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RADIOISOTOPE STUDY OF SKELETAL AND CARDIAC MUSCLE METABOLISM AND IONIC FLUXES IN RATS WITH EXPERIMENTAL HYPERTHYROIDISM. THE IMPACT OF GEROVITAL H3

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Abstract

The aim of this study was to see the effect of Thyroxine administrated in excess upon striated and cardiac muscle metabolism as well as upon $^{45}CaCl_2$ transport in aging rats. Another objective was related with investigation of Gerovital H3 effect upon ^{3}H Tryptophane uptake. Our studies have been pointed out that Thyroxine administrated in excess has an influence upon proteic metabolism and membrane permeability. Our data regarding the effect of in vitro incubation with Gerovital H3 upon ^{3}H Tryptophane uptake. The rats treated with different doses of Thyroxine may have a clinical significance regarding Gerovital H3 therapy in the case of patients with hyperthyroidism, conducting to an improvement in metabolic function of cardiac muscle.

Key words*: Gerovital H3, cardiac muscle, striated muscle, hyperthyroidisnm.*

INTRODUCTION

It is well known that thyroid hormones modify the muscle metabolism and ionic content of muscle fibre as well as blood supply of skeletal and cardiac muscle. Literature data (Davis, 1993) pointed out that thyroid hormones administrated in excess have a direct cardiostimulatory action by increasing cAMP (cyclic adenosine monophosphate) content in myocardium with a positive effect on Ca^{++} dependent ATP-ase in myocyte`s sarcolemma (Agostini et al., 1987). On the other hand, thyroid hormones have the ability of increasing the number of beta adrenergic receptors by intensifying the direct action of cathecolamines upon myocardium and to stimulate adenylat cyclase action, which can explain the increase in myocardial sensitivity to catecholamines (Nwoye, 1981). Thyroid hormones do not affect directly cAMP concentration, but they may modulate the target cell response to stimulatory and inhibitory of cAMP, respectively catecholamine, glucagon and parathormon favouring the action upon adrenergic receptors.

The molecular mechanism implicated was related with a change in sensitivity of beta adrenergic receptor unit, but this fact has not been completely confirmed (Celsing, 1986). Even, the answer to beta adrenergic agents is increased in hyperthyroidism and decrease in hypothyroidism.

It is possible that thyroid hormones to produce a change in regulatory unit of beta adrenergic receptor, respectively of membrane proteins (Gs stimulatory, Gi inhibitory). The excess of thyroid hormones intensify the lipolysis by a modulatory effect of intracellular answer of cAMP, on G regulatory protein action, resulting in sensitization of adipose tissue to catecholamine, growth hormone and glucagon.

At the level of striated muscle, the excess of thyroid hormones induce changes in muscle fibres because of the liberation of lysosomal enzymes, resulting in in muscle atrophy and an increased catabolism of muscle proteins (Koreney, 1981).

Our previous studies (Revnic, 1990) pointed out that during ageing, there is a progressive decline in the active shortening capacity of sarcomere from papillary muscle of left

ventricle from white Wistar rats. This fact may be the result of a diminished protein synhesis at cellular level and of a reduction in the ability of myosin ATP-ase to liberate Pi due to a decrase in total number of SH groups at the level of enzyme situs, due to a progresive decline in thyroxine secretion.

The aim of study was related with evaluation of thyroxine treatment in different dose on skeletal and cardiac muscle metabolism and on $^{45}Ca^{++}$ transport, as well as evaluation of Gerovital H3 effect on 3H tryptophane uptake in the rat heart.

MATERIALS AND METHODS

Thyroxine treatment was performed using the following dosage: 0.25 µm/kg body weight, 0.50 µm/kg body weight, and 1.0 µm/kg b.wt. for 12 days according to the published method (Revnic et al., 1990). Our study was performed on 28 white Wistar rats aged 27 month old divided into 4 groups: 7 rats in control group, 7 rats treated with 25 µm thyroxine/kg b.wt. (experimental group 1), 7 rats treated with 0.50 µm thyroxine/kg b.wt. (experimental group 2) and 7 rats treated with 1 µm thyroxine/kg b.wt. (experimental group 3). The animals were weighted before and after finishing the tretment.

The animals were sacrificed by cervical dislocation, the heart and fragments from Sartorius muscle have been removed and washed in in physiological salt solution on ice bath, the heart has been weighted then placed again on ice bath in physiological salt solution.

Experimental protocol:

For radioisotope experiments of 3H Tryptophane incorporation and for investigation of $^{45}CaCl_2$ transport, were used left ventricle and Sartorius muscle tissue fragments between 50-100 mg each.

After weighting, tissue fragments were reintroduced in Hanks (1 ml for each test tube) and set for preincubation for ½ hour at 37°C.

In each test tube were placed 10 µl 3H Tryptofane from 500 mCi/ml solution, the specific activity was 26 mCi/mg. Incubation of biological samples with radioisotopes was done for 1½ hour at 37°C.The next step was concerned with extraction with HCl 2N. Biological samples were removed from the incubation solution and placed in test tubes containing 1 ml HCl 2N each. The samples were kept in extraction solution for 24 hours. The next day, biological samples were processed for radioactive uptake measurement in incubation and extraction solution. Radioactivity has been assessed in vials with scintillation liquid and incubation solution and extraction solution, respectively by means of Beta Berthold Scintillation Counter with 3 channels for 3H Tryptofane.

The same procedure was used also for the uptake experiments of $^{45}CaCl_2$.

A set of biological samples for each radioisotope was prepared using Gerovital H3, 10 mg/ml in incubation medium.

RESULTS AND DISCUSSIONS

A significant increase in ATP-ase activity in treated rats with Throxine ($P<0.05$) for concentration of 0.5 mM and 1mM and very significant ($P<0.001$) for concentration of 2mM ATP were observed (Table 1).

