REVISTA ESPAÑOLA DE FISIOLOGIA, 39, 197-202. 1983

Influence of Certain Fish Meals on $(Na^+ + K^+)$-ATPase and Ca^{2+}-ATPase Activity in Rat Small Intestine

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(Received on October 1, 1982)

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The effect of high-protein content fish meal on $(Na^+ + K^+)$-ATPase and Ca^{2+}-ATPase activity in rat small intestine was studied. 5 groups of Wistar rats, weighing between 40-60 g, were fed diets with 12 % protein content of dry matter for 10 days. The protein source was casein for the control group and fish meal derived from *Coryphaenoides rupestris, Chimaera monstruosa* and *Merluccius merluccius* for the test group. The results show a decrease in $(Na^+ + K^+)$-ATPase and a rise in Ca^{2+}-ATPase activity in animals fed with fish meal protein compared to those fed on casein. No significant variations were observed between the groups fed on fish meal derived from *C. rupestris* and *Ch. monstruosa.*

The calcium ion, which is abundant in fish, may be a factor responsible for these variations which produce inhibition of the $(Na^+ + K^+)$-ATPase and stimulation of the Ca^{2+}-ATPase.

Intestinal enzymes play an important part in the digestion and absorption of dietary nutrient and the role of $(Na^+ + K^+)$-ATPase, discovered by SKOU (18), is fundamental to the active transport absorption process. Apart from being responsible for the elimination of Na^+ and the accumulation of K^+ within animal cells (19), it is also involved in the transport of amino acids and sugars (17).

Intestinal cells also contain a Ca^{2+}-ATPase responsible for the transport of Ca^{2+} through these cells (3).

Both $(Na^+ + K^+)$-ATPase and Ca^{2+}-ATPase need the presence of Mg^{2+} and ATP ions for their activity to develop.

Several authors have reported that mucosal enzymes (maltase, sucrase, leucineaminopeptidase) modify their activity in response to dietary change (7-9, 16). HEITANEN (5) showed that the slightest change in the dietary content is of great importance in the regulation of intestinal ATPase activity and that, furthermore, such dietary changes can be detected statistically after 4 weeks of feeding.

Following this line of work and owing to the scant amount of research carried

out on changes in intestinal ATPase activity produced by dietary variations, this present paper studies the effect of certain fish meals *Coryphaenoides rupestris*, *Chimaera monstruosa* and *Merluccius merluccius* on the $(Na^+ + K^+)$-ATPase and Ca^{2+}-ATPase activity in the small intestine of the rat and compares the results with a casein fed control group.

Materials and Methods

Animals and diets. 5 groups of female Wistar rats weighing from 40-60 g were used. They were fed for 10 days on a diet whose composition in percentage of dry matter was: protein 12; fats 4; raw fibre 8; salt mixture 5 (14); vitamin mixture 5 (14) and vitamin A + D 30 and 10 UI/rat/day. The remainder was made up to 100 of equal parts of starch and saccharose. Microcrystaline cellulose was used as fibre and olive oil as fat. The protein source was fish meal from *C. rupestris*, *Ch. monstruosa* and *M. merluccius* for the treated animals and a casein supplement with 0.6 % DL-methionine for the controls. The fish meal was prepared by decapitating and gutting the fish, boiling them for three minutes and then drying them in an oven at 80 ± 2° C. When dried they were ground in an electric grinder and the resulting meal was homogenised and then analysed.

Tissue preparation. After the feeding period the animals were sacrificed and the mucosa of the thin intestine was removed according to the technique of MILLER and CRANE (13) and the procedure described by BERG and CHAPMAN (2). 0.5 grams of the mucosa was homogenized in 4.5 ml of 20 mM Tris-HCl buffer (pH = 7.5), containing 250 mM of saccharose and 1 mM of EDTA. Homogenization was carried out using a Potter-Elvehjem (200-300 r.p.m., 17 strokes) with the teflon point at 4° C.

The intestinal homogenate was then centrifuged at 432.6 g_{av} for 10 min in a table model centrifuge (Selecta-P). The following supernatants (fractions I, II, III) were obtained by successive periods of centrifuging in a Sorval OTD-2 centrifuge at 2° C for 10 min at 8.428 g_{av}, 10 min at 27.052 g_{av} and 60 min at 107.640 g_{av} respectively. The final supernatant corresponded to fraction IV.

The different fractions obtained were suspended in 5 ml of 20 mM Tris-HCl buffer (pH = 7.5) containing 250 mM of saccharose. The enzymatic activity of each fraction was determined and their values added to obtain the total.

Enzyme assays. $(Na^+ + K^+)$-ATPase activity was defined as the difference between the inorganic phosphate liberated in the presence and absence of sodium and potassium. Total ATPase was determined in a 2.8 ml reaction mixture containing NaCl 90 mM, KCl 10 mM, Tris-HCl buffer (pH = 7.8) 50 mM, $MgCl_2$ 3 mM and ATP disodium salt (Sigma) 3 mM, and enough enzyme suspension to bring the final protein concentration to 30-60 μg/0.1 ml. The reaction was started by the addition of ATP and $MgCl_2$ shaken in a water bath at 37° C for 20 minutes and stopped by the addition of 0.5 ml of 10 % (wt/vol.) trichloroacetic acid. After centrifugation the inorganic phosphate liberated was measured by the method of LE BEL *et al.* (11).

