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INCORPORATION OF GLYCINE-2-C$^{14}$ IN ACID-INSOLUBLE PROTEINS OF RAT BONES AND TEETH DURING HYPOKINESIA AND ADMINISTRATION OF THYROCALCITONINE

BY

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Incorporation of Glycine-2-C\(^{14}\) in Acid-Insoluble Proteins of Rat Bones and Teeth during Hypokinesia and Administration of Thyrocalcitonine

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**Abstract:**
A forced limitation of the motor activity in rats (from 5 to 60 days) results in a reduced incorporation of glycine 2-C\(^{14}\) in the total acid-insoluble proteins of limb bones and its increase in the teeth and mandibular-maxillary bones. Daily administration to these rates of five of thyrocalcitonine together with polyvinylpyrrolidone normalizes the protein metabolism in the bone tissues during all the 40 days of experiment.

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INCORPORATION OF GLYCINE-2-C\(^{14}\) IN ACID-INSOLUBLE PROTEINS OF RAT BONES AND TEETH DURING HYPOKINESIA AND ADMINISTRATION OF THYROCALCITONINE

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A forced limitation of the motor activity in rats (from 5 to 60 days) results in a reduced incorporation of glycine-2-C\(^{14}\) in the total acid-insoluble proteins of limb bones and its increase in the teeth and mandibular-maxillary bones. Daily administration to these rats of 5 mg of thyrocalitonine together with polyvinylpyrrolidone normalizes the protein metabolism in the bone tissues during all the 40 days of experimentation.

The thyroid gland hormone thyrocalcitonine (TCT) can be used to prevent disruption in the metabolic processes in the bone tissue during prolonged limited motor activity (Alliapoulos et al., 1966), which, as is known, is accompanied by osteoporosis and a negative calcium balance (Ye. N. Biryukov et al., 1966; Isscutz and Blizzard, 1967). We studied the effect of TCT on certain aspects of the protein exchange in bone and teeth tissues of animals in a normal motor pattern and in hypokinesia.

Methods of Study

Experiments were conducted on 95 albino mongrel male rats with initial weight 120-130 g. All the animals were divided into 4 groups: first--control, second--under conditions of hypokinesia, third--received TCT, fourth--received TCT together with polyvinylpyrrolidone.

*Numbers in margin indicate pagination in original foreign text.
fourth — received TCT on the background of hypokinesia. Hypokinesia was created by placing the rats in close cages in which movement of the animals was sharply limited. Here the dimensions of the cages did not prevent normal consumption of water and food by the rats. Rats in the third and fourth groups were daily given a subcutaneous administration of 5 μg of a complex preparation of TCT with polyvinylpyrrolidone (PVP) obtained by the method of L. I. Stekol’nikov et al. (1969a) and which has a prolonged hypocalcemic effect (L. I. Stekol’nikov et al., 1969b).

Within 5, 10, 20, 40 and 60 days after the start of the experiment the rats (4-5 from each group) were given subcutaneous injections of 2 μCi each of glycine-2-C14 diluted in a physiological solution. In a day after administration of the isotope the animals were decapitated and the total protein content was determined in the blood plasma according to Lowry. The molars, incisors, lower and upper maxillary, humeral, femoral and tibial bones were removed, thoroughly cleaned of soft tissues, washed in running water, dehydrated, degreased and decalcified in 1 n. of HCl solution according to the accepted technique (A. A. Prokhonchukov and Lyu Din'sin', 1961).

The acid-insoluble bone protein that was dried to a constant weight was exposed to hydrolysis by concentrated HCl on backings made of fluoroplastic and dried. Radiometry was conducted on a gas-flow counter SOT-30-BFL. The magnitude of radiation attenuation was computed in the mass of the preparation. The C14 content in the protein of bones and teeth was expressed in percents of the administered isotope dose. The results were processed by the method of variation statistics according to Student and were considered reliable with D ≤ 0.05.

