

Human Thyroid Gland HCO_3^- -ATPase Activity

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ABSTRACT. In tissue of the human thyroid gland the existence of the Mg^{2+} -dependent, HCO_3^- -stimulated ATPase (HCO_3^- -ATPase) has been investigated. Distribution of the enzyme in the subcellular fractions of the gland tissue has been evaluated. It was found that especially high enzymatic activity is characteristic of mitochondria, plasma membranes, and endoplasmic reticulum. The correlation between various pathologies of the thyroid and the HCO_3^- -ATPase activity was demonstrated. The data obtained point out that during pathologies, uneven alterations of the HCO_3^- -ATPase activity occur in the subcellular fractions of the gland. On the basis of investigation of the enzyme kinetic parameters, a suggestion was made that in different pathologies alterations of both enzyme activity and its affinity towards the bicarbonate ions do occur, which putatively might be due to the changes in the gland tissue, which, in its turn, represents a basis of various pathologies.

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Key words: thyroid gland, adenoma, carcinoma, diffuse-nodular goiter; HCO_3^- -ATPase.

The reference data show that in the studies of different pathologies, determining the normal activity values for certain enzymes is implemented in the clinical laboratories with an ever-increasing intensity [1-3].

Considering the above-mentioned, the subject of our investigation was Mg^{2+} -dependent, HCO_3^- -stimulated ATPase or for short - HCO_3^- -ATPase. For the first time, HCO_3^- -ATPase was discovered in the pancreas tissue of the mammalian animals [4]. Later its presence was confirmed in the liver and kidney tissues, in the gastric mucosal cells, in plasma membrane of erythrocytes [5-7], in the brain tissues, e.g. in the C-6 cells of the glioma. It was determined that maximal HCO_3^- -ATPase activity was characteristic of the mitochondria of various tissues and the plasma membranes. Later, fairly high activity of this enzyme was shown in the glial cells of the brain as well. It was suggested that it is just the HCO_3^- -ATPase, which participates in the process of intracellular pH regulation, that maintains the buffer

functions in the glial cells [8-11]. Therefore, the mentioned enzyme might have a significant impact on the intracellular pH in the thyroid gland and hence sharply change the cell metabolism. Considering that there are no data concerned with the effect of this ATPase on the thyroid gland and in changes of the functioning process therein, the goal of our study was investigation of HCO_3^- -ATPase activity in the human thyroid tissue and determination of the correlation between this enzyme activity and different pathologies of the gland.

Materials and Methods. Activity of HCO_3^- -ATPase was evaluated according to the difference between the active (in the presence of HCO_3^- ions) and passive (when instead of HCO_3^- ions, identical concentration of NaCl was introduced into the reaction medium) ATPase. The reaction medium contained the following constituents: NaHCO_3 – 60 mM, MgCl_2 – 3 mM, ATP – 3 mM, the protein suspension under study – 500 mg.

Results and Discussion

At initial stage of the study the distribution of HCO_3^- -ATPase enzyme activity in the subcellular fractions of the thyroid was assessed. The results obtained are presented in table 1. As shown in the table, the highest enzyme activity in the studied fractions was evident in the mitochondrial fraction, while the lowest HCO_3^- -ATPase activity was found in the cellular nucleus fraction. The results obtained are in complete agreement with the reference data, according to which high activity of the HCO_3^- -ATPase is a characteristic of the mitochondrial fraction.

