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STUDIES ON A $Na^+ + K^+$-DEPENDENT, OUABAIN-SENSITIVE ADENOSINE TRIPHOSPHATASE IN THE AVIAN SALT GLAND

MABEL R. HOKIN

Department of Physiological Chemistry, University of Wisconsin, Madison, Wisc. (U.S.A.)

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SUMMARY

1. A $Na^+ + K^+$ — dependent, ouabain-sensitive ATPase was found in homogenates of the avian salt gland. Some of its properties have been investigated.

2. The levels of ouabain-sensitive and ouabain-insensitive ATPase activities were the same in homogenates of tissue from gulls maintained on fresh water and from gulls maintained on 1.5% salt water.

3. The levels of ouabain-sensitive and ouabain-insensitive ATPase activities were the same in homogenates of slices which had been incubated in the absence of acetylcholine and slices which had been incubated in the presence of acetylcholine. (Acetylcholine stimulates NaCl secretion in this tissue.)

4. The oxygen uptake in unstimulated salt-gland slices was reduced about 25% in the presence of 10^{-4} M ouabain. The increase in oxygen uptake which occurs in response to acetylcholine did not occur if ouabain was also present.

5. The level of 7-min acid-hydrolyzable phosphate esters in salt-gland slices fell in the presence of acetylcholine but this fall did not occur if ouabain was also present.

6. These results, and the relationship between the rates of Na^+ transport and ATPase activity in the salt gland, are discussed.

INTRODUCTION

Following on the observation by SKOU[1] of the presence of a $Na^+ + K^+$ — dependent ATPase in crab nerve, reports from several laboratories have shown the presence of a similar ATPase activity in many other tissues. Most of the $Na^+ + K^+$ — dependent ATPase activity is inhibited by relatively low concentrations of ouabain and other cardiac glycosides. This $Na^+ + K^+$ — dependent ATPase activity shows many of the same parameters as the Na^+ transport system, which has led to the suggestion that it is closely linked to the process of active Na^+ transport. Part of the present report deals with some properties of a $Na^+ + K^+$ — dependent, ouabain-sensitive ATPase which has been found in homogenates of the avain salt gland. This gland is an organ which excretes NaCl in high concentration; this enables aquatic birds to obtain their water from hypertonic saline solutions. In the gull, the glands secrete a 0.7–0.8 M solution of NaCl in response to salt loading; 0.04 M KCl is also present in the secre-

tion[2]. Secretory activity is under the control of the parasympathetic nervous system and the glands secrete NaCl in response to stimulation by acetylcholine or other cholinergic agents[3]; ouabain blocks the secretion[4]. In the present work, the levels of ouabain-sensitive and ouabain-insensitive ATPase activity in non-secreting and secreting glands were compared. The effects of ouabain on oxygen uptake and on ATP levels in salt-gland slices have also been studied. The relationship of the rate of Na^+ transport to the rates of ATPase activity in the salt gland is discussed.

METHODS

Animals

Domestic geese were given 1.5% NaCl as their drinking water for one week before sacrifice.

Gulls were captured in spring, 3–4 weeks after they had hatched, in the Great Lakes region. They were given canned dog food (whale meat) and canned cat food (fish) to eat and either fresh water or 1.5% NaCl, as indicated in the Tables, as their drinking water. They were killed in the autumn and winter following their capture.

Preparation of salt-gland homogenates and cytoplasmic particulate fractions

The birds were killed by decapitation and the salt glands were removed and placed in 0.31 M sucrose at 0°. In both geese and gulls, the weight of the glands averaged about 600 mg wet wt. per bird. The glands were trimmed of connective tissue and were then sliced with a Stadie-Riggs slicer. The slices were homogenized in 0.31 M sucrose in batches of not more than 2 ml volume with not more than 150 mg of tissue/ml. The homogenization was carried out in an all-glass conical Potter-Elvejem homogenizer and the homogenization time was held to 1 min or less. The preparation at this stage, made up to a suitable volume in 0.31 M sucrose, is refered to as the whole homogenate.

To prepare cytoplasmic particulate fractions from goose salt gland, the whole homogenate, made up to give a tissue: volume ratio of 1 g fresh weight in 15 ml, was centrifuged at 500 × *g* for 5 min. The residue was discarded. The supernatant fluid was usually stored at 0° overnight. It was then centrifuged at 20 000 × *g* for 15 min; this procedure brought down almost all of the particulate material—both mitochondria and microsomes. The supernatant fluid was water-clear; it was removed by aspiration and was discarded. The residue was resuspended in 0.31 M sucrose with very brief homogenization and was made up to the starting volume of the whole homogenate. If necessary, it was poured through a small amount of glass wool to remove any lumps. These preparations contained about 2.5 mg of protein/ml, which represents about 40 mg of protein/g fresh weight of tissue. The whole homogenate contained about 100 mg of protein/g of fresh tissue. (Protein was determined by the method of LOWRY *et al.*[5]). The preparations remained stable with respect to ATPase activity for several days when kept at 0°.

