Biochimica et Biophysica Acta, 330 (1973) 173–185

BBA 76488

STUDIES ON (Na^+–K^+)-ACTIVATED ATPase XXXII.

OCCURRENCE AND PROPERTIES OF (Na^+–K^+)-ATPase IN IMMATURE, LACTATING AND INVOLUTED GUINEA PIG MAMMARY GLAND

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(Received July 31st, 1973)

SUMMARY

In homogenates of immature, lactating and involuted mammary gland tissue of the guinea pig the presence of (Na^+–K^+)-ATPase activity (ATP phosphohydrolase, EC 3.6.1.3) has been established in activities of 0.09, 0.23 and 0.20 mole/kg dry weight per h, respectively.

The properties of the enzyme have been determined in a microsomal fraction of the lactating mammary gland tissue. Half-maximal activities are 1.6 mM K^+, 8 mM Na^+ and 1 mM Mg^{2+}, respectively. Optimal activities are obtained at 7.8 and 2 mM ATP and K^+, Na^+ and Mg^{2+} concentrations of 4 mM 40 mM and 4 mM, respectively. The (Na^+–K^+)-ATPase activity is totally inhibited by 10^{-4} M ouabain, while 50% inhibition occurs with $2.5 \cdot 10^{-6}$ M ouabain.

In the immature, lactating and involuted gland the DNA, phospholipid and protein contents have been determined on a dry weight basis. All three parameters are lowest in the immature gland due to the presence of a large amount of fat tissue. During involution the DNA content increases by 60%, while the phospholipid content and to a lesser extent the protein content decrease, indicating cell shrinkage and loss of membrane material.

The (Na^+–K^+)-ATPase activity, expressed on a DNA basis (proportional to activity per cell) is the same in the immature and in the lactating gland. It is 43% lower in the involuted gland, indicating a lower cation pump activity in the resting cell. The Mg^{2+}-ATPase activity on DNA basis is highest in the lactating gland, and about double that in the immature and involuted gland.

Total phospholipids and individual phospholipids, excluding phosphatidylserine and sphingomyelin, decrease by 60–75% upon involution, indicating a decrease in mitochondrial membrane content and little change in the plasma membrane content of the cell.

Mg^{2+}-ATPase activity, which is present in plasma membranes and mitochondrial membranes, is about the same in all three states when expressed on a total phospholipid basis. During involution (Na^+–K^+)-ATPase activity, which is present in plasma membranes only, decreases to the same extent when expressed on a DNA basis as on a phosphatidylserine or sphingomyelin basis.

INTRODUCTION

The presence of a Na^+- and K^+-stimulated ATPase [ATP phosphohydrolase, EC 3.6.1.3, (Na^+–K^+)-ATPase] activity and its function as a cation pump system have been demonstrated in a great number of tissues of different species[1]. Its role has been shown in various secretory processes, as the exocrine pancreatic secretion of fluid and electrolytes[2], the secretion of aqueous humor[3], the secretion of cerebrospinal fluid[4] and gastric acid secretion[5].

There are some indications that it might also play a role in the secretory function of the mammary gland. In milk of all mammals investigated the Na^+ concentration is lower and the K^+ concentration is higher than in plasma. During the late period of lactation there is an increase in Na^+ and a decrease in K^+ concentration in the milk of several species[6]. Mastitis also causes a rise in milk Na^+ and a fall in K^+ (ref. 7). These observations suggest that active cation transport plays a role in the process of milk secretion. The presence of the Na^+–K^+-activated ATPase system in the lactating mammary gland has, however, not yet been demonstrated by biochemical assay. On the basis of a histochemical staining technique Kinura[8] has claimed its presence in the mammary gland cell, but the reliability of the histochemical method is subject to serious doubt (ref. 1, p. 276).

In the present study the presence of (Na^+–K^+)-ATPase activity and its properties have been investigated in lactating mammary glands of the guinea pig by biochemical methods. The (Na^+–K^+)-ATPase activity has also been determined in the immature and involuted gland, since in these conditions the gland is functionally less active. In view of the morphological changes occurring in these states, the (Na^+–K^+)-ATPase activity has not only been compared on the basis of dry weight, but also on the basis of the DNA, protein and phospholipid contents of the mammary tissue in the various morphological states.

MATERIALS AND METHODS

Chemical reagents

Adenosine 5′-triphosphate disodium salt (ATP), Boehringer, Mannheim (Germany). Ouabain (strophanthin g), E. Merck AG, Darmstadt (Germany). Bovine serum albumin, Sigma Chemical Company, St. Louis, Mo. (U.S.A.). Deoxyribonucleic acid-sodium salt (DNA) type III from salmon, Sigma Chemical Co. Silica gel H.R., Merck Darmstadt (Germany).