In order to evaluate the involvement of HCO_3^- -ATPase in the functioning of the thyroid gland, in the next series of experiments alterations of the enzyme activity was studied in different pathologies of the thyroid. To this end, the post-surgery tissue materials of the patients with thyroid adenoma, carcinoma, and diffuse-nodular goiter were investigated. Like in the case of the normal tissue, changes of the enzyme activity were studied in different subcellular fractions. The results obtained are presented in table 1. For instance in the post-surgery subcellular fractions, certain changes of HCO_3^- -ATPase activity are manifested. Reliable alteration of the enzyme activity, against the subcellular fractions of the normal tissue, was noted in the endoplasmic reticulum and mitochondria. Specifically, decrease of the enzyme activity by, respectively, about 50% and 10%, was found. In the Golgi apparatus and in the plasma membrane an insignificant alteration of the enzyme activity was noted, whereas the cellular nucleus showed no change of the enzyme activity. Unlike the adenoma-afflicted gland, the subcellular fractions of the thyroid tissue of the carcinoma-diagnosed patients showed increased enzyme activity, as compared to the norm. Specifically, the elevation index in the mitochondria totalled about 30%, in the endoplasmic reticulum – 100%, in the nucleus fraction – 100%, in the plasma membrane – 70%. Insignifi-

cant, unreliable increase of the HCO_3^- -ATPase activity was found in the Golgi apparatus. Alteration of HCO_3^- -ATPase activity was noted also in the post-surgery tissue material of the patients with diffuse-nodular goiter. Specifically, in comparison to the other studied pathologies, in this disease the enzyme activity is decreased in the mitochondria (~20%) and in the endoplasmic reticulum (~50%), whereas in the Golgi apparatus, contrariwise, a 30% increase of HCO_3^- -ATPase activity was the case. As to the plasma membrane and nuclei, the enzyme activity in these structures was unchanged.

Thus, as shown in the data presented, relatively higher enzyme activity is characteristic of the mitochondrial and endoplasmic reticulum fractions. The least enzyme activity was found in the cellular nuclei, whereas intermediate activity was found in the Golgi apparatus and the plasma membranes.

As to the thyroid pathologies, it was found that the gland carcinoma, against the adenoma, and diffuse-nodular goiter, is most sensitive to alterations of the HCO_3^- -ATPase activity. Namely, patients with gland carcinoma show significant increase of the HCO_3^- -ATPase activity, as compared to the norm. Notably, even in this case, the nuclear fraction is an exception.

Therefore, considering the data obtained, a link between the HCO_3^- -ATPase activity and different diseases of the thyroid gland could be suggested, which might be due to the importance of the enzyme under study in normal functioning of the gland.

In order to determine which parameter of the enzymatic reaction produces alterations in the HCO_3^- -ATPase activity in different disorders of the thyroid gland, the kinetic properties of the enzyme, specifically, maximal velocity of the enzymatic reaction (V_{\max}) and the enzyme affinity to the bicarbonate ions (K_m) were investigated. The enzyme reaction indices were established in both norm and in various pathologies. The subcellular fractions, the HCO_3^- -ATPase activity of which

Table 1

The HCO_3^- -ATPase activity in the subcellular fractions of the tissues in the thyroid gland

Subcellular fraction	Healthy thyroid gland	Adenomatous thyroid gland	Carcinoma-afflicted thyroid gland	Thyroid gland with diffuse-nodular goiter
Mitochondria	17.7± 1.4	15.8 ± 1.8	23.9 ± 3.1	14.3 ± 2.4
Endoplasmic reticulum	16.0± 0.8	8.0 ± 1.1	35.2 ± 2.9	8.0 ± 1.4
Nuclei	3.5± 0.2	2.0 ± 0.4	2.5 ± 0.6	4.3 ± 0.9
Golgi apparatus	11.4 ± 2.1	10.2 ± 1.5	21.4 ± 3.9	14.3 ± 3.3
Plasma membrane	12.0 ± 1.5	12.3 ± 1.9	19.7 ± 1.3	12.4 ± 2.6