Assay of ATPase activity

The composition of the incubation medium in each experiment is given in the Tables and Figures.

The incubations were carried out in glass tubes, with shaking, in a water bath. The reaction was stopped by the addition of trichloroacetic acid and the mixtures were centrifuged. Inorganic phosphate in the supernatant fluid was determined by the method of FISKE AND SUBBARROW[6]. All values are corrected for the amount of inorganic phosphate found in unincubated samples. The assays were always carried out in duplicate and the values given are the averages of at least two estimations, which agreed closely.

RESULTS

$Na^+ + K^+$ — dependent and ouabain-sensitive ATPase activities in cytoplasmic particulate fractions from goose salt gland

In the experiments shown in Table I, cytoplasmic particulate fractions from the goose salt gland in the absence of added $Na^+ + K^+$ showed no ouabain-sensitive ATPase activity. Addition of Na^+ gave about a 50% increase in ATPase activity and

TABLE I

THE DEPENDENCE ON $Na^+ + K^+$ AND THE OUABAIN-SENSITIVITY OF SALT GLAND ATPASE ACTIVITY

Cytoplasmic particulate fractions were prepared from goose salt glands as described in the text. The incubation medium contained, in mmoles/l, the following: Tris–chloride buffer (pH 7.4), 8; $MgSO_4$, 0.8; Tris–ATP, 0.13; sucrose, 126. NaCl and KCl were added as indicated; when either NaCl, KCl, or both of these were omitted sucrose was added in their place. The tissue protein concentration was 0.03 mg/ml in Expt. 1 and 0.07 mg/ml in Expt. 2. The incubation was carried out at 38° for 5 min with shaking. 3 ml of reaction mixture were incubated in each tube; the reaction was stopped by the addition of 0.5 ml of 50% trichloroacetic acid.

Additions			*P_i liberated (mμmoles/mg protein/min)*	
Na^+ (70 mM)	*K^+ (10 mM)*	*Ouabain (10^{-4} M)*	*Exp. 1*	*Expt. 2*
—	—	—	108	98
—	—	+	86	98
+	—	—	170	144
+	—	+	134	125
—	+	—	120	102
—	+	+	118	100
+	+	—	210	202
+	+	+	158	134

half of this increase was abolished by ouabain. K^+ gave little or no increase in ATPase activity. $Na^+ + K^+$ together doubled the ATPase activity; about 60% of the increase was abolished by ouabain. In view of the fact that these preparations were not washed exhaustively, it is possible that the effect with Na^+ alone might be dependent on traces of K^+ left in the preparations. In most of the work presented below, $Na^+ + K^+$ were added to all the vessels and the ouabain-sensitive and ouabaininsensitive ATPase activity was measured.

TABLE II

THE EFFECT OF Mg^{2+} CONCENTRATION ON ATPASE ACTIVITIES

The ATP concentration was 0.2 mM; it was added as the Tris salt. The tissue protein concentration was 0.17 mg/ml. Other concentrations, in mmoles/l were: Tris–chloride buffer (pH 7.4), 10; sucrose, 91. Added as indicated were: NaCl, 85; KCl, 10; or sucrose in their place. Other conditions of incubation were as in Fig. 1.

Mg^{2+} concentration (mM)	*P_i liberated (mμmoles/mg protein/min)*					
	–	*Na^+*	*K^+*	*$Na^+ + K^+$*	*$Na^+ + K^+$ + Ouabain (10^{-4} M)*	*Ouabain-sensitive activity*
0	43			62		
0.1	140	104	121	182	94	88
0.2	140	140	153	232	141	91
0.6	138	179	157	262	167	95
1.2	138	171	132	270	144	126

Table II shows that most of the ATPase activity in these preparations is dependent on the addition of Mg^{2+}. The lowest concentration of Mg^{2+} (0.1 mM) gave a maximum effect on ATPase activity in the absence of monovalent cations; 0.6 mM Mg^{2+} gave a maximum effect in the presence of $Na^+ + K^+$. In this experiment almost all of the increase in ATPase activity observed on addition of $Na^+ + K^+$ was abolished by ouabain. There appeared to be some competiton between the two types of ATPase activities at the lowest Mg^{2+} concentration - the ouabain-insensitive activity was lower in the presence of $Na^+ + K^+$ with 0.1 mM Mg^{2+} than the activity in the absence of $Na^+ + K^+$, which would suggest that the ouabain-insensitive activity had been suppressed under these conditions, probably by competition for the substrate.

