Biochimica et Biophysica Acta, 601 (1980) 229—234

BBA Report

BBA 71480

THE INDUCTION OF (Na^++K^+)-ATPase IN THE SALT GLAND OF THE DUCK

RUSSELL B. LINGHAM, DAVID J. STEWART and AMAR K. SEN

Department of Pharmacology, University of Toronto, Toronto, Ontario M5S 1A8 (Canada)

(Received February 29th, 1980)

Key words: (Na^++K^+)-ATPase; Salt adaptation; Salt gland; Induction; (Duck)

Summary

Hypertonic saline was administered to ducks for 24 h. Protein synthesis was measured in salt-gland slices by a double-isotope technique. Salt-gland slices from saline-treated animals incorporated more radioactivity than those from control animals. Sodium dodecyl sulphate polyacrylamide gel electrophoresis of proteins in the heavy microsomal fraction of the salt glands revealed that the greatest radioactive amino acid incorporation occurred in the catalytic subunit of (Na^++K^+)-ATPase. The results support the tenet that saline treatment increases (Na^++K^+)-ATPase synthesis.

Marine birds can survive on a regimen of saline drinking water by excreting excess electrolytes through the nasal salt glands [1—9]. Although the domestic duck is not a marine animal it does have functional salt glands. The salt glands of control animals are small and appear to be inactive whilst saline-treated ducks possess large actively secreting glands.

A large body of evidence exists indicating that saline administration to ducks for an extended period of time results in an increase in salt-gland size, (Na^++K^+)-ATPase (EC 3.6.1.3) activity, as well as membrane differentiation [2, 3, 5, 7, 11, 12]. These changes are preceded by increases in RNA and polyribosomal content [10]. In 1976, Stewart et al. [12] observed that the salt gland content of the catalytic subunit of (Na^++K^+)-ATPase increased commensurately with the total activity of (Na^++K^+)-ATPase after adaptation of ducks to salt water.

The increased glandular content of (Na^++K^+)-ATPase can be due to ei-

ther a reduction in the rate of degradation of existing protein or an increase in the rate of synthesis of new protein. In the present study, we examined the rate of synthesis of $(Na^+ + K^+)$-ATPase by measuring the incorporation of radioactive leucine into the subunits of $(Na^+ + K^+)$-ATPase in salt-gland slices.

Male Pekind ducks, 7 weeks old, were obtained from a local supplier and maintained on tap water and an ad libitum supply of Purina duck chow for 3 days before the start of the experiment.

At time zero, ducks were stomach-loaded with 100 ml of a solution containing 284 mM NaCl, 6 mM KCl. The drinking water was removed and replaced with the above solution. The ducks were stomach-loaded again 8 h later with the same volume of saline solution. Control animals were treated as above except that the ducks received fresh water instead of saline. The saline-treated animals were killed 24 h after the first saline load and the nasal salt glands rapidly dissected out and placed in Krebs-Ringer bicarbonate buffer, pH 7.4, on ice oxygenated with 95% O_2 /5% CO_2 . Adhering connective tissue was removed and the salt glands were sliced free-hand using a frosted-glass slide and a hand-held razor blade. The slices were approx. 0.3 mm thick. Salt-gland slices were transferred to a flask containing 5.0 ml of the above buffer and incubated for 10 min at 37° C while being oxygenated. The reaction was initiated by the addition of 5 μCi of [^{3}H]leucine and allowed to proceed for 2 h. The glands from control animals were treated in the same manner except that slices were incubated with 5 μCi of [^{14}C]leucine. In some experiments, the control slices received [^{3}H]leucine and the slices from salt-treated ducks received [^{14}C]leucine.

At the end of the incubation period, the incubating medium was poured off and the slices frozen in liquid nitrogen. The slices were thawed and washed four times with ice-cold unlabelled 1.0 mM leucine in 0.9% NaCl. The slices were blotted dry, weighed and homogenized in 19 vols. (w/v) of a solution containing 250 mM sucrose, 20 mM Tris-HCl, 1.0 mM Na_2EDTA, pH 7.4, using a conical ground-glass homogenizer at 0° C.

Equal volumes of each crude homogenate were mixed and the combined homogenates were centrifuged at 5100 × *g* for 10 min. The supernatant was carefully decanted and placed on ice. The pellet was resuspended in the Tris/sucrose buffer and centrifuged as before. The supernatants were combined and centrifuged at 48 000 × *g* for 60 min. The 48 000 × *g* pellet was resuspended in the above solution and an aliquot was subjected to electrophoresis on 7.5% SDS-polyacrylamide gels as described by Stewart et al. [12]. The absorbance of the stained protein bands was monitored by scanning the gels in a Gilford Spectrophotometer at 560 nm. The gel rods were then sliced into 2-mm discs and solubilized prior to counting in a Nuclear Chicago Mark II Liquid Scintillation Spectrophotometer. Radioactivity was measured by the external standard ratio method.