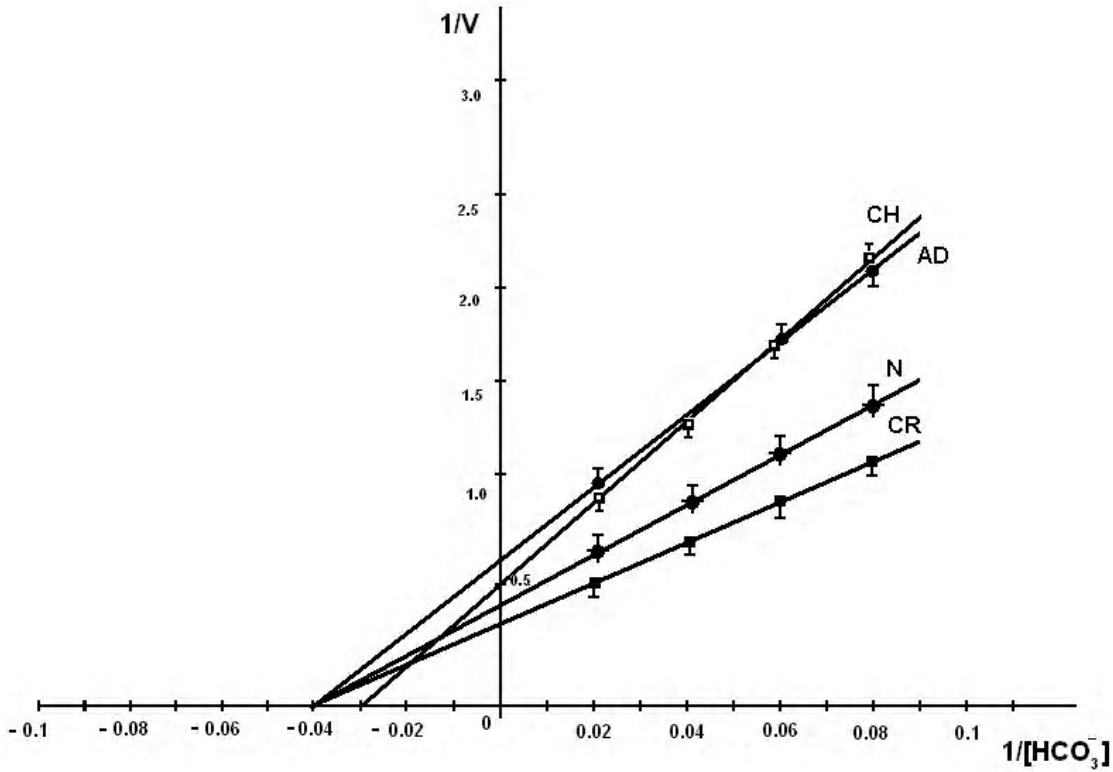


Fig. 1. Alteration of the HCO₃⁻-ATPase kinetic parameters in the mitochondrial fraction of the thyroid gland during different pathologies. Ordinate: Alterations of the enzyme activity; Abscissa: Change in HCO₃⁻-ion concentration (mM). N – health; CR – carcinoma; AD – adenoma; CH – diffuse-nodular goiter.