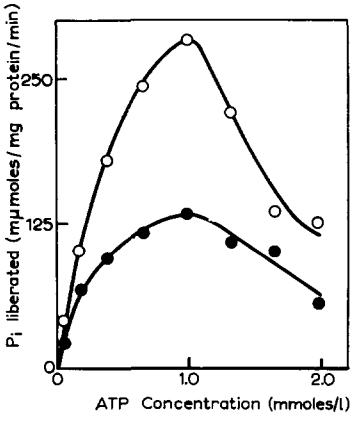

Fig. 1. ATPase activity with different concentrations of ATP. A cytoplasmic particulate fraction of goose salt gland, which contained 0.17 mg protein/ml of final incubation mixture, was incubated in a medium which contained, in mmoles/l, the following: Tris–chloride buffer (pH 7.4), 10; $MgSO_4$, 1; NaCl, 55; KCl, 7; sucrose, 150; and ATP as shown; alternate vessels contained 10^{-4} M ouabain. The incubation volume was 1.5 ml and the samples were incubated with shaking for 5 min at 38°. The reaction was stopped by the addition of 1 ml of 12.5% trichloroacetic acid. ○—○ the ouabain-insensitive ATPase activity; ●—● the ouabain-sensitive ATPase activity.

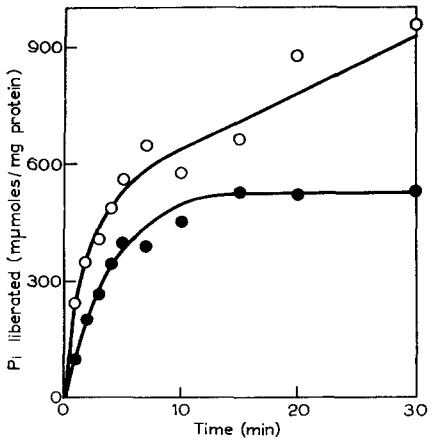

Fig. 2. Time course of ATPase activity in the presence of 0.2 mM ATP. Other conditions of incubation were as in Fig. 1. ○—○, the ouabain-insensitive ATPase activity; ●—●, the ouabain-sensitive ATPase activity.

The concentration of ATP which gave the maximum total ATPase activity was about 1 mmole/l; however, both lower and higher concentrations of ATP gave a higher ratio of ouabain-sensitive to ouabain-insensitive activity (Fig. 1). In the presence of 0.2 mM ATP. the ouabain-sensitive and the ouabain-insensitive ATPase activities were linear over the first 5 min of incubation time (Fig. 2); an incubation time of 5 min was routinely used in all the assays. The fall-off in ATPase activity at

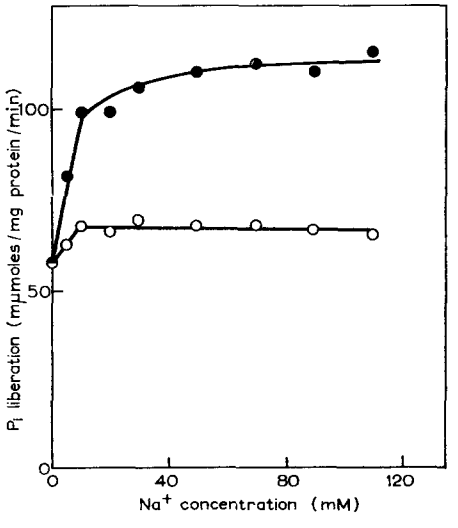

Fig. 3. The effect of Na^+ concentration on ATPase activity. The concentration of ATP was 0.2 mM and of KCl was 6 mM. By combining 0.155 M NaCl and 0.31 M sucrose in the required proportions the medium was kept isotonic in each sample. Other conditions were as in Fig. 1. ●—●, the total ATPase activity; ○—○, the ATPase activity in the presence of 10^{-4} M ouabain.