The experimental data pointed out an increase in 3H Tryptophane uptake in treated rats versus control group (Table 2). The optimum dose for the highest increase in 3H Tryptophane uptake was 0.25 µg/ml thyroxine. 3H Tryptophane uptake is inversely proportional with Thyroxine concentration used (Table 3).

It can be concluded that 0.25 µg Thyroxine has the highest stimulatory effect upon protein synthesis.

It can be observed the stimulation effect of GH3 of protein synthesis in rat heart treated with different concentrations of Thyroxine (Table 4).

A significant increase in quantity of myosin SH groups in treated rats with Thyroxine was recorded in rat heart (Table 5).

Literature data (Florini, 1985) pointed out that protein metabolism is stimulated by thyroide hormones, stimulation of protein synthesis being partialy in relation with calorigenic effect and with an increase in synthesis of other enzymes which may explain these effects.

The effects of thyroid hormones upon protein metabolism depends on metabolic state of the organism and on the administated dosage at thyroidectomised rats, and that higher concentrations of hormone administrated in rats

inhibit protein synthesis and increase the aminoacid concentration in plasma, liver and muscle.

It can be observed a reduction in the uptake of 3H Tryptophane in treated rats with different concentrations of Thyroxine in comparison with control group (Table 6) which are in accordance with literature data (Florini, 1985) who pointed out that thyroid hormones administrated in excess have an inhibitory effect on protein synthesis.

A characteristic discovery is that florid hyperthyroid condition incudes a significant increase in final dyastolic volume of left ventricle and an increase in heart index and in beat volume. As it was expected, thyroxine treatment resulted in heart hypertrophy (50% increase in HW/BW - heart weight/body weight).

Another objective of this study was related with investigation of changes in $^{45}Ca^{++}$ transport in rat heart treated with different concentrations of thyroxine (Table 7). It is well known that Ca^{++} together with other ions are implicated in regulation of neuromuscular excitability and in transmision of nerve influx, for triggering muscle contraction (skeletal, heart and smooth muscle).

Hyperthyroid condition is associated with abnormal metabolism of Ca^{++} (i.e. hypercalcemia and hyperphosphatemia).

The reduction of $^{45}Ca^{++}$ uptake is inversely proportional with Thyroxine concentration used for treatment; this can be correlated with the fact that the experimental hyperthyroidism induced with thyroxine in excess leads to an increase of $^{45}Ca^{++}$ uptake in rat heart in such a way that all sites for $^{45}Ca^{++}$ are occupied by the existent Ca^{++} from heart tissue.

Table 1. The values of body and heart weight in controls and thyroxin treated rats

Age (months)	No	Thyroid status	BW (g)	RV (mg)	RV/BW (mg/g)	LV (mg)	LV/BW (mg/g)
27	7	Control	52.1±20*	62±3	0.120±0.08*	274±0.2	0.158±0.04**
27	21	Treated	479±20*	94±15	0.18±0.03	323±5*	0.687±0.08

BW = body weight, RV = right ventricle, LV = left ventricle
*p<0.01 Control/Treated (BW)
**p<0.001 Control/Treated (RV/BW)

Table 2. Activity of myosin ATPase of Pi release in relation with age and Thyroxin treatment (mM Pi/100mg) in 27 months old rat heart

Group	[ATP] 0.5 mM	[ATP] 1 mM	[ATP] 2 mM
Control	5	8	38.5
Treated	21*	27*	87**

*p<0.05; **p<0.001

Table 3. The mean values of 3H Tryptophan uptake in heart of Thyroxin treated rats

Age (months)	RA (cpm/g tissue) in control group	RA (cpm/g tissue) in treated rats		
		Thyroxine [0.25ug]	Thyroxine [0.50ug]	Thyroxine [1.0ug]
27	348	454	410	373

RA = radioactive uptake (cpm/g tissue)

Table 4. Mean values of 3H Tryptophan uptake in control rat heart and in rat heart treated with Thyroxin and incubated with GH3

Age (months)	RA (cpm/g tissue) in control group	RA (cpm/g tissue) in Thyroxin treated rats (incubated with GH3)		
		Thyroxine [0.25ug]	Thyroxine [0.50ug]	Thyroxine [1.0ug]
27	679	830	975	1070

Table 5. Mean values of SH myosin content in relation with Thyroxin treatment (mM/g tissue)

Age (months)	SH myosin (mM/g tissue) in control group	SH myosin (mM/g tissue) in treated rats
27	94±1	178±2*

Table 6. Mean values of 3H Tryiptophan (cpm/g tissue) uptake in skeletal muscle from rats treated with different concentrations of Thyroxin

Age (months)	RA (cpm/g tissue) in control group	RA (cpm/g tissue) in treated rats		
		Thyroxine [0.25ug]	Thyroxine [0.50ug]	Thyroxine [1.0ug]
27	679	418	366	298

RA = radioactive uptake (cpm/g tissue)

Table 7. The uptake of 45Ca++ in heart from Control and thyroxin treated rats with different concentrations

Age (months)	RA (cpm/g tissue) in control group	RA (cpm/g tissue) in treated rats		
		Thyroxine [0.25ug]	Thyroxine [0.50ug]	Thyroxine [1.0ug]
27	324	276	238	179

RA = radioactive uptake (cpm/g tissue)

CONCLUSIONS

Thyroxine administrated in excess in rat influences protein metabolism and ATP-ase activity as well as membrane permeability.
The effect of Gerovital H3 from the incubation media of heart fragments from rats treated with thyroxine in excess may have a clinical relevance concerning the utilization of Gerovital H3 therapy in the patients with hyperthyroidism in order to improve the heart metabolical functions.

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