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ABSORPTION OF THIAMINE AND NICOTINIC ACID IN THE RAT INTESTINE DURING FASTING AND IMMobilIZATION STRESS

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**Title and Subtitle:** Absorption of Thiamine and Nicotinic Acid in the Rat Intestine During Fasting and Immobilization Stress

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**Abstract:**
By perfusion of isolated sections of intestine with a solution containing thiamine at a concentration of 3.1 μmole, it was established that thiamine absorption in animals fasted for 72 hours decreased by 28%, whereas absorption increased by 12% in rats after 24 hour immobilization. Besides, after immobilization, absorption of label in the intestinal mucosa increased. Na-K-ATPase activity in the intestinal mucosa increased. Na-K-ATPase activity in the intestinal mucosa decreased by 10% during fasting, and it increased with immobilization of the animals. Evidently, Na-K-ATPase activity in the intestinal mucosa cells determined the absorption rate of thiamine and nicotinic acid at the level of vitamin transport through the plasma membranes of the enterocytes.
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One possible cause of endogenous vitamin deficiency is disturbance of the absorption of vitamins in the intestine [10, 20]. Clinically, the syndrome described is absorption deficiency, which is associated with many diseases [1, 9]. However, the characteristics of vitamin absorption in the intestine in pathologies, as well as the role of separate processes which provide vitamin transport through the intestinal cell membranes, have been studied insufficiently.

We studied the characteristics of absorption of thiamine and nicotinic acid in the small intestine of the rat during fasting and in animals under immobilization stress. At the same time, the Na\(^+\)-K\(^+\) ATPase activity of the intestines of the experimental animals, as a membrane enzyme, was studied [6, 13]. This provides active transport of some substances through the membranes of the epithelial cells of the intestine [4, 12].

Method

The studies were conducted on 50 white male rats, weighing 200±20 g, which were maintained on normal vivarium rations. The characteristics of thiamine and nicotinic acid absorption were studied under conditions of immobilization stress and fasting.

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The animals were immobilized for 24 hours in the horizontal position, to prevent numerous ulcers in the stomachs of the animals. Upon incision, the stomachs frequently are filled with blood, which is characteristic of immobilization stress [17].

Rats of the other group were not fed for 72 hours (unlimited water) before the experiment. After this time, the weight of the rats decreased by an average of 15%, but the weight of the intestine was unchanged.

The absorption of thiamine in the rat intestine was studied, by means of our modification of a method of perfusion of the intestine [14]. Under hexenal anesthesia, the intestinal wall of the rat was cut, and a section of the intestine 5 cm long was isolated, at the level of the upper third of the small intestine, without damaging the mesentery. The isolated section was perfused with a 0.9% sodium chloride solution, which contained thiamine at a concentration of 3.1 μM. The perfusion rate was 0.75 ml/min. The perfusate was collected fractionally every 2 min for a period of 10 min. Thiamine absorption was determined from the decrease in thiamine in the perfusate. The thiamine was determined in the tests according to [3].

Nicotinic acid absorption was determined from the degree of disappearance of the $^{14}$C label, in administration of $^{14}$C labelled nicotinic acid to a 5 cm long section of the small intestine, isolated at the level of the upper third of the small intestine. A 0.9% solution of sodium chloride which contained $^{14}$C nicotinic acid at a concentration of 40 μM (activity of the preparation, 10 mC/g) was administered to the section in the amount of 1 ml. After 10 and 60 min, the solution was
separated from the lumen of the intestine, and the degree of decrease of the label in the contents of the section of intestine and the amount of label in the mucosa were determined. The radioactivity of the samples was determined in a PST-100 end counter. The results were expressed in count/g/min.

The Na\(^+\)-K\(^+\) ATPase activity was determined by the method proposed by Govorova et al [2]. The results were expressed in \(\mu g\) phosphorus/g tissue/hour.

The results were processed biometrically [5].

Results

Study of the absorption of thiamine by a section of the small intestine of the rat, during its perfusion for 10 min with a thiamine solution (3.1 \(\mu M\)), permitted determination that, after 24 hour immobilization of the animals, absorption of thiamine from the perfusate increases by 12\% (Fig. 1). While, in the control group of animals, of the total amount of thiamine (7.5 \(\mu g\)), 4.25 \(\mu g\) of thiamine was absorbed in 10 min, it was 5.25 \(\mu g\) in the rats which were immobilized. On the contrary, in the animals which were fasted for 72 hours, thiamine absorption decreased by 28\% and, of the total amount of thiamine (7.5 \(\mu g\)), only 2.25 \(\mu g\) was absorbed (Fig. 1).