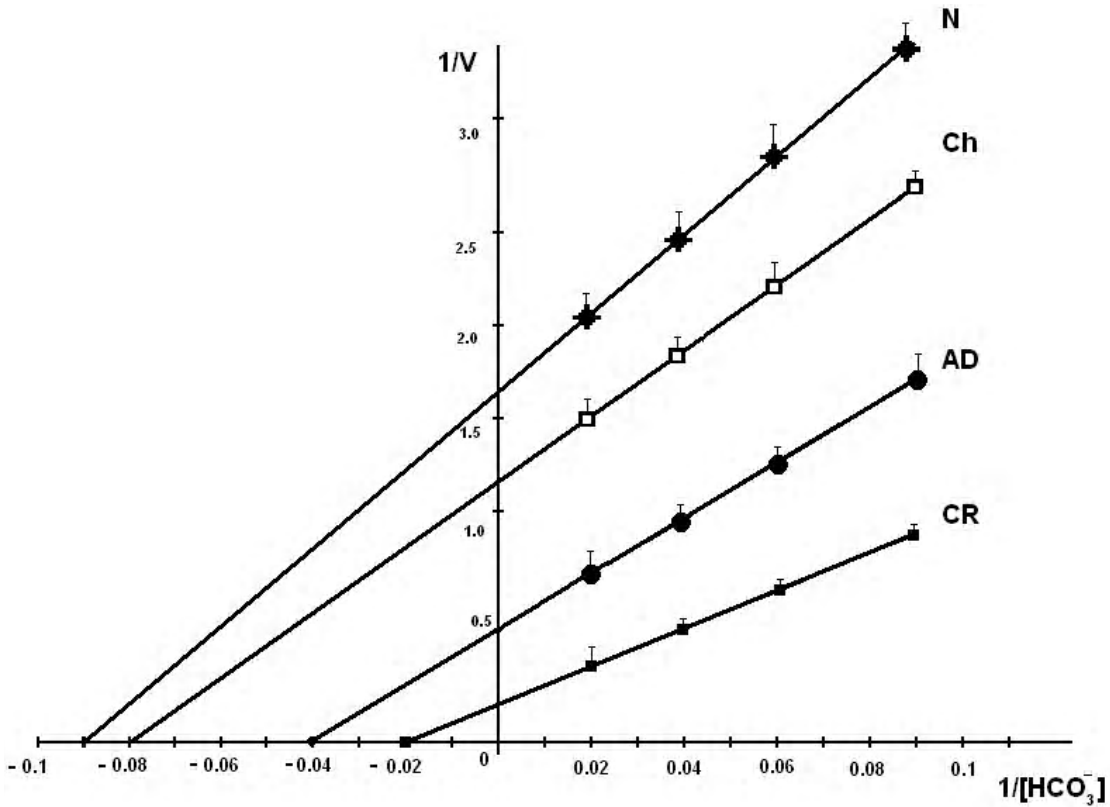


Fig. 2. Alteration of the HCO₃⁻-ATPase kinetic parameters in the endoplasmic reticulum fraction of the thyroid gland during different pathologies. Ordinate: Alterations of the enzyme activity; Abscissa: Change in HCO₃⁻-ion concentration (mM). N – health; CR – carcinoma; AD – adenoma; CH – diffuse-nodular goiter.