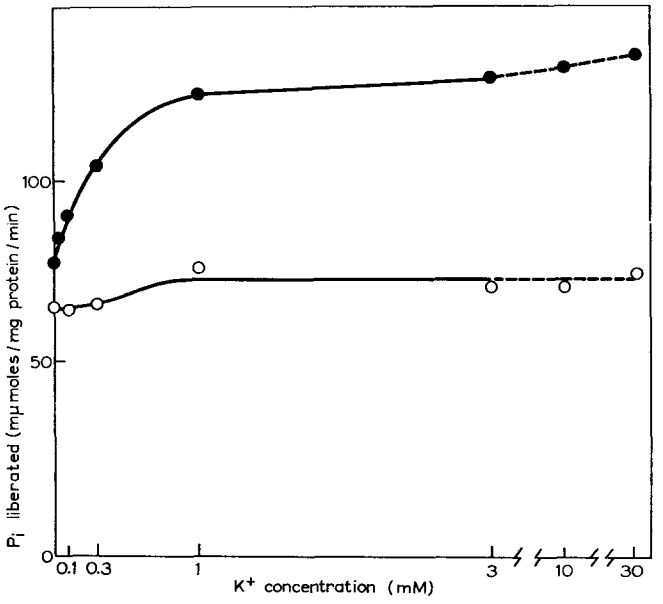

Fig. 4. The effect of K^+ concentration on ATPase activity. The concentrations, in mmoles/l were: Tris–chloride buffer (pH 7.4), 10; ATP, 0.6; $MgSO_4$, 1; NaCl, 70. By combining 0.155 M KCl and 0.31 M sucrose in the required proportions the medium was kept isotonic in each sample. Other conditions were as is Fig. 1. ●—●, the total ATPase activity; ○—○, show the ATPase activity in the presence of 10^{-4} M ouabain.

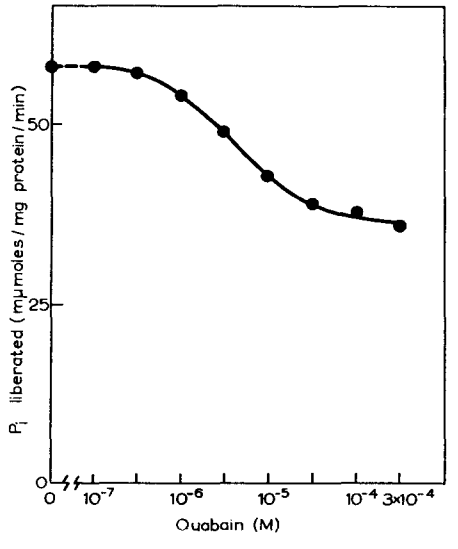

Fig. 5. The inhibition of ATPase activity by different concentrations of ouabain. The concentrations of other components of the assay system, in mmoles/l, were: Tris–chloride buffer (pH 7.4), 10; $MgSO_4$, 1; ATP, 0.13; NaCl, 55; KCl, 7; sucrose, 160. Other conditions were as in Fig. 1.

TABLE III

THE SUBSTRATE SPECIFICITY OF THE OUABAIN-SENSITIVE ATPase

In Expt. 1, a cytoplasmic particulate fraction from goose salt gland (0.17 mg protein/ml) was incubated in a medium which contained, in mmoles/l, the following: Tris–chloride buffer (pH 7.4), 9; $MgSO_4$, 0.9; sucrose, 151; NaCl, 60; KCl, 7 and, where indicated, CTP, 0.2; GTP, 0.2; or ATP, 0.2. The incubation was carried out at 38° for 5 min. 1.5-ml aliquots were used and the reaction was stopped by the addition of 1 ml of 12.5% trichloroacetic acid. In Expt. 2, a whole homogenate of gull salt gland (0.22 mg protein/ml) was incubated in a medium which contained, in mmoles/l, the following: Tris–chloride buffer (pH 7.4), 14; $MgSO_4$, 1.2; NaCl, 119; KCl, 12; sucrose, 21 and, where indicated, AMP, 1; ADP, 1 or ATP, 1. Other conditions were as in Expt. 1.

Expt. (No.)	*Additions*	*P_i liberated (mμmoles/mg protein/min)*	
		Control	*+ Ouabain (10^{-4} M)*
1	None	3	1
	CTP	58	28
	GTP	42	41
	ATP	246	102
2	AMP	0	0
	ADP	30	16
	ATP	292	128

later times in Fig. 2 may be due to the fact that a high proportion of the total ATP added is broken down under these conditions.

In the presence of 6 mM KCl, concentrations of Na^+ between 30 mM and 120 mM gave the same ATPase activity (Fig. 3). In the presence of 70 mM NaCl, concentrations of K^+ between 1 mM and 30 mM gave the same ATPase activity (Fig. 4).