Results and Their Discussion

The concentration of total blood protein in the rats (fig. 1) in hypokinesia (second group) had a tendency to drop, which corresponds to our previous data (A. I. Volozhin, 1971). The administration of TCT preparation to animals of the third group resulted in an increase in the blood protein level on the fifth and tenth days, and then a decrease on the 40th and 60th days of the experiment. In rats of the fourth group the
changes in the level of blood plasma protein did not significantly differ from those in the third group.

The content of acid-insoluble protein in the molars and incisors of rats in the second group increased on the 20th and 40th days respectively. In the humeral bones the quantity of protein was increased on the 40th day and considerably dropped on the 60th day of hypokinesia (78%). A decrease in the amount of protein was also noted in the femoral (10th and 60th days) and in the tibial bones (on the 20th and 60th days).

The administration of TCT to the freely placed rats (third group) produced a reduction in the protein content in the molars (on the fifth and tenth days) and an increase of it in the incisors and maxillary bones (on the 40th day). The quantity of acid-insoluble protein in the humeral (on the 60th day), tibial (on the 60th day) and in the femoral bones (on the fifth, tenth and 60th days of TCT administration) was reduced. In the humeral, femoral and tibial bones of rats in the fourth group the quantity of protein exceeded that in the control, as well as in rats of the second and third groups for the entire length of the experiment.

Data on incorporation of glycine-2-C$^{14}$ in the acid-insoluble proteins of bones and teeth of rats under the conditions of our experiment are presented in figure 2.

It is important to study the reactivity (according to indices for incorporation of glycine-2-C$^{14}$ in proteins) of the bone tissue and teeth of rats under conditions of limited motor function. We speak of the reactivity
Figure 2. Incorporation of Glycine-2-\(^{14}\)C (in % of Control taken as 100) in Proteins of Bones and Teeth of Rats during Hypokinesia and Administration of Thyrocalcitonine.

A--molars; B--incisors; C--lower maxilla; D--upper maxilla; E--shoulder; F--femur; G--tibia. 1,2,3--the same as in fig. 1.

of the bone tissue in relation to TCT on that basis that the bone apparently is the "critical" organ on which the indicated hormone acts (O'Riordan and Aurbach, 1968).

To clarify this question the indices of protein exchange in the mineralized tissues obtained in the second group of rats were compared with the corresponding indices obtained in the fourth group. Here it was found
that in all the periods of observation (except the 60th day) the incorporation of C$^{14}$ into the teeth and bones of the rats of the fourth group was considerably higher than in the "untreated" animals (second group). In contradiction to this the level of incorporation of C$^{14}$ into the proteins of the calcified tissues of the rats in the third group is higher than in the animals of the first group, only in the teeth and maxillary bones, and in the humeral, femoral and tibial bones is lower than in the control, on the fifth, tenth and 20th days. Thus, a change in the reactivity of the bone tissue to the TCT in rats under conditions of hypokinesia occurs in the bones of the skeleton whose function is significantly diminished, i.e., in the bones of the extremities.

The protein matrix of the mineralized tissues consists by 95% of an acid-insoluble collagen in which about 1/3 of the amino acid residues are glycine. Therefore recording of the incorporation of glycine in the acid-insoluble proteins of the bones and teeth is an important indicator of the synthetic processes in the protein matrix of these tissues.

During hypokinesia in rats a considerable inhibition occurs of the incorporation of glycine-2-C$^{14}$ in the acid-insoluble proteins of the bones of the extremities, apparently indicating the decrease in the synthetic processes in the protein matrix of the bones. The use of TCT under conditions of limited movement has a significant normalizing effect on the incorporation of glycine-2-C$^{14}$ in the bones of rats. It is possible that the difference in the effect of TCT on the freely placed rats and animals with limited movement is linked to the change in the initial condition of the bone tissue emerging under the influence of hypokinesia.

Conclusions

1. Limited mobility in rats for 5-60 days results in a suppression of the incorporation of glycine-2-C$^{14}$ into the total acid-insoluble proteins of the bones of the extremities.

2. Administration of thyrocalcitonin to rats during hypokinesia normalizes the incorporation of glycine into the total acid-insoluble proteins of the bone tissue.
References