Tris–ATP was prepared by converting the disodium salt of ATP into the Tris salt by ion-exchange chromatography on Amberlite IR120 (Tris form).
All other reagents were of analytical grade.

Animals

Female albino guinea pigs (Central Institute for breeding of laboratory animals, T.N.O. Zeist, the Netherlands) were killed by cervical dislocation and the mammary glands were removed within 5 min and homogenized. Immature glands were taken from adult, female guinea pigs, which had never been pregnant. Lactating mammary glands were obtained from suckling guinea pigs, taken from their litter 5–10 days after giving birth. Involuted mammary glands were obtained from suckling guinea

pigs, which had been taken from their litters 15 days after giving birth and had been kept isolated for 20 days.

Tissue preparation

Aqueous homogenates, usually 10% (w/v), were prepared with double-distilled water at 0–4 °C in Potter–Elvehjem glass tissue grinders. Aliquots of 0.5 ml of this homogenate were shell-frozen and lyophilized at −20 °C and stored at this temperature *in vacuo*.

A microsomal fraction of the lactating mammary gland tissue was prepared by centrifuging tissue homogenates in 0.3 M sucrose for 10 min at $10000 \times g$ in a Sorvall RC 2B centrifuge. The supernatant was centrifuged for 1 h at $144000 \times g$ in a Spinco L 50 ultracentrifuge. The resulting pellet was washed three times with twice-distilled water. This pellet was shell-frozen and lyophilized at −20 °C and stored at this temperature *in vacuo*.

Determinations

The ATPase assay method, described by Bonting (ref. 1, p. 260), was used with the substrate media listed in Table I.

The protein content of the lyophilized homogenates was determined according to the method of Lowry *et al.*[9], using bovine serum albumin as standard. The DNA content of lyophilized homogenates was determined by means of the diphenylamine reaction according to Schneider[10].

Lipid extraction was carried out according to Folch *et al.*[11], except that a chloroform–methanol–water mixture (60:30:35, by vol.) was used in order to replace the water, previously removed through lyophilization. The total lipid extract was washed with 0.2 vol. 0.1 M KCl and concentrated by evaporation under N_2. The concentrated extract was dissolved in 0.2 ml benzene–ethanol (4:1, by vol.). This solution was used for phospholipid analysis by thin-layer chromatography and for determination of total lipid phosphate content.

Phospholipid analysis was carried out by two-dimensional thin-layer chromatography, according to the method of Broekhuyse[12].

TABLE I

COMPOSITION OF SUBSTRATE MEDIA

All concentrations are expressed in mmoles/l. The only other anion present is Cl^-.

	Medium				
	A	*B*	*C*	*D*	*E*
ATP*	2	2	2	2	2
Mg^{2+}	4	4	4	4	4
K^+	4	—	4	4	—
Na^+	50	54	—	50	54
EDTA	0.1	0.1	0.1	0.1	0.1
Tris buffer (pH 7.5)	100	100	100	100	100
Ouabain	—	—	—	0.1	0.1

* Media A, B, D and E the disodium salt was used, but in Medium C Tris–ATP was used.

The phosphate content of the total lipid extract and of the chromatographic spots was determined by means of a modified Fiske–SubbaRow method[12]. The phospholipid content was calculated from phosphate content by multiplying with 750/31. For the phospholipid analysis the sum of the phosphate contents of the phospholipid spots was set at 100%.

RESULTS

Presence of (Na^+–K^+)-ATPase activity

ATPase activities in guinea pig lactating mammary gland homogenates were determined in the Media A (complete), B (no K^+), C (no Na^+), D ($+10^{-4}$ M ouabain) and E (no K^+ + 10^{-4} M ouabain). Table II (Column 2) shows that there is no significant difference between the activities in Media B–E, which may thus be taken to represent the Mg^{2+}-ATPase activity. The additional activity in Medium A by definition should represent the (Na^+–K^+)-ATPase activity. In further experiments the (Na^+–K^+)-ATPase activity has been determined as the difference between the activities in Media A and E, unless otherwise indicated.

TABLE II

ATPase ACTIVITY OF THE LACTATING MAMMARY GLAND OF THE GUINEA PIG

Mean values with standard errors of the mean; *n* represents number of assayed samples of the same gland in upper part of the table, and number of assayed mammary glands in lower part of the table.