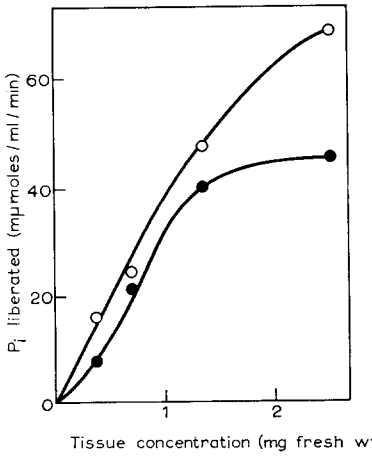

Fig. 6. The effect of tissue concentration on ATPase activity in whole homogenates of gull salt gland. Aliquots of the homogenate were incubated in an assay medium which contained, in mmoles/l, the following: Tris–chloride buffer (pH 7.4), 14; $MgSO_4$, 1.2; NaCl, 119; KCl, 12, sucrose, 20; ATP, 1. The incubation was carried out at 38° for 5 min. The ouabain concentration was 10^{-4} M. ●—●, the ouabain-sensitive ATPase activity; ○—○, the ouabain-insensitive ATPase activity.

The concentration of ouabain required to achieve half-maximal inhibition was about $5 \cdot 10^{-6}$ M; $3 \cdot 10^{-5}$ M gave near-maximum inhibition (Fig. 5).

The liberation of inorganic phosphate from ATP was much greater than the liberation of inorganic phosphate from equivalent amounts of CTP, GTP, AMP, or ADP (Table III).

ATPase activity in gull salt-gland homogenates

The ouabain-sensitive ATPase activity in whole homogenates of gull salt gland was quite sensitive to tissue concentration under these assay conditions (Fig. 6).

The levels of ATPase in homogenates of salt glands from gulls which had been raised on salt water and from gulls which had been raised on fresh water were compared. Assays were carried out at two different concentrations of tissue. No differences in either the ouabain-sensitive or the ouabain-insensitive ATPase activities were found between these two groups of gulls (Table IV).

TABLE IV

THE LEVELS OF ATPASE ACTIVITY IN SALT GLANDS FROM GULLS MAINTAINED ON FRESH WATER AND ON SALT WATER

Gulls were watered from the age of about 1 month for about 6 months with either fresh water or 1.5% NaCl, as indicated. The animals were killed by decapitation and the salt glands were removed. They were then sliced and homogenized in 0.31 M sucrose. Aliquots of the whole homogenate were used. The concentration of ATP was 1 mM; other conditions were as in Expt. 2 of Table II.

Gull number	*Dietary water*	*Amount of tissue per assay (mg fresh weight/ml)*	*P_i liberated (mμmoles/mg fresh tissue/min)*			
			Ouabain sensitive		*Ouabain insensitive*	
1	Salt	0.66	10.5		6.8	
2	Salt	0.66	31.5	18.8	35.0	24.5
3	Salt	0.66	14.4		31.6	
4	Fresh	0.66	16.7		13.0	
5	Fresh	0.66	23.0	19.9	29.2	22.8
6	Fresh	0.66	20.1		26.3	
1	Salt	1.93	35.0		26.7	
2	Salt	1.33	33.3	32.4	32.7	31.2
3	Salt	1.33	28.9		34.2	
4	Fresh	1.93	32.9		25.2	
5	Fresh	1.33	35.7	30.4	31.8	29.0
6	Fresh	1.33	22.6		30.0	

The ATPase levels in homogenates from slices which had been incubated in the presence and absence of acetylcholine (which stimulates NaCl secretion in the salt gland) were compared with each other and with the ATPase activity in homogenates of unincubated tissue (Table V). In each of the two experiments shown in this Table the total ATPase activity was not affected by prior incubation of the slices; however, the amount of ouabain-sensitive ATPase activity was reduced and the amount of ouabain-insensitive ATPase activity was increased, by a proportionate amount, after

TABLE V

ATPASE ACTIVITY IN HOMOGENATES OF SALT-GLAND SLICES PREVIOUSLY INCUBATED WITHOUT AND WITH ACETYLCHOLINE PLUS ESERINE

Gulls were watered as is Table IV. Slices were incubated in Krebs–Henseleit bicarbonate saline[7] with added glucose (1 mg/ml) and with O_2–CO_2 (95:5) as the gas phase at 38° for 60 min before the addition of acetylcholine ($3 \cdot 10^{-6}$ M) plus eserine ($3 \cdot 10^{-5}$ M) and for 20 min after this addition. Homogenates from unincubated and incubated tissue were prepared as described in the text. Tissue which had been incubated with acetylcholine plus eserine was homogenized in sucrose to which acetylcholine ($3 \cdot 10^{-6}$ M) and eserine ($3 \cdot 10^{-5}$ M) had been added; the same concentrations of acetylcholine and eserine were also present in the assay of ATPase activity in these slices. The tissue concentration for ATPase assay was 2 mg fresh wt./ml; the ATP concentration was 1 mM and the ouabain concentration was 10^{-4} M. Other conditions were as in Expt. 2 of Table II.