Ca^{2+}-ATPase activity was defined as the difference between the inorganic phosphate liberated in the presence and absence of calcium. $(Ca^{2+} + Mg^{2+})$-ATPase was determined in a 2.8 ml reaction containing $CaCl_2$ 1 mM, Tris-HCl buffer (pH = 7.6) 50 mM, $MgCl_2$ 3 mM and ATP disodium salt 3 mM. Mg^{2+}-ATPase was determined in 2.8 ml reaction mixture containing EGTA 1 mM, Tris-HCl buffer (pH = 7.8) 50 mM, $MgCl_2$ 3 mM and ATP 3 mM. The same procedure

used for $(Na^+ + K^+)$-ATPase was then followed.

The enzymatic activity was expressed as micromoles of inorganic phosphate liberated per milligram of protein per minute.

Protein assay. Protein estimation was performed according to the method of LOWRY *et al.* (12) using bovine serum albumin (Sigma) as standard.

Statistical methods. The differences between groups were analysed statistically with Student's t-test.

Results

The $(Na^+ + K^+)$-ATPase and Ca^{2+}-ATPase activity in the small intestine of rats fed on fish meal derived from *C. rupestris* and *Ch. monstruosa* is shown in table II, and for those rats fed on fish meal derived from *M. merluccius* in table III.

Table I. *Composition (%) of casein and fish meals derived from* Coryphaenoides rupestris,, *Chimaera monstruosa* and *Merluccius merluccius.*

	Casein	Fish meals from «C. rupestris»	«Ch. monstruosa»	«M. merluccius»
Water	10.50	11.25	13.53	19.62
Protein	82.27	75.00	76.12	66.25
Fats	1.67	2.22	3.78	4.80
Ash	1.55	10.46	5.73	7.03
Nitrogen free material	4.01	1.07	0.84	2.30
Ca^{2+} in ash	0.32	2.84	2.65	2.70

Table III. *$(Na^+ + K^+)$-ATPase and Ca^{2+}-ATPase activity of the small intestine of rats fed on casein and fish meal derived from* Merluccius merluccius.

Values are means ± SE (n = 8). Units are μmoles Pi/mg protein/min.

	Casein	Fish meal from «M. merluccius»
$(Na^+ + K^+)$-ATPase	0.68 ± 0.01	0.61 ± 0.02 **
Ca^{2+}-ATPase	0.16 ± 0.008	0.18 ± 0.007 **

** $P < 0.005$.

$(Na^+ + K^+)$-ATPase activity varied with the type of fish meal, there being a fall of 9.2 %, 6.1 % and 10.3 % in animals fed with fish meal derived from *C. rupestris, Ch. monstruosa* and *M. merluccius* respectively, against the casein fed controls. This fall is statistically significant for the probabilities studied.

Ca^{2+}-ATPase activity also underwent modification according to the diet which in this case produced an increase in activity of 20 %, 13.3 % and 12.5 % in animals fed on fish meal from *C. rupestris, Ch. monstruosa* and *M. merluccius* respectively, against the control group. The statistical significance is only $P < 0.005$ for animals fed on fish meal from *C. rupestris* and *M. merluccius.*

No statistical variations were observed between groups fed on fish meal from *C. rupestris* and *Ch. monstruosa.*

Table II. *$(Na^+ + K^+)$-ATPase and Ca^{2+}-ATPase activity of the small intestine of rats fed on casein and fish meals derived from* Coryphaenoides rupestris *and* Chimaera monstruosa.

Values are means ± SE (n = 8). Units are μmoles Pi/mg protein/min.

	Casein	Fish meals from «C. rupestris»	«Ch. monstruosa»
$(Na^+ + K^+)$-ATPase	0.65 ± 0.03	0.59 ± 0.01 **	0.61 ± 0.03 *
Ca^{2+}-ATPase	0.15 ± 0.01	0.18 ± 0.01 **	0.17 ± 0.007

* $P < 0.01$; ** $P < 0.005$.

Discussion

It has been shown that variations in animal diets produced by amino acids (10), carbohydrates (1), fat and vitamins (5) modify the activity of intestinal disaccharidases, ATPases and leucineaminopeptidase.

On the other hand, HIETANEN (5) reports that changes in dependent ($Na^+ + K^+$)-ATPase is statistically demonstrated after four weeks of feeding.

In this paper, the results obtained show marked changes in the ATPases in animals fed on fish meal compared to those fed on casein. These variations are significant for ($Na^+ + K^+$)-ATPase in general and for Ca^{2+}-ATPase in the case of those animals fed on fish meal from *C. rupestris* and *M. merluccius* (tables II and III) after 10 days of feeding.