Medium	*Whole-homogenate relative activity* (%)	*Microsomal-fraction relative activity* (%)
A (complete)	100	100
B (no K^+)	79.0 ± 2.9 ($n=10$)	59.3 ± 1.9 ($n=8$)
C (no Na^+)	72.1 ± 3.5 ($n=8$)	57.0 ± 3.2 ($n=8$)
D (10^{-4} M ouabain)	77.5 ± 2.5 ($n=10$)	62.2 ± 1.1 ($n=8$)
E (no K^+, 10^{-4} M ouabain)	77.9 ± 3.4 ($n=9$)	57.3 ± 2.1 ($n=21$)
Average B–E	76.6 ± 1.5	59.0 ± 1.2
	Absolute activity (moles/kg dry weight per h)	*Absolute activity (moles/kg dry weight per h)*
Mg^{2+}-ATPase	1.23 ± 0.09 ($n=6$)	1.29 ± 0.07 ($n=4$)
(Na^+–K^+)-ATPase	0.23 ± 0.02 ($n=6$)	1.28 ± 0.22 ($n=4$)

The average (Na^+–K^+)-ATPase activity in homogenates of several glands amounts to 0.23 MKH which is 15.8 (± 1.8)% of the total ATPase activity at pH 7.5. Since this low relative activity makes it rather difficult to determine the properties of this enzyme in homogenates, a microsomal fraction was prepared in which the absolute and the relative (Na^+–K^+)-ATPase activity were increased to 1.28 MKH and 50%, respectively (Table II, Column 3).

Properties of (Na^+–K^+)-ATPase

The effect of the K^+ concentration on the (Na^+–K^+)-ATPase activity is shown

in Fig. 1. The ATPase activity was measured in Medium B, to which KCl was added in final concentrations ranging from 0–10 mM, while the Na^+ concentration was kept constant at 54 mM. The activity in Medium E was deducted. Optimal activity was obtained at a concentration of 4 mM K^+ and half-maximal activation at 1.6 mM K^+. This value falls within the range of 0.4–1.8 mM observed in a variety of animal tissues (ref. 1, p. 267).

Fig. 2 shows the effect of the Na^+ concentration on the (Na^+–K^+)-ATPase activity. The enzyme activity was measured in Medium C, to which NaCl was added

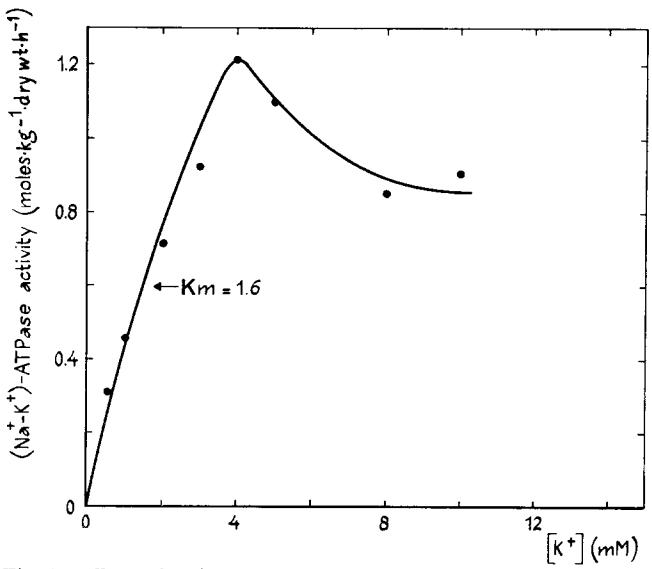

Fig. 1. Effect of K^+ concentration on (Na^+–K^+)-ATPase activity in microsomal fractions of lactating mammary gland of the guinea pig. 50% activation of the enzyme was found at 1.6 mM K^+.

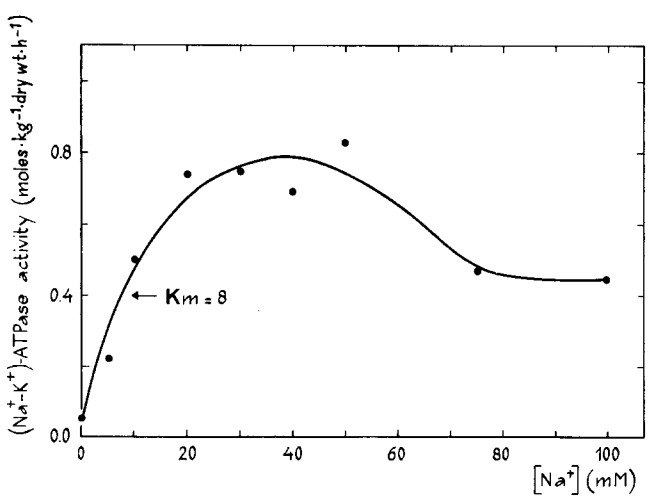

Fig. 2. Effect of Na^+ concentration on (Na^+–K^+)-ATPase activity in microsomal fractions of lactating mammary gland of the guinea pig. At 8.0 mM Na^+, 50% activation of the enzyme was found.

in final concentrations ranging from 0 to 100 mM, with the K^+ concentration kept constant at 4 mM. Optimal activity is reached at 40 mM Na^+ and half-maximal activation at 8 mM Na^+. The latter value also falls within the range of 4.5–12.5 mM observed for a variety of animal tissues (ref. 1, p. 267).

The effect of Mg^{2+} concentration on the activities of both Mg^{2+}-ATPase and (Na^+–K^+)-ATPase was determined by measuring total activity in Medium A with $MgCl_2$ added in final concentrations from 0 to 6 mM (ATP level 2 mM in all cases), while Mg^{2+}-ATPase activity was measured in Medium E with $MgCl_2$ again added in final concentrations from 0 to 6 mM. The (Na^+–K^+)-ATPase activity was calculated as the difference between the activities in Media A and E. For (Na^+–K^+)-ATPase an optimum at 4 mM Mg^{2+} and for Mg^{2+}-ATPase an optimum at 2.5 mM Mg^{2+} was obtained. An activity of 50% was found for (Na^+–K^+)-ATPase at 1.0 mM Mg^{2+} and for Mg^{2+}-ATPase at 0.5 Mg^{2+} (Fig. 3). Hence, the optimal Mg^{2+}/ATP ratio for (Na^+–K^+)-ATPase activity was about 2, which is at the upper level of the range observed in a variety of animal tissues (ref. 1, p. 267).

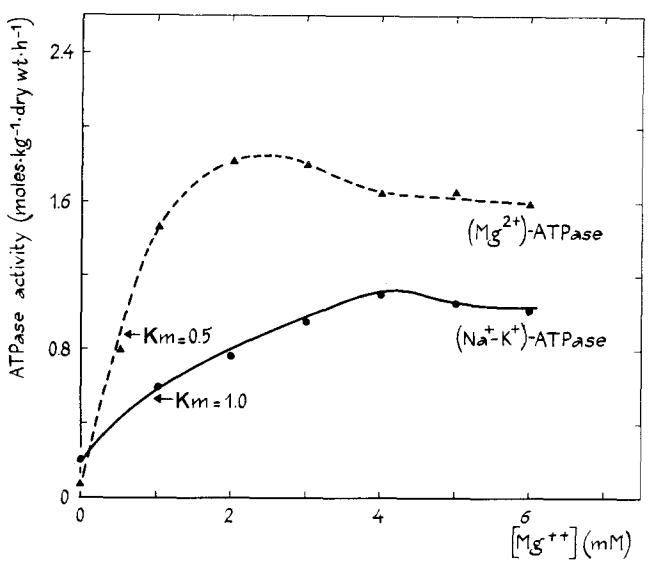

Fig. 3. Effect of Mg^{2+} concentration on the activities of Mg^{2+}-ATPase (▲---▲) and (Na^+–K^+)-ATPase (●—●) in microsomal fractions of lactating mammary gland of the guinea pig. 50% activation of Mg^{2+}-ATPase was found at 0.5 mM Mg^{2+} and of (Na^+–K^+)-ATPase at 1.0 mM.

Fig. 4 shows the effect of the pH of the incubation medium on the two enzyme activities. For the pH range from 6.0–7.5 a Tris–Histidine-HCl buffer (50 mM each) and for the range from 7.5–9.3 Tris–HCl buffer (100 mM) was used. ATPase activity was measured at each pH in Media A and E. The Mg^{2+}-ATPase activity was calculated from the activity in Medium E and the (Na^+–K^+)-ATPase activity from the difference between the activities in Media A and E. An optimum for Mg^{2+}-ATPase was found at pH 9.0, for (Na^+–K^+)-ATPase at pH 7.8. Both pH optima are on the high side of the range of pH optima for both enzymes (7.0–8.0 and 8.4–8.9) observed in a variety of animal tissues (ref. 1, p. 267).

