Scientific Papers. Series D. Animal Science. Vol. LVI
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN-L 2285-5750

# MOLECULAR BIOLOGY STUDIES OF PROTEIN SYNTHESIS AND $^{45}$CA TRANSPORT IN STRIATED AND CARDIAC MUSCLE OF RABBITS WITH EXPERIMENTAL GLANDULARDYS FUNCTIONS

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***Abstract***

*The aim of our study was related with investigation of hormone excess treatment (Thyroxine and Hydrocortisone) upon heart and skeletal muscle metabolism, looking for modifications in nucleic acid and protein synthesis by means of radioisotope methods of 45Ca,3H Tryptohane and 3H Uridine uptake. In young heart rabbits treated with 0.625mg Hydrocortisone, a reduction in 45Ca uptake has been recorded ,while in old rabbits there is a progressive increase in 45Ca uptake as a function of dose of hormone used. As far as skeletal muscle is concerned, both in young and old rabbits treated with Hydrocortisone there is a progressive decline in cellular receptors for 45Ca affinity. An increase in the uptake of 3H Uridine for 0.15 and 1.50 mg thyroxine in rabbit heart has been recorded while at 0.75mg there is a decrease in the uptake. In skeletal muscle, there is a progressive increase in RNA synthesis under hormone impulse as a function of admitted dose in comparison with Controls.*

***Key words:*** *aging rabbit heart,aorta, thyroxine, hydrocortisone,45 Ca, skeletal muscle.*

## INTRODUCTION

Hypertrophy is an adaptive response of ventricular myocardium to a variety of physiological and pathological stimuli. Removal of the stimulus responsible for the growth process usually results in a return of cardiac mass to normal. For example, regression of thyroxine administration is a reproducible and extensively studied model for the development of cardiac hypertrophy. We have previously shown that thyroxine-induced cardiac hypertrophy is associated with both increased fractional and absolute rates of in vivo protein synthesis, as well as increases in the efficiency and capacity for protein synthesis (Parmacek et al., 1986). Measurements of protein degradative rates in this hormone induced model of hypertrophy vary, but in all cases, hypertrophy occurs because the accelerated rate of new protein synthesis exceeds the rate of degradation (Morgan et al., 1987).

Corticosteroids also may increase vascular tone by trophic effects, i.e., hypertrophy or hyperplasia of VSMCs. More VSMCs or larger VSMCs in a given vessel may allow enhanced contractile responses to angiotensin II or norepinephrine (Michael, 1999).

The study of molecular basis of hormone action represents an important place in fundamental and applicative research. A great interest is represented by this study in Gerontology field, taking into account the fact that ageing process evolves with a series of modifications at endocrine level (Peeters, 2008).

Many hormones affect transmembranar transport of ions and molecules modifying target tissue metabolisms (Peeters, 2008).

Thyroid hormones affect muscle metabolism, the ionic content of muscle fiber as well as

blood irrigation at the level of cardiac and skeletal muscle (Moriun et al., 1983).
Adrenal steroids modify muscle metabolism and Na/K balance (Bonnin et al., 1983)
These hormones not only modulates molecular processes at the level of cardiac and skeletal muscle but they play a decisive role in human pathology of these tissues (Carter et al., 1982).
The aim of study was related with the investigation of the effects of hormone excess (hydrocortisone and thyroxine) upon skeletal and cardiac muscle metabolism from rabbits of different ages, following modifications in cellular metabolism(nucleic acid and protein synthesis with radioisotope methods of uptake $^{3}$H Uridine and $^{3}$H Tryptophan.

## MATERIALS AND METHODS

Our study has been done on fresh striated and cardiac muscle from rabbits treated in excess with hormones and also, from age matched controls as well as in glycerinated muscle.

**Animal groups:**

Our study has been done on 36 male rabbits (24-37 months old) out of these 18 have been treated in excess with: Thyroxine (9 treated and 9 controls) and 18 with Hydrocortisone (9 treated and 9 controls).

**Methods:** Two weeks treatment with Thyroxine has been done with 0.25 ug/kg body weight, 0.50 ug/kg body weight, 1 ug/kg body weight.
Hydrocortisone treatment has been applied for two weeks in the following doses: 0.125 mg, 0.625 mg, 1.25 mg.

The biology of cardiac and striated muscle tissue have been investigated with radioisotope methods using $^{3}$H Tryptophane and $^{45}$Ca uptake.

**Experimental protocol**

For experiments, $^{3}$H Tryptophane uptake and $^{45}$Ca transport, tissue fragments between 50-100 mg each have been used. Tissue fragments have been introduced in Hanks medium (1 ml in a test tube) and left for preincubation at 37$^{o}$C for ½ hour according to the published technique (Revnic, 1993).
In each test tube we placed 10ul 3H Tryptophane from 500 mCi/ml solution. The specific activity was 26 mCi/mg.
The next step is related with incubation of biological samples with isotope for 1 ½ hour at 37$^{o}$C.
Next step was related with extraction of samples with 2N HCl. Tissue fragments have been taken out from incubation medium,being placed into test tubes with 1 ml HCl 2N.
Biological samples have been kept in extraction medium for 24 hours.
Next day the radioactivity in incubation and extraction media has been measured 96 vials with 5 ml scintillation liquid. In each vial 0.2 ml from incubation and extraction medium has been placed. The radioactivity has been estimated with a Beta Berthold Liquid Scintillation Counter for $^{3}$H on three channels (Revnic, 1993).
The same procedure has been used for the uptake of $^{3}$H Uridine and $^{45}CaCl_2$.

## RESULTS AND DISCUSSIONS

Table 1. The values of body weight and heart from controls and thyroxin treated rabbits

| Age (months) | Nr. | Thyroid status | Body weight | RV | RV/BW | LV | LV/BW |
|---|---|---|---|---|---|---|---|
| 24 | 9 | Control | 531+/-23 | 64+/-3 | 0.120+/-0.08* | 276+/-2 | 0.519 |
| 24 | 9 | Treated | 478+/-20 | 94+/-15 | 0.196+/-0.03 | 324+/-5 | 0.677+/-8 |

p<0.01C/T(BW)
*p<0.001 C/T (VD/BW)

Table 2. The mean values of $^{3}$H Tryptophane uptake in rabbit heart from control and thyroxine treated rabbts

| Heart | Age | Control | Thyroxine treated rabbits | | |
|---|---|---|---|---|---|
| | | | 0.250ug | 0.50ug | 1.0ug |
| | 24 | 678 | 414 | 360 | 290 |

In table I are presented the values of body weight and heart from controls and thyroxin treated rabbits.

We can observe a decrease in 3H Tryptophane in rabbits treated with different doses of thyroxine versus controls, accounting for a reduction in protein synthesis. Our data are in accordance with the literature (Peeters, 2008) which have shown that thyroxine hormone administered in excess inhibits protein synthesis.

Concerning the uptake of $^{3}H$ Uridine an increase in $^{3}H$ Uridine uptake has been recorded in rabbits treated with 0.50 ug Thyroxine. We can conclude that for 0.50 ug thyroxine a stimulatory effect on mRNA synthesis.

Another objective of our study was related with pointing out of modifications in $^{45}Ca$ transport in rabbits heart of treated rabbits with different doses of Thyroxine.

It is known that $Ca^{++}$ together with other ions are implicated in regulation of neuromuscular sensitivity and in transmