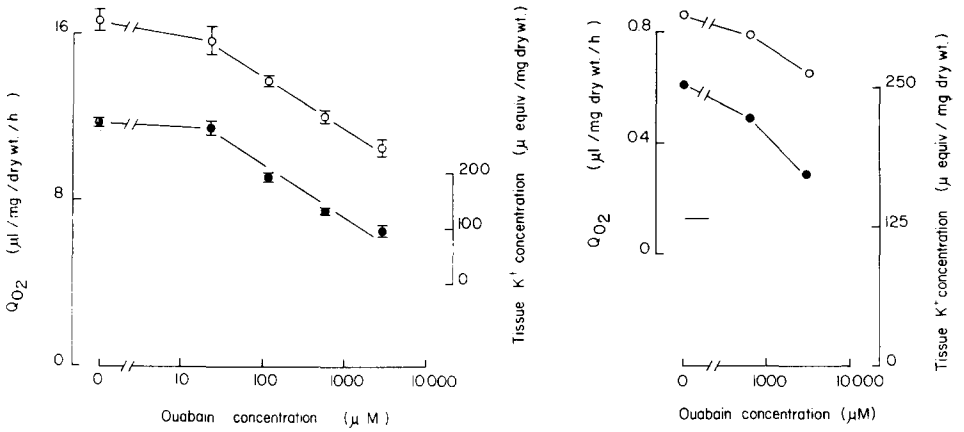

Fig. 4. Effect of ouabain in hamster renal cortical slices. Procedure same as in Fig. 1. ○, O_2 consumption; ●, final tissue K^+ concn. Tissue K^+ concn. (μequiv/g dry wt.) after leaching was 124 ± 6 (17 cases). Means ± S.E. are shown for 6–13 cases.

Fig. 5. Response of hamster kidney cortex to ouabain at 5°. Procedure same as Fig. 2. Horizontal line indicates concentration of K^+ in slices after leaching, before incubation (131 μequiv/g dry wt.; 5 cases). Means for four pairs of slices are shown.

DISCUSSION

The specificity of ouabain as an inhibitor of cation transport and its lack of direct effect on glycolysis and oxidative metabolism are well established[2,7]. As in earlier studies[1,2,8], therefore, the close correspondence between the inhibition of K^+ and Na^+ transport by ouabain and the accompanying reduction in O_2 consumption may be taken to indicate the existence of a regulatory effect of ion transport on cellular respiratory mechanisms in kidney cortex of both hamsters and ground squirrels. The persistence of this parallelism at 5° especially in ground squirrel kidney slices is an added confirmation that the relationship between K^+ transport and suprabasal O_2 consumption is not merely fortuitious. In addition it is a further indication that the nature of cation transport at that temperature in hibernator kidneys is normal except for the feature of cold resistance.

An observation peculiar to these studies was the partial reversal of ouabain effect at higher concentrations at 38°. Further experiments would be required to determine whether this represents a sort of 'substrate inhibition' at the active site or the interaction of two different effects of ouabain. In the latter category is the possibility that ouabain may reduce passive leakage of K^+ or Na^+. The finding (Table II) that slices leached in N_2 with ouabain lost significantly less K^+ than those leached

without may add some credence to this suggestion, but at the same time the hypothesis would not explain the reversal of the ouabain effect on O_2 consumption. It is worth remembering also that even slices of kidney cortex retain some degree of tissue organization so that a secondary effect of ouabain, such as swelling, might tend to reduce access of the inhibitor to the active site.