A comparison of the different protein sources (table I) show that the ash content of the fish meal is greater than that of casein, from which it follows that the calcium content is also greater.

From experiments carried out *in vitro* and *in vivo*, it has been demonstrated that ($Na^+ + K^+$)-ATPase, which is inhibited by ouabain (4, 6), is also inhibited by Ca^{2+} ions, and that this inhibition is due to competition between the Mg^{2+} and Ca^{2+} for the ATP, which strongly binds both ions (15).

The Ca^{2+} ion, which abounds in fish, might be one of the factors responsible for these differences by stimulating the Ca^{2+}-ATPase and inhibiting the ($Na^+ + K^+$)-ATPase.

For this reason, in the present paper, the statistical variations appear earlier thant that reported by HIETANEN (5).

These findings suggest that only by also changing the ash content of these diets can changes in intestinal ATPases be produced, for the dietary composition is similar for both the test and control animals in terms of protein content, fat, fibre, minerals and vitamins. The only variant was the protein source which also contributed the calcium ion.

From this one may conclude that fish meal derived from *C. rupestris*, *Ch. monstruosa* and *M. merluccius* modify the ($Na^+ + K^+$)-ATPase and Ca^{2+}-ATPase of the thin intestine of the rat.

Resumen

Se estudia el efecto de algunas harinas de pescado, con alto contenido en proteína, sobre la ($Na^+ + K^+$)-ATPasa y Ca^{2+}-ATPasa del intestino delgado de rata. Se utilizan 5 grupos de ratas, raza Wistar, con un peso comprendido entre 40-60 g alimentadas, durante 10 días, con dietas cuyo contenido proteico es del 12 % y el cual procede de caseína para los grupos controles y de las harinas de *Coryphaenoides rupestris*, *Chimaera monstruosa* y *Merluccius merluccius* para los tratados.

Los resultados muestran un descenso y un aumento en las actividades de la ($Na^+ + K^+$)-ATPasa y Ca^{2+}-ATPasa, respectivamente, en los animales alimentados con dietas procedentes de harinas de pescado con respecto a los que ingieren caseína, mientras que no se observan variaciones significativas entre los grupos alimentados con harinas de *C. rupestris* y *Ch. monstruosa*. Quizá sea el ion calcio, abundante en los pescados, uno de los factores responsables de estas variaciones, produciendo inhibición de la ($Na^+ + K^+$)-ATPasa y estimulación de la Ca^{2+}-ATPasa.

References

1. ADAMS, J. L. and LEICHTER, J.: *J. Nutr.*, 103, 1716-1722, 1973.
2. BERG, G. G. and CHAPMAN, B.: *J. Cell. Comp. Physiol.*, 65, 361-372, 1965.
3. COBURN, J. W., HARTENBOWER, D. L. and MASSRY, S. G.: *Kidney International*, 4, 96-104, 1973.
4. GRISHAM, C. M. and BARNETT, R. E.: *Biochim. Biophys. Acta*, 266, 613-624, 1972.
5. HIETANEN, E.: *Comp. Biochem. Physiol.*, 50 A, 41-46, 1975.
6. HILDEN, S., RHEB H. M. and HOKIN, L. E.: *J. Biol. Chem.*, 249, 7432-7440, 1974.

7. KIMURA, T., SHIOSAKA, S. and SAKAKIBARA, A.: *Nutr. Rep. Int.*, **14**, 657-670, 1976.
8. KIMURA, T., SETO, A. and YOSHIDA, A.: *J. Nutr.*, **108**, 1087-1097, 1978.
9. KIMURA, T., KATO, T., TSUKAZAKI, K. and YOSHIDA, A.: *J. Nutr. Sci. Vitaminol.*, **25**, 195-204, 1979.
10. KIMURA, T., SHIOSAKA, S. and YOSHIDA, A.: *J. Nutr.* **108**, 1098-1103, 1978.
11. LE BEL, D., POIRIER, G. G. and BEAUDOIN, A. R.: *Anal. Biochem.*, **85**, 86-89, 1978.
12. LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. and RANDALL, R. J.: *J. Biol. Chem.*, **193**, 265-275, 1951.
13. MILLER, D. and CRANE, R. K.: *Biochim. Biophys. Acta.*, **52**, 293-298, 1961.
14. REBOLLEDO-VARELA, E. and FERNÁNDEZ-OTERO, M.ª P.: *Rev. esp. Fisiol.*, **38**, 321-326, 1982.
15. RICHARDSON, S. H.: *Biochim. Biophys. Acta*, **150**, 572-577, 1968.
16. SAITO, M. and SUDA, M.: *J. Nutr. Sci. Vitaminol.*, **21**, 313-315, 1975.
17. SCHULTZ, S. G. and CURRAN, P. F.: *Physiol. Rev.*, **50**, 637-718, 1970.
18. SKOU, J. C.: *Biochim. Biophys. Acta*, **23**, 394-401, 1957.
19. SKOU, J. C.: *Bioenergetics*, **4**, 14, 1972.