Expt. (No.)	*Treatment of animal*	*Treatment of tissue before homogenization*	*P_i liberated (mμmoles/mg fresh tissue/min)*		
			Ouabain-sensitive	*Ouabain-insensitive*	*Total*
1	Gull maintained on salt water	Unincubated	35.0	26.7	61.7
		Incubated	26.6	38.1	64.7
		Incubated with acetylcholine plus eserine	29.2	34.5	63.7
2	Gull maintained on fresh water	Unincubated	32.9	25.2	58.1
		Incubated	22.8	36.5	59.3
		Incubated with acetylcholine plus eserine	23.1	37.7	60.8

TABLE VI

THE EFFECT OF OUABAIN ON OXYGEN UPTAKE IN GULL SALT-GLAND SLICES

Gull salt-gland slices were incubated at 38° in Medium III of KREBS[9] with added glucose (1 mg/ml) and with O_2 as the gas phase in Warburg vessels. Ouabain was added at the beginning of the incubation and acetylcholine (plus 10^{-4} M eserine) was tipped in after 40 min. The values given are for the average rate during 45 min after tipping. All values are the averages of duplicates which showed good agreement.

Additions					*Oxygen uptake (mμmoles/mg fresh weight/min)*		
Acetylcholine		*Ouabain*					
(10^{-6} M)	*(10^{-5} M)*	*(10^{-6} M)*	*(10^{-5} M)*	*(10^{-4} M)*	*Total*	*Increase with acetylcholine*	*Decrease with ouabain*
−	−	−	−	−	1.6		
−	−	+	−	−	1.5		0.1
−	−	−	+	−	1.5		0.1
−	−	−	−	+	1.2		0.4
+	−	−	−	−	2.6	1.0	
+	−	−	+	−	1.7	0.2	0.9
−	+	−	−	−	2.5	0.9	
−	+	+	−	−	2.6	1.1	−0.1
−	+	−	+	−	2.6	1.1	−0.1
−	+	−	−	+	1.4	0.2	1.2

incubation of the slices. There was no effect of incubation with acetylcholine on the ATPase activities.

The effect of ouabain on oxygen uptake in gull salt-gland slices

The oxygen uptake in unstimulated gull salt-gland slices was reduced about 25% by 10^{-4} M ouabain; 10^{-6} M and 10^{-5} M ouabain had little or no effect on the unstimulated oxygen uptake. Acetylcholine stimulates oxygen uptake in salt-gland slices[8]; this stimulated oxygen uptake was much more sensitive to ouabain. The increase in oxygen uptake which occured in the presence of 10^{-6} M acetylcholine did not occur if 10^{-5} M ouabain was also present; the increase in oxygen uptake with 10^{-5} M acetylcholine was not much affected by 10^{-6} M or 10^{-5} M ouabain but was almost completely inhibited by 10^{-4} M ouabain. These results are shown in Table VI. WHITTAM[10] has previously reported that ouabain reduces the rate of O_2 uptake in slices of rabbit brain and kidney.

The effect of ouabain on ATP levels

Table VII shows that the level of 7-min acid-hydrolysable phosphate esters in goose salt-gland slices was much lower than the controls when the slices were exposed to 10^{-6} M acetylcholine but was maintained at the control level if 10^{-4} M ouabain

TABLE VII

THE EFFECT OF ACETYLCHOLINE AND OF OUABAIN ON THE LEVEL OF 7-MIN ACID-HYDROLYSABLE PHOSPHATE ESTERS IN SALT-GLAND SLICES

Goose salt-gland slices were incubated as in Table V except that acetylcholine and ouabain were both added after 60 min of incubation; the incubation was continued for a further 20 min. Eserine (10^{-4} M) was added with the acetylcholine. The level of Norite adsorbable, 7-min acid-hydrolysable phosphate esters was determined as described by CRANE AND LIPMANN[11].