The decreased sensitivity of the tissues to ouabain at 5° was a striking result of this study (Fig. 3). The fact that this effect was supervened in ground squirrel kidneys by pretreatment with ouabain at 38° (Table II) strongly suggests that it is due to a diminished access or attachment of the inhibitor to its site of action. Since the subsequent observations indicated that the effects of ouabain are not easily reversed once the tissue has been exposed to ouabain (Tables III and IV), one would expect on the basis of that hypothesis to find that exposure of slices to 25 μM ouabain at 5° has the slight effect characteristic of that temperature when they are returned to 38° in ouabain-free medium. The results do in fact bear out this expectation, when precautions are taken against transferring excess ouabain with the slice. Thus, in Table II the minimal K^+ concentration is 66 μequiv/g dry wt., while the concentration of K^+ in slices incubated at 5° with 25 μM ouabain, then washed and incubated at 38° is 146 μequiv/g dry wt. The 'maximal' K^+ content of slices treated in a similar way but without ouabain is between 161 μequiv/g dry wt. (Table III) and 178 μequiv/g dry wt. (Table IV, tissues from largely the same animals as in Table III). The relative, residual effect of the ouabain is therefore between 15 and 29%, a range which is in reasonably good agreement with the 12% effect observed at 5° (Fig. 2). One interpretation of the parallel effect of ouabain on respiration and transport is that the drug acts by inhibiting the (Na^+-K^+)-activated ATPase. It will be of interest therefore to compare the inhibition of this enzyme with that of intact renal cortical cells in terms of the three peculiarities of ouabain action observed in this study: species specificity, reduced binding at low temperature, and reversal of effect at high concentration.

The effects of ouabain on Na^+ transport are an important consideration. The detailed investigation of KLEINZELLER AND KNOTKOVA[9] has indicated that a large fraction of Na^+ extrusion from kidney slices of rabbit kidney cortex treated in a manner comparable to those in this study is not inhibited by 0.3 mM ouabain. Partly on the basis of the observation that that concentration totally inhibited K^+ uptake the authors concluded that the excess removal of Na^+ (accompanied by water and Cl^- loss) was related to some mechanism other than ordinary cation transport, such as perhaps cellular contraction.

The independent findings of WHITTEMBURY[10] and of WILLIS[5], however, that about half of Na^+ transport in kidney slices occurs in the absence of K^+ raises two other possibilities. On the one hand, the 'ouabain-insensitive' fraction of Na^+ extrusion observed by KLEINZELLER AND KNOTKOVA may simply represent the activity of some other membrane mechanism not dependent upon the binding of K^+ to an external site and therefore not inhibitable by the attachment of ouabain to that site. Alternatively, the partial inhibition of Na^+ extrusion either by K^+ removal or by ouabain may be a reflection of the possibility that the active surfaces of the proximal tubular cells are located upon highly folded surfaces of these cells and are in contact with solutions which are somewhat separated from the bathing medium. Concentrations of K^+ and ouabain at the active sites might therefore be quite different from those prevailing in the external medium.

The results in Table I showing that incubation of kidney slices of ground squirrels with 25 μM ouabain inhibited only half the extrusion of Na^+ at 38° agree with those of KLEINZELLER AND KNOTKOVA in rabbit kidney slices at 25°. On the other hand, those authors found that with 1 mM ouabain a total inhibition of Na^+ extrusion could be achieved, whereas in the ground squirrel kidney at 38° the partial inhibition with 25 μM ouabain was also maximal (*i.e.* was greater than that in 3 mM ouabain).

A corollary of this difference in the two sets of findings is that at 38° in the ground squirrel kidney, reduction in Na^+ extrusion (168 μequiv/g dry wt.) was quantitatively the same as in K^+ uptake (161 μequiv/g dry wt.) with maximal ouabain inhibition (25 μM). This finding taken at face value would be in conformity with the notion that the ouabain was inhibiting only the K^+-activated Na^+ extrusion. At 5°, however, the results with ground squirrel kidney resembled those of KLEINZELLER AND KNOTKOVA in that at high concentrations of ouabain there was virtually no extrusion of Na^+. Such comparisons are limited by the fact that possible alterations in extracellular space have not been taken into account, which might influence the estimate of Na^+. In any case, a more comprehensive evaluation requires a comparison of effects of removal of K^+ from the medium and the effects of ouabain in K^+-free medium with those in this study.

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