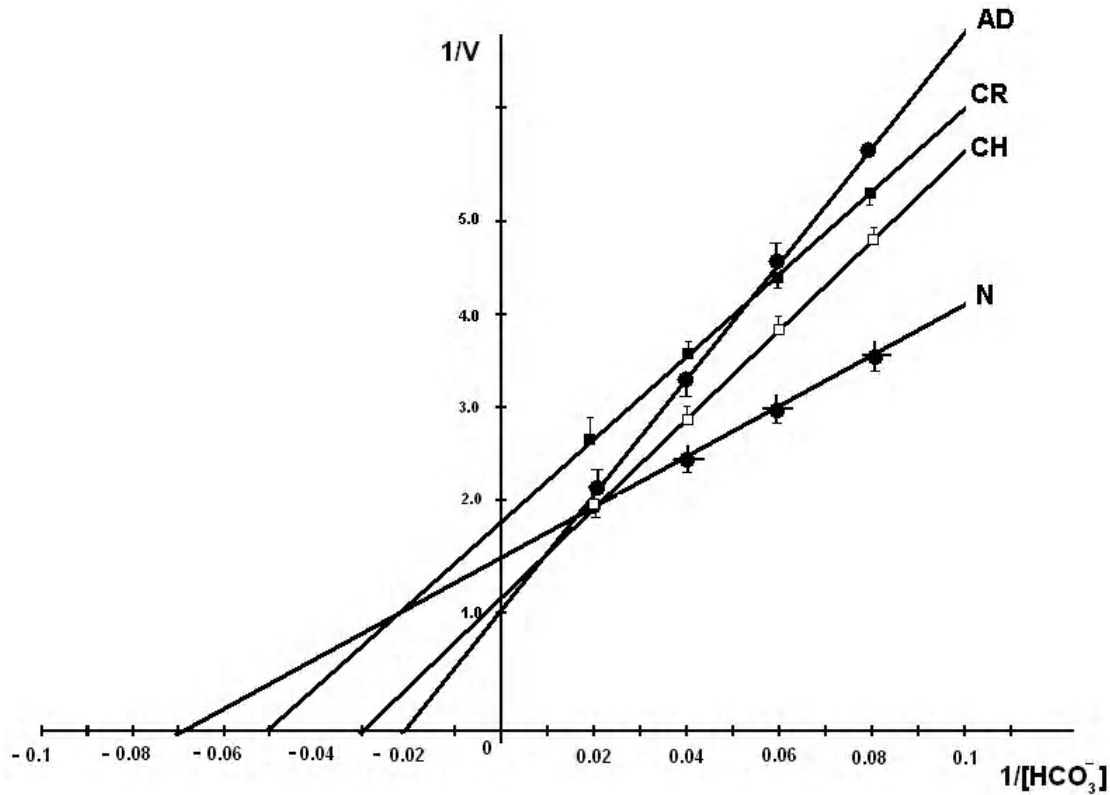


Fig. 3. Alteration of the HCO_3^- -ATPase kinetic parameters in the plasma membranes during different pathologies. Ordinate: Alterations of the enzyme activity; Abscissa: Change in HCO_3^- ion concentration (mM). N – health; CR – carcinoma; AD – adenoma; CH – diffuse-nodular goiter.

showed special sensitivity to a disease, were chosen as the experimental material. These are endoplasmic reticulum, plasma membranes, and mitochondria.

The results obtained are presented in Figures 1, 2 and 3. As shown in Fig. 1, changes of the HCO_3^- -ATPase in the mitochondrial fraction of the thyroid gland, during different pathologies, are determined by altered V_{\max} of the enzyme. The diffuse-nodular goiter represented an exception, during which only the enzyme affinity to the HCO_3^- ion did change. Radically different results were obtained in the cases of the endo-

plasm reticulum and plasma membranes (Figs. 2,3). These fractions were characterized by dramatic changes in both K_m and V_{\max} . The figures clearly show that during the pathologies significant alterations of both K_m and V_{\max} were obvious, as compared to the controls. Therefore, the data obtained show that during different pathologies of the thyroid (diffuse-nodular goiter, adenoma, carcinoma), changes of the HCO_3^- -ATPase activity are manifested against the healthy tissue, which are determined by the changes in kinetic parameters of the enzyme.

ბიოქიმია

ადამიანის ფარისებრი ჯირკვლის HCO_3^- -ატფ-აზური აქტივობა

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ადამიანის ფარისებრი ჯირკვლის ქსოვილში ავტორთა მიერ შესწავლილია Mg^{2+} -დამოკიდებული, HCO_3^- -ით სტიმულირებული ატფ-აზას არსებობა. დადგენილია ფერმენტის განაწილება ჯირკვლის ქსოვილის სუბუჯრედულ ფრაქციებში.

დადგენილია კორელაცია ფარისებრი ჯირკვლის სხვადასხვა პათოლოგიასა და HCO_3^- -ატფ-აზას აქტივობას შორის. ნანახია, რომ პათოლოგიების შემთხვევაში ჯირკვლის სუბუჯრედულ სტრუქტურებში ადგილი აქვს HCO_3^- ატფ-აზური აქტივობის ცვლილებას. ფერმენტის კინეტიკური პარამეტრების (V_{\max} , K_m) შესწავლით დადგენილია, რომ სხვადასხვა პათოლოგიისას აღინიშნება როგორც ფერმენტის აქტივობის, ასევე HCO_3^- -იონისადმი მისი თვისების შეცვლა, რაც, სავარაუდოდ, განპირობებული უნდა იყოს ჯირკვლის ქსოვილში მიმდინარე პათოლოგიური ცვლილებით.

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Received July, 2008