Additions	*7-min acid-hydrolysable phosphate esters (μmoles P/g fresh weight)*
None	1.9
Ouabain (10^{-4} M)	1.7
Acetylcholine (10^{-6} M)	0.9
Acetylcholine (10^{-6} M) + Ouabain (10^{-4} M)	1.7

was also present; in the absence of acetylcholine, ouabain had no significant effect on the level of 7-min acid-hydrolyzable phosphate esters. Most of the 7-min acid-hydrolyzable phosphate is in the ATP:ADP system; the fall from 1.9 to 0.9 μmoles/g of tissue suggests that, in the presence of acetylcholine, the ATP is converted to ADP as soon as it is made and that virtually all of the 7-min acid-hydrolyzable phosphate is present as ADP under these conditions. Such a change in the steady-state ratio of ATP:ADP could account for the increase in respiration observed in the presence of acetylcholine since the rate of respiration is governed by the concentration of ADP.

The change in ratio of ATP:ADP is presumably due to the increased utilization of ATP for the active transport initiated by acetylcholine. The maintenance of the 7-min acid-hydrolyzable phosphate level at the control level when ouabain is added with the acetylcholine indicates that the ATPase activity initiated by acetylcholine is ouabain-sensitive. The fact that the increase in O_2 uptake in response to acetylcholine does not occur in the presence of ouabain can be accounted for by the lack of change in the ATP:ADP ratio under these conditions.

DISCUSSION

After establishing the presence of a $Na^+ + K^+$ dependent, ouabain-sensitive ATPase in the salt gland, one of the aims of the present work was to determine the relationship between the ATPase activity as measured in homogenates and the rates of Na^+ transport and O_2 uptake in the intact tissue. The question arose as to what proportion of the total ATPase activity might be involved in Na^+ transport. GLYNN[12] and JARNEFELDT[13] have both pointed out that there is no real justification for assuming that only the $Na^+ + K^+ -$ dependent ATPase activity (as measured under laboratory conditions) is associated with ion transport.

Data for ATPase activity in homogenates and for O_2 uptake in slices of gull salt gland have been presented above, and data are available from FANGE *et al.*[3] on the rates of transport in stimulated gull salt gland *in vivo*. These data, together with the data available concerning these activities in the dog kidney, where the relationship between O_2 uptake and Na^+ transport has been measured *in vivo*[14], are summarized in Table VIII. Assuming a P:O ratio of 3 for ATP formation, the ratio of Na^+ transported to ATP used in the dog kidney is about 4.6. If the salt gland has the same ratio of Na^+: ATP as the dog kidney, the ouabain-sensitive ATPase activity in

TABLE VIII

THE RELATIONSHIP BETWEEN ATPASE ACTIVITY AND Na^+ TRANSPORT

Tissue	*Activity*	*Rate of activity (μmoles/g fresh weight/min*	*Source of data*
Dog kidney	Na^+ transport *in vivo*	128	LASSEN *et al.*[14]
Dog kidney	Basal O_2 uptake *in vivo*	1.0 = 6 ATP	LASSEN *et al.*[14]
Dog kidney	Total working O_2 uptake *in vivo*	5.6 = 34 ATP	LASSEN *et al.*[14]
	Total ATP from working O_2 uptake *minus* ATP from basal O_2 uptake = 34 − 6 =	28 ATP	
	Ratio 128 Na^+ : 28 ATP = 4.6 Na^+ : 1 ATP		
Gull salt gland	Na^+ transport *in vivo*	260 (range 210–320)	FANGE *et al.*[3]
Gull salt gland	Ouabain-insensitive O_2 uptake in unstimulated slices	1.2 = 7 ATP	Table VI, above
Gull salt gland	Ouabain-sensitive ATPase activity in homogenates	30 ATP	Table V, above
	Ratio 260 Na^+ : 30 ATP = 8.7 Na^+ : 1 ATP		
Gull salt gland	Total ATPase activity in homogenates	62 ATP	Table V, above
	Total ATP hydrolyzed minus ATP from resting O_2 = 62 − 7 =	55 ATP	
	Ratio 260 Na^+ : 55 ATP = 4.7 Na^+ : 1 ATP		
	The P : O ratio for ATP formation was assumed to be 3		