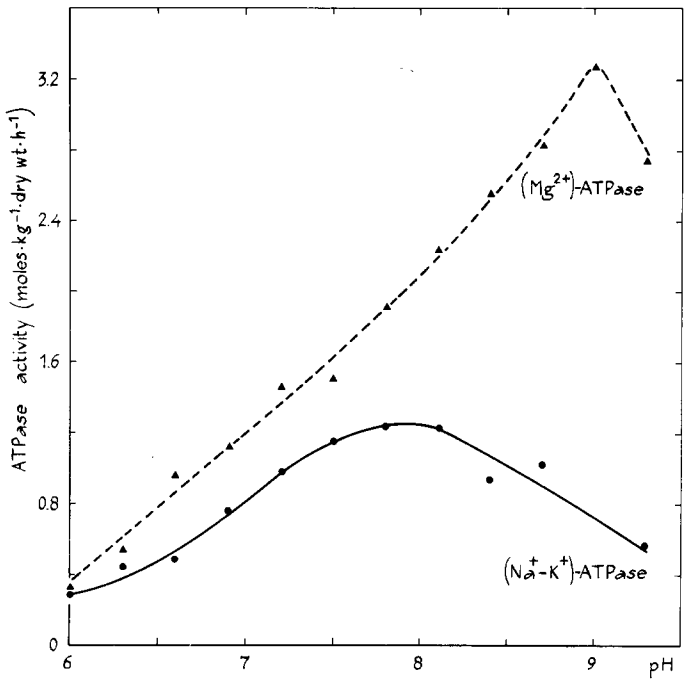

Fig. 4. Effect of pH on Mg^{2+}-ATPase activity (▲---▲) and (Na^+–K^+)-ATPase activity (●—●) in microsomal fractions of the lactating mammary gland of the guinea pig.

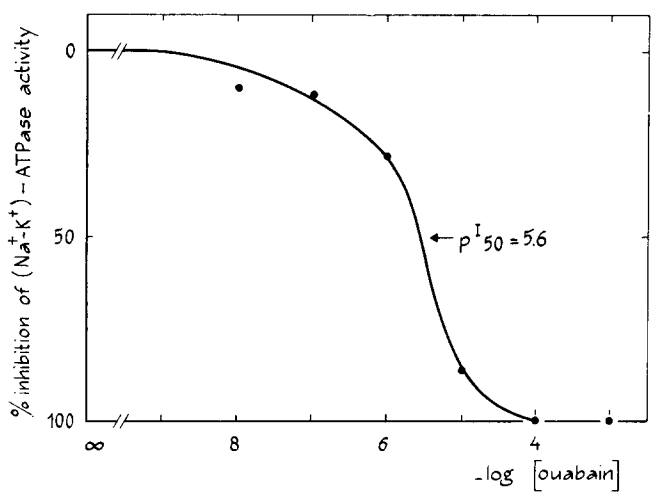

Fig. 5. Effect of ouabain on (Na^+–K^+)-ATPase activity in microsomal fractions of the lactating mammary gland of the guinea pig. The pI_{50} value (5.6) is the negative logarithm of the ouabain concentration giving 50% inhibition.

Fig. 5 shows the inhibitory effect of ouabain on the (Na^+–K^+)-ATPase activity. Ouabain was added to Medium A in final concentrations ranging from 10^{-8} to 10^{-3} M. The inhibition is expressed as a percentage of the uninhibited (Na^+–K^+)-ATPase activity, which was calculated from the difference between the activities in

Media A and E. Half-maximal inhibition occurs at $2.5 \cdot 10^{-6}$ M ouabain ($pI_{50} = 5.6$) and complete inhibition at 10^{-4} M ouabain. The pI_{50} value falls within the range of 3.9–6.8 observed for a variety of animal tissues (ref. 1, p. 267).

TABLE III

SEVERAL COMPONENTS IN HOMOGENATES OF GUINEA PIG MAMMARY GLANDS

Mean values with standard errors of the mean. In parentheses number of assayed mammary glands. ATPase activities are expressed in moles ATP hydrolysed/h per kg dry weight, or per kg DNA, or per kg protein, or per kg phospholipid (PL). The other components are expressed as g per 100 g dry weight.

	Immature gland	*Lactating gland*	*Involuted gland*
Mg^{2+}-ATPase	0.27 ± 0.06 (8)	1.23 ± 0.09 (6)	0.75 ± 0.07 (8)
$(Na^+–K^+)$-ATPase	0.09 ± 0.02 (8)	0.23 ± 0.02 (6)	0.20 ± 0.03 (8)
Protein	16.5 ± 3.4 (10)	52.5 ± 3.4 (6)	46.8 ± 3.6 (7)
DNA	0.97 ± 0.28 (6)	2.3 ± 0.23 (5)	3.6 ± 0.19 (6)
Phospholipids	1.8 ± 0.36 (7)	8.8 ± 1.0 (5)	5.0 ± 0.36 (6)
Mg^{2+}-ATPase/DNA	28 ± 10	53 ± 6.5	21 ± 2.3
$(Na^+–K^+)$-ATPase/DNA	9 ± 3.4	10 ± 1.3	5.6 ± 0.90
Mg^{2+}-ATPase/protein	1.6 ± 0.50	2.3 ± 0.23	1.6 ± 0.19
$(Na^+–K^+)$-ATPase/protein	0.55 ± 0.17	0.44 ± 0.05	0.43 ± 0.07
Mg^{2+}-ATPase/PL	15 ± 4.4	14.1 ± 1.9	15 ± 1.8
$(Na^+–K^+)$-ATPase/PL	5 ± 1.5	2.6 ± 0.37	4 ± 0.67

Activity in various functional states

The ATPase activities were also determined in the immature and the involuted gland. Since change of functional state is accompanied by morphological changes, it is not sufficient to determine the enzyme activities on a dry weight basis only. Therefore, protein, DNA and phospholipid contents have also been determined.

Table III presents the ATPase activities and these chemical parameters on a dry weight basis in the three functional states. Both ATPase activities on dry weight basis are significantly lower in the immature gland, while in the involuted gland only the Mg^{2+}-ATPase activity has decreased significantly.

There is no significant difference between the protein content of the lactating and involuted mammary gland, while in the immature gland the protein content is only 30% of these values. The DNA content is 52% higher in the involuted gland and 57% lower in the immature gland than in the lactating gland. The total phospholipid content is highest in the lactating gland, as is also observed for the Mg^{2+}-ATPase activity.

Expressed on the basis of DNA, protein and phospholipid content, the ATPase activities show striking differences in the three functionally different states of the mammary gland (Table III, Fig. 6). On a DNA basis, Mg^{2+}-ATPase activities are the same in the immature and the involuted gland, while in the lactating gland this activity is much higher. $(Na^+–K^+)$-ATPase activity on a DNA basis in the involuted gland has decreased to 50% of its activity in the lactating gland, while in the immature gland its activity is the same as in the lactating state. Expressed on a protein basis the Mg^{2+}-ATPase activity is also significantly higher in the lactating gland,

while the (Na^+–K^+)-ATPase activities are not different in the three functional states. Expressing the activities on a phospholipid basis leads to a remarkable result. While the Mg^{2+}-ATPase activity is the same in all three states, the (Na^+–K^+)-ATPase activity in the lactating gland is about 60% of the activities in the immature and involuted gland.

The phospholipid composition is given in Table IV, both in percent of total phospholipids and in percent of dry weight. The most striking differences are the high phosphatidylserine content in the involuted gland, the low phosphatidylinositol content in the immature gland and the low sphingomyelin content in the lactating gland, when expressed in percent of total phospholipids. Expressed in percent of dry weight, all phospholipid components are very low in the immature gland, while in the involuted gland compared with the lactating gland there is a decrease in phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and cardiolipin, but an increase in phosphatidylserine and sphingomyelin.

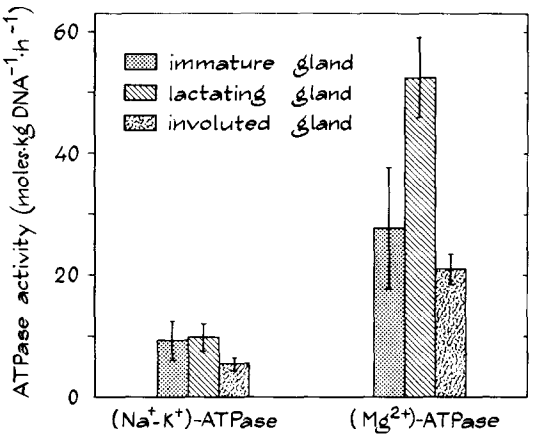

Fig. 6. (Na^+–K^+)-ATPase and Mg^{2+}-ATPase activities in immature, lactating and involuted mammary gland, expressed as moles splitted ATP/kg DNA per h.

TABLE IV

PHOSPHOLIPID COMPOSITION OF GUINEA PIG MAMMARY GLAND HOMOGENATES

Mean values with standard errors of the mean. Figures in parentheses, number of assayed glands.

	Percentage of total phospholipid			*g per 100 g dry weight*		
	Immature gland (4)	*Lactating gland (5)*	*Involuted gland (6)*	*Immature gland (4)*	*Lactating gland (5)*	*Involuted gland (6)*
Phosphatidylcholine	50.3 ± 6.4	50.5 ± 1.7	43.2 ± 3.2	0.92 ± 0.22	4.46 ± 0.54	2.14 ± 0.22
Phosphatidylethanolamine	29.4 ± 1.8	32.7 ± 1.0	25.3 ± 0.8	0.54 ± 0.11	2.89 ± 0.34	1.25 ± 0.10
Phosphatidylserine	4.4 ± 1.3	2.9 ± 0.8	8.3 ± 0.4	0.08 ± 0.03	0.26 ± 0.08	0.41 ± 0.04
Phosphatidylinositol	3.5 ± 1.3	7.7 ± 1.0	8.3 ± 2.2	0.06 ± 0.03	0.68 ± 0.12	0.41 ± 0.11
Sphingomyelin	9.0 ± 2.1	4.2 ± 0.7	13.6 ± 0.7	0.16 ± 0.05	0.37 ± 0.08	0.67 ± 0.06
Cardiolipin	3.4 ± 1.4	2.0 ± 1.3	1.3 ± 0.8	0.06 ± 0.03	0.18 ± 0.12	0.07 ± 0.04

DISCUSSION

The presence of the (Na^+-K^+)-ATPase system in the lactating mammary gland of the guinea pig has been established. The activity of 0.23 mole ATP hydrolyzed/kg dry weight per h is comparable to that found in other secreting tissues[1-4], and represents about 16% of the total Mg^{2+}-activated ATPase activity.

The properties of the (Na^+-K^+)-ATPase system in the lactating mammary gland of the guinea pig are similar to those of the enzyme system in other tissues and species[1]. All parameters fall within the ranges found in a variety of animal tissues. Half-maximal activities are 1.6 mM K^+, 8 mM Na^+ and 1 mM Mg^{2+}, respectively. Optimal activities are obtained at pH 7.8 and 2 mM ATP and K^+, Na^+ and Mg^{2+} concentrations of 4 mM, 40 mM and 4 mM, respectively. The (Na^+-K^+)-ATPase activity is totally inhibited by 10^{-4} M ouabain, while 50% inhibition occurs with $2.5 \cdot 10^{-6}$ M ouabain.

There is no significant difference between (Na^+-K^+)-ATPase activity on a dry weight basis in the lactating gland (0.23 ± 0.02 MKH) and the involuted gland (0.20 ± 0.03 MKH), but in the immature gland it is only 50% of this activity. However, considering the morphological change during pregnancy and, in the reverse direction, during involution it is not rational to compare the (Na^+-K^+)-ATPase activity of the mammary gland in the three different states of activity on a dry weight basis only. Therefore, other components have been determined.

The DNA content of the mammary gland represents the number of cells, because the DNA content per nucleus in this tissue does not change during pregnancy, lactation and involution[13]. Hence, the higher DNA content per g dry weight in the involuted gland relative to that in the lactating gland indicates cell shrinkage during involution. During the reverse process in the period of pregnancy and particularly at the time of parturition there is a fall in the number of alveoli per unit area and a corresponding rise in alveolar diameter, while the number of nuclei in the average alveolus remains relatively constant. This indicates an increase in cell volume during evolution of the mammary gland cell and during lactation, in agreement with electron microscopic investigations[14]. Thus ATPase activities in the mammary gland expressed on a DNA basis are proportional to ATPase activities per mammary gland cell.

Both ATPase activities expressed on DNA basis decrease upon involution of the gland: 60% for Mg^{2+}-ATPase and 43% for (Na^+-K^+)-ATPase (Table III, Fig.6). This suggests that the secreting mammary gland cell requires a higher cation pump activity than the resting cell. On the other hand, the (Na^+-K^+)-ATPase activity on a DNA basis in the immature gland is the same as that in the lactating gland, while the Mg^{2+}-ATPase activity is only 53% of that in the lactating gland.

The protein content on a dry weight basis does not change during involution, while in the immature gland it is only 30% of the protein content in the lactating gland. This result is understandable, since the bulk of immature mammary gland tissue is fatty tissue. It should, however, be noted that the protein content per g DNA in the involuted gland (13.0 g/g DNA) is 43% lower than in the lactating gland (22.4 g/g DNA), while in the immature gland (17.0 g/g DNA) it is 25% lower, suggesting a loss of cellular protein during involution and an increase during pregnancy. Results of electron microscopic investigations of the mammary gland cells during pregnancy and secretion[15] support this conclusion. During involution there may

also be an increase in connective tissue protein. Hence, expressing ATPase activities on a protein basis is not useful, because the protein content in the mammary gland does not represent the same tissue component in the several states of activity.

The total phospholipid content of 8.8 g/100 g dry weight, observed in the lactating guinea pig mammary gland is about the same value as found in the lactating gland of the sow[16] and the cow[17]. In the involuted gland the total phospholipid content is 43% less and in the immature gland 80% less than in the lactating gland. The analysis of the phospholipid composition per g DNA in the mammary gland during the three states permits the following observations (Fig. 7). Total phospholipid, phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and cardiolipin per cell decreased by 65, 72, 59, 69 and 75%, respectively, during involution. However, phosphatidylserine and sphingomyelin contents per cell do not change. The same phospholipid composition, at least not significantly different, is found in the immature gland. The loss of total phospholipid content and of the four components during involution are roughly of the same size. These four components are the major components of the mitochondrial and microsomal membranes. In mitochondria of guinea pig liver[18] and beef heart[19] no phosphatidylserine and sphingomyelin are found, while phosphatidylcholine and phosphatidylethanolamine are present in roughly equal amounts and comprise about 76–78% of total phospholipid and cardiolipin (20%) and phosphatidylinositol (3–5%) are the only other phospholipids. The percentage of phosphatidylserine and sphingomyelin in microsomes differs considerably for different organs[18]. Our phospholipid analysis suggests

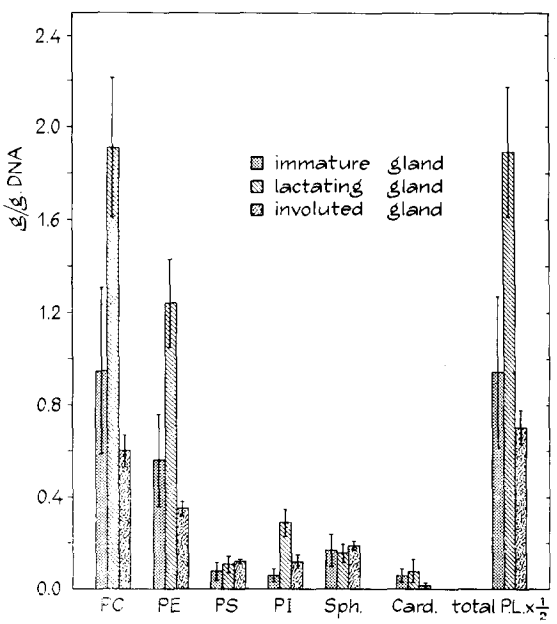

Fig. 7. Content of total phospholipid (PL) and individual phospholipid components in immature, lactating and involuted mammary glands, respectively, expressed as g phospholipid per g DNA. PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; Sph, sphingomyelin; Card., cardiolipin.

that there is a major loss of intracellular membranes, particularly of mitochondria and endoplasmic reticulum during mammary gland involution, but a relatively minor decrease in plasma membrane material.

When Mg^{2+}-ATPase activity is expressed on a total phospholipid basis no difference is found between the three states of the mammary gland (Table III). The loss of 60% of Mg^{2+}-ATPase activity, when expressed on a DNA basis (Table III, Fig. 6) and its constancy on a total phospholipid basis are in agreement with our conclusion that there is a major loss of mitochondrial membranes, since Mg^{2+}-ATPase is present in mitochondria as well as in plasma membrane. (Na^+–K^+)-ATPase activity, expressed on a total phospholipid basis, increases during involution and the activity in the involuted gland is the same as found in the immature gland. This result is understandable, since (Na^+–K^+)-ATPase is a plasma membrane enzyme, while the phospholipids represent all membranes. When (Na^+–K^+)-ATPase is expressed on a phosphatidylserine or sphingomyelin basis, which phospholipids occur primarily in plasma membranes, a loss of 52% is found after involution of the gland. This loss is about equal to the loss of (Na^+–K^+)-ATPase activity expressed on DNA basis. Similarly, there is no significant difference between (Na^+–K^+)-ATPase activity on sphingomyelin basis in immature and lactating mammary gland, as is also the case for the (Na^+–K^+)-ATPase activity on a DNA basis.

Thus, it can be concluded that a comparison of (Na^+–K^+)-ATPase activities in the mammary gland in the three different states is only meaningful, when the activities are expressed on the basis of cell number (DNA basis), or of plasma membrane content (sphingomyelin basis). It then becomes clear that (Na^+–K^+)-ATPase activity is about equal in the immature and the lactating mammary gland cell, while the cell of the involuted gland has only about half of this activity. Moreover, the lactating cell has more intracellular organelles than the immature and involuted cell[15], which agrees with the conclusion drawn from the phopholipid composition on DNA basis and indicates that the metabolic activity of the lactating cell is higher than that in the other two states.

Linzell and Peaker[2] (suggest that during lactation (Na^+dK^+)-ATPase is absent from the apical cell membrane, but that it would be present there in the involuted state. The decrease in (Na^+–K^+)-ATPase activity on a DNA basis, which we find during involution, does not support their hypothesis, since this would require an even larger decrease in activity in the basal and lateral membrane simultaneously with an increase in the apical membrane. While our biochemical investigations definitely show the presence of the enzyme in the mammary gland, knowledge of the localization of the enzyme and its exact role in the process of milk secretion requires further investigation.

ACKNOWLEDGEMENT

The excellent technical assistance of Miss Yvonne Lie is gratefully acknowledged.

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