The ouabain-sensitive $(Na^+ + K^+)$-ATPase activity was assayed as described by Post and Sen [13] with the exception that the reaction was terminated after 5.0 min. Protein content was determined as described by Lowry et al. [14] using bovine serum albumin as a standard. Oxygen consumption of salt-gland slices was measured by the procedure described elsewhere [15].

[^{3}H] Leucine (spec. act. 129 Ci/mmol) and [^{14}C]leucine (spec. act. 290

TABLE I

THE EFFECT OF 24 h OF SALINE TREATMENT ON THE SIZE AND THE $(Na^+ + K^+)$-ATPase ACTIVITY OF NASAL SALT GLANDS

Group	Wet weight of glands (mg)	$(Na^+ + K^+)$-ATPase activity[c] (μmol P_i/mg protein per h)
Control	394.0 ± 63[a] (11)	10.6 ± 5.0[b] (4)
Saline-treated	593.0 ± 121[d] (11)	18.6 ± 8.0[e] (4)

[a]Values are mean ± S.D.
[b]Values are mean ± S.E.
[c]$(Na^+ + K^+)$-ATPase activity in the crude homogenate fraction. Values in parentheses indicate number of experiments.
[d]$P < 0.005$.
[e]Not significant.

mCi/mmol) were obtained from New England Nuclear. Na_2ATP (containing vanadium) was obtained from Sigma Chemical Co. All other chemicals were of ACS reagent grade.

Statistical analysis, when performed, was carried out according to the Student's *t*-test.

Table I shows the effect of 24 h of saline treatment on the size and the $(Na^+ + K^+)$-ATPase specific activity of the salt glands. The wet weight of the salt glands from treated animals was increased significantly by 51% ($P < 0.005$). The specific activity of $(Na^+ + K^+)$-ATPase did not significantly change between control and treated animals. The total amount of the $(Na^+ + K^+)$-ATPase, however, was greater in the salt-treated group reflecting the increase in total gland weight.

A dual-label isotope technique was used to detect changes in protein synthesis induced by hypertonic saline administration. Such a procedure would be more sensitive to changes in protein synthesis and less suspectible to artefactual differences of isolation between treated and control enzyme preparations.

To assess the viability of the tissues slices during the incubation period, oxygen consumption of the slices was monitored and found to remain relatively constant at approx. 1.4 μM O_2 /g wet wt. tissue per min over the 2 h period. Radioactive-leucine incorporation was linear over the same period.

To test for possible differences in labelling between the two isotopes ([^{14}C] - or [^{3}H]leucine), salt-gland slices were prepared from control animals only. The slices were divided into two portions. One portion was incubated with [^{3}H]leucine and the other portion with [^{14}C]leucine. The slices were treated as described above. The heavy microsomes (48 000 × *g* pellet) were isolated and subjected to electrophoresis as described. The results are shown in Fig. 1A. No difference in the distribution between the two isotopes was apparent, as expected.

To verify the positions of the catalytic and glycoprotein subunits of $(Na^+ + K^+)$-ATPase, the enzyme was purified to a specific activity of 2200 μmol P_i/mg protein per h by using the method of Jorgensen [16] as described by Hopkins et al. [17]. The two subunits are clearly visible (Fig. 2) with R_F values of 0.328 and 0.606 for the catalytic and glycoprotein subunits, respectively. One minor contaminant of low molecular weight is also present, contrary to the report by Hopkins et al. [17]; this may be due to a better resolution of our electrophoresis system. The catalytic subunit had an apparent

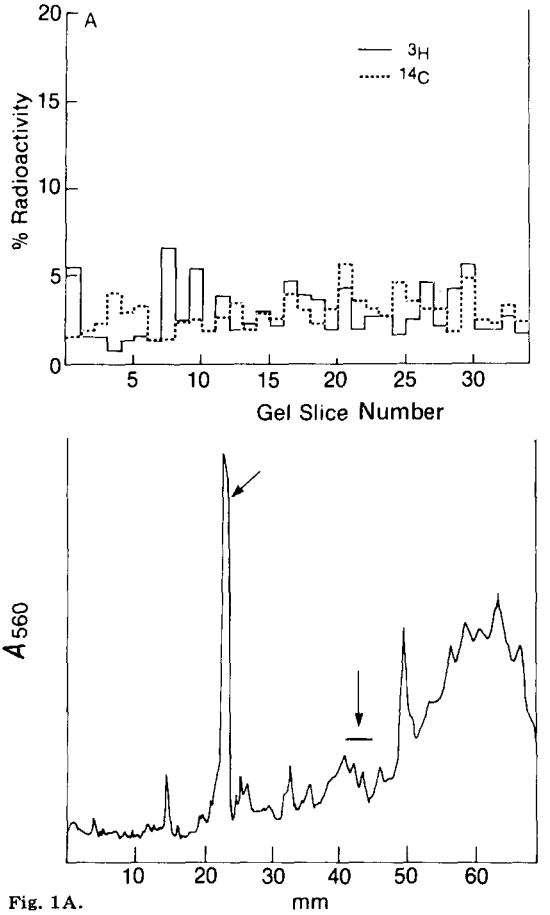

Fig. 1A.