The change in the thiamine absorption rate, which was determined at 2 min intervals over 10 min, is in the same direction as that of the data presented (Fig. 2). In immobilization stress, the thiamine absorption rate increases to 0.55 \(\mu g/min\), and it decreases to 0.23 \(\mu g/min\) in the animals which were fasted, with a standard 0.45 \(\mu g/min\) of thiamine in the perfused section of the small intestine.
We determined nicotinic acid absorption in the small intestine of normal rats and in animals which endured immobilization stress. The study results are presented in Fig. 3. They characterize the degree of decrease of the $^{14}$C label in the luminal contents, 10 and 60 min after administration of the $^{14}$C nicotinic acid solution to the lumen of the intestine. A higher degree of absorption of nicotinic acid from the lumen of the intestine of the rat during immobilization stress was found. The difference in the decrease in label was a little higher in 10 than in 60 min.

Fig. 4 presents the results of study of the accumulation of the $^{14}$C label of nicotinic acid in the mucosa of the section of intestine of normal rats and those which endured immobilization stress. A sharp increase in accumulation of label in 10 min was traced in the intestinal mucosa of the rats which endured immobilization. Besides, the difference in accumulation of label in the mucosa was less pronounced in 60 min, although the direction of the process is the same.

Study of Na$^+$-K$^+$ ATPase showed that the activity of the enzyme in the intestinal mucosa of the rat depends on the condition of the body.
Fig. 3. Nicotinic acid absorption from rat intestinal lumen: conventional designations as in Fig. 1.

Key: a. count/min/ml  
b. min

Fig. 4. Nicotinic acid accumulation in rat intestinal mucosa upon absorption from intestinal lumen: conventional designations as in Fig. 1.

Key: a. count/min/g  
b. min

of the animal. Fig. 5 presents the results of determination of Na\(^+\)-K\(^+\) ATPase activity in the intestinal mucosa of the rat under immobilization stress and in fasted animals, compared with normal rats. In 24 hour immobilization, the activity of the enzyme under study increased sharply, and it is nearly 50% above the Na\(^+\)-K\(^+\) ATPase activity of the mucosa of the small intestine of normal animals. In 72 hour fasting, a tendency develops toward a decrease in activity of the enzyme, compared with the group of control animals.

Discussion

It was determined from the study that, under immobilization stress conditions, thiamine and nicotinic acid absorption in the rat intestine increases. At the same time, Na\(^+\)-K\(^+\) ATPase activity in the mucosa of the upper third of the small intestine of the rat increases.

According to the data of Tursunkhodzhayeva et al [8], the consumption of group B vitamins increases under stress. It follows from the data we obtained that the increase of vitamin consumption is produced by increase
in absorption in the intestine, due to an increase in activity of Na\(^+\)-K\(^+\) ATPase, which participates in transmembrane transport of matter [4].

It is known that Na\(^+\)-K\(^+\) ATPase is a membrane fragment [5], localized chiefly in the lateral and basal plasma membranes of the enterocytes [7, 13]. The entry of thiamine into the blood and lymph apparently is connected with the functioning of this enzyme [18]. It is known that the activity of this enzyme is under hormonal control, since the possibility of activation of Na\(^+\)-K\(^+\) ATPase in some tissues of animals by corticosteroids [13], the content of which increases under stress [11], has been established.

The decrease in thiamine absorption in fasting animals must be explained by decreases in Na\(^+\)-K\(^+\) ATPase activity in the intestinal mucosa. It must be noted that some authors point out a decrease in activity of the intestinal enzymes which participate in digestion and absorption, when peroral intake of foodstuffs to the body is stopped [15], or in parenteral feeding [16], which is accompanied by a decrease in the absorptive capacity of the intestine.

Therefore, the absorption of thiamine and nicotinic acid in the rat intestine in small physiological concentrations (3.1 and 40 μM) depends on the activity of membrane Na\(^+\)-K\(^+\) ATPase, which provides active transport through the plasma membrane of the cells of the intestine [12]. A reduction in activity of this enzyme by hormones or other actions (anorexia, exclusion of the functional activity of the intestine)
affects the level of absorption of the coenzyme vitamins thiamine and nicotinic acid in the intestine which, in the final analysis, can affect the provision of the body with these vitamins.

REFERENCES


8. Tursunkhodzhayeva, M.S. and A. Shadmanov (cited in [10]).