homogenates is insufficient to account for the rate of transport. It is possible that the assay conditions do not give a correct measure of all of the ouabain-sensitive ATPase present; alternatively, some of the ouabain-sensitive ATPase may be completely inactivated during the homogenization and assay. However, the calculations in Table VIII show that the total ATPase activity is sufficient to account for the rate of transport. The basal O_2 uptake in the salt gland would give about 7 μmoles ATP/g/min; this is presumably not associated with Na^+ transport. Subtraction of this figure from the total amount of ATP which is hydrolyzed per gram of tissue in the homogenate in this time gives an ATP utilization of 55 μmoles/g/min, of which 30 μmoles is ouabain-sensitive. Na^+ transport in the gull salt gland averages 260 μmoles/g/min (see ref. 3) so that, using only the ouabain-sensitive ATPase activity, the Na^+ : ATP ratio would be 8.7, which is much higher than in the kidney; however, using the ratio of 55 μmoles of ATP to 260 μmoles of Na^+, the Na^+ : ATP ratio is 4.7, which is very close to the ratio found in the kidney, based on the relationship between O_2 uptake and Na^+ transport in that tissue.

The $Na^+ + K^+$ — dependent ATPase activity in dog kidney, as measured by BONTING *et al.*[15], also is too small to account for the O_2 uptake associated with Na^+ transport in the dog kidney. These authors found a $Na^+ + K^+$ — dependent ATPase activity of 5–8 μmoles ATP/g/min whereas the O_2 uptake associated with Na^+ transport in the experiments of LASSEN *et al.*[14] would give 28 μmoles ATP/g/min. The total ATPase activity observed by BONTING *et al.*[15] was 16–22 μmoles ATP/g/min, so that in dog-kidney homogenates also, the total ATPase activity appears to more nearly approach the rate required for Na^+ transport than does the $Na^+ + K^+$ — dependent ATPase activity.

Since Na^+ transport can be completely abolished be ouabain, it is likely that all of the ATPase activity associated with Na^+ transport is ouabain-sensitive and $Na^+ + K^+$ — dependent in the intact cell. However, it is possible that the laboratory procedures used in making preparations for assay of ATPase activity may produce structural changes which result in the uncoupling of some of the ATPase from its ouabain-sensitivity and from its $Na^+ + K^+$ — requirement. There are other suggestions that such an uncoupling may happen. The data in Table V suggest that if slices of salt gland are incubated before homogenization, some of the ATPase which is ouabain-sensitive in the homogenate of the unincubated slice is converted to ouabain-insensitive ATPase. Also, the data in Tables I and II show that in different preparations a different proportion of $Na^+ + K^+$ — dependent activity is inhibited by ouabain; possibly in some preparations some of the ATPase has lost ouabain sensitivity but not $Na^+ + K^+$ — dependence. Other ATPase activity may have lost both of these properties.

Both unstimulated and stimulated salt-gland tissues show the same level of ATPase activity. The ATPase activity in homogenates of non-secreting tissue is of the magnitude which would be expected in the active, secreting gland (see Table VIII). Prior to homogenization, there is more utilization of ATP for Na^+ transport in the stimulated than in the unstimulated glands. It would seem therefore that the rate of breakdown of ATP which is measured in homogenates of unstimulated tissue is not the rate which was occuring in the cells *in vivo* at the time of sacrifice or in the unstimulated slices, but is the rate of breakdown of which the tissue is capable if stimulated. It seems likely therefore that the process of homogenization and incu-

bation involved in the assay activates the ATPase in the tissue. Much of this activity is presumably suppressed in the intact cells of the resting gland and is normally activated in response to acetylcholine when the tissue is stimulated to secretory activity.

The ouabain-insensitive O_2 uptake in salt-gland slices is approximately the same as the basal O_2 uptake in the dog kidney *in vivo* (see Table VIII). There is no reason therefore to suppose that this ouabain-insensitive O_2 uptake in salt gland slices does not reflect the basal rate of O_2 uptake in the salt gland *in vivo*. In stimulated slices, however, there is some discrepancy. If the Na^+ transported : O_2 consumed ratio is the same in the salt gland as in the dog kidney, and if the stimulated slices secrete Na^+ at the same rate as the stimulated gland *in vivo*, then there would be a requirement for an extra 9 μmoles O_2/g/min to cope with the stimulated rate of secretion. However, when the slices are stimulated maximally with acetylcholine, the observed increase is only about 1 μmole/g/min. A possible explanation for this discrepancy might be that the availability of O_2 under these incubation conditions was only enough to give this amount of increase – the data in Table VII indicate that the stimulated slices use up the ATP as soon as it is formed, which would support the idea that the availability of O_2 may be the limiting factor. Alternatively, or in addition to this, it is not unlikely that the sliced, incubated tissue loses some of its responsiveness and that the actual amount of NaCl which can be secreted under these conditions is much less than the maximum amount which can be secreted *in vivo*.

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