molecular weight of 96 000 and the glycoprotein subunit, which runs as a broad band, had an apparent molecular weight of approx. 54 000. The arrows in Fig. 1 mark the position of the subunits.

We then applied the dual-isotope technique to salt-gland slices from control or treated animals which were incubated with [^{14}C]- or [^{3}H]leucine, respectively, and the heavy microsomes were treated as described above. The results are shown in Fig. 1B. The salt-gland slices from saline-treated animals incorporated more radioactive leucine into the two subunits of (Na^++K^+)-ATPase than did slices from control animals.

When salt-gland slices from control or treated animals were incubated with [^{3}H]- or [^{14}C]leucine, respectively, the results were quantitatively similar to those in Fig. 1B.

The results in Fig. 1B clearly indicate that the two subunits of (Na^++K^+)-ATPase incorporated more radioactive leucine than most other proteins of the microsomal fraction of salt glands from salt-loaded animals. The results are consistent with our hypothesis that osmotic stress can cause de novo synthesis specifically of (Na^++K^+)-ATPase and generally of other membrane components in duck salt glands.

Previous studies on other tissues also indicate that an important mechanism for altering sodium-pump activity is through the synthesis of new en-

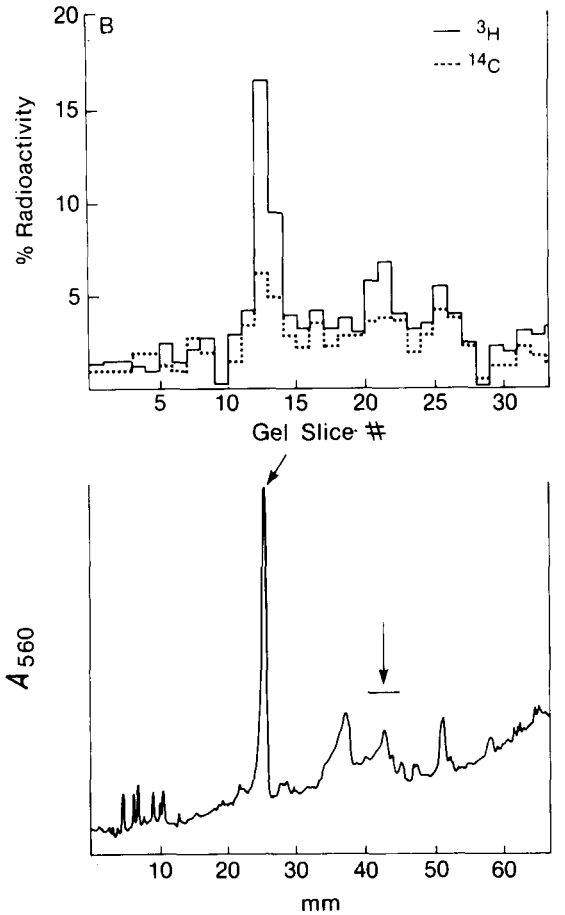

Fig. 1. (A) Salt-gland slices from control animals were incubated with [^{3}H]- or [^{14}C]leucine. The slices were homogenized separately and equal volumes of the homogenates were combined and treated as described in the text. SDS-polyacrylamide gel electrophoresis was performed as described by Stewart et al. [12]. The stained gels were scanned at 560 nm, in the linear-transport attachment for the Gilford 2400 spectrophotometer. The gels were sliced into 2-mm discs, solubilized with 30% H_2O_2 and counted in a Nuclear Chicago Liquid Scintillation Spectrophotometer. Radioactivity was measured by the external standard ratio method. The arrows indicate the positions of the two subunits of (Na^++K^+)-ATPase. Statistical analysis of the double-isotope incorporation data was carried out according to the method of Smith [21]. Several gel slices which did not correspond to either subunit of the (Na^++K^+)-ATPase, showed significant differences at the 5% level. The results shown are representative of at least four separate experiments. (B) Salt-gland slices from control and salt-loaded animals were incubated with [^{14}C]- or [^{3}H]-leucine, respectively, and treated as described in the text, and in Fig. 1A. The arrows indicate the positions of the two subunits of (Na^++K^+)-ATPase. Statistical analysis of the incorporation data as described by Smith [21] indicated that the gel slices corresponding to the catalytic subunit of (Na^++K^+)-ATPase showed a significant increase in labelling (at the 5% level), whilst the increase in labelling in the glycoprotein subunit was not significant. The results shown are representative of at least four separate experiments.

zyme. Evidence of de novo synthesis of (Na^++K^+)-ATPase as indicated by radioactive amino-acid incorporation has been obtained in rat kidney after aldosterone [18] and triiodothyronine [19] treatment. Similar data have also been obtained in brine shrimp using a labelled sodium carbonate amino-acid precursor [20].

In other studies in this laboratory, a mechanism for activating (Na^++K^+)-ATPase, possibly through cyclic GMP, has been described [15]. This mechanism appears to modulate ion secretion by the salt glands in response to an

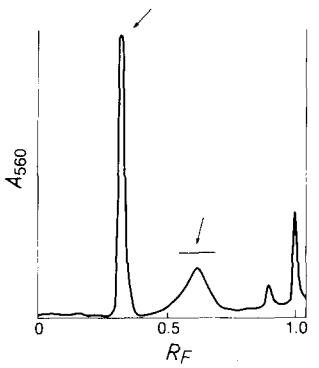

Fig. 2. $(Na^+ + K^+)$-ATPase was purified from duck salt glands to a specific activity of 2200 μmol P_i/mg per h according to the procedure of Jorgensen [16] as described by Hopkins et al. [17]. The catalytic and glycoprotein subunits had R_F values of 0.328 and 0.606, respectively.

acute salt load. However, this mechanism is restricted in that it can only operate under a ceiling imposed by the amount of enzyme present in the salt glands. The low initial $(Na^+ + K^+)$-ATPase activity present in salt glands from control animals would not allow these animals to excrete sufficient amounts of electrolytes to survive on a daily regimen of salt-water. Therefore, it seems that, in response to chronic salt-water loading, the large increases in $(Na^+ + K^+)$-ATPase activity that do occur in this tissue are an adaptive mechanism necessary for survival. The report by Holmes and Stewart [10] of increased changes in RNA and polysome content and the results of this study indicate that one of the initial steps in development of this adaptive mechanism is the de novo synthesis of $(Na^+ + K^+)$-ATPase.

This study was supported in part by Medical Research Council of Canada Grant MT-2485 and National Institute of Health Grant 1-R01-AM 20425-01.

References

1 Holmes, W.N. (1965) Arch. Anat. Microsc. Morphol. Exp. 54, 491—513
2 Ernst, S.A., Goertemiller, C.C. and Ellis, R.A. (1967) Biochim. Biophys. Acta 135, 681—692
3 Fletcher, G.L., Stainev, J.M. and Holmes, W.N. (1967) J. Exp. Biol. 47, 375—392
4 Holmes, W.M., Fletcher, G.L. and Stewart, D.J. (1968) J. Exp. Biol. 48, 487—508
5 Ballantyne, B. and Woods, W.G. (1968) J. Physiol. 196, 125 p.
6 Stewart, D.J., Holmes, W.N. and Fletcher, G.L. (1969) J. Exp. Biol. 50, 527—539
7 Ernst, S.A. and Ellis, R.A. (1969) J. Cell Biol. 40, 305—321
8 Hokin, M. (1963) Biochim. Biophys. Acta 77, 108—116
9 Bonting, S.L., Canady, L. and Hawkins, N. (1964) Arch. Biochem. Biophys. 106, 49—56
10 Holmes, W.N. and Stewart, D.J. (1968) J. Exp. Biol. 48, 509—519
11 Karlsson, K.A., Samuelson, B.E. and Steen, G.O. (1971) J. Membrane Biol. 5, 169—184
12 Stewart, D.J., Semple, E.W., Swart, G.T. and Sen, A.K. (1976) Biochim. Biophys. Acta 419, 150—163
13 Post, R. and Sen, A.K. (1967) Methods Enzymol. 10, 762—776
14 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
15 Stewart, D.J., Sax, J., Funk, R. and Sen, A.K. (1979) Am. J. Physiol. 237, C200—C204
16 Jorgensen, P.L. (1974) Biochim. Biophys. Acta 356, 36—52
17 Hopkins, B., Wagner, H. and Smith, T. (1976) J. Biol. Chem. 251, 4365—4371
18 Knox, W. and Sen, A.K. (1974) Ann. N.Y. Acad. Sci. 242, 471—488
19 Lo, C. and Edelman, J.S. (1976) J. Biol. Chem. 251, 7384—7840
20 Peterson, G.H., Ewing, R.D. and Conte, F.P. (1978) Dev. Biol. 67, 90—98
21 Smith, K. (1976) Anal. Biochem. 76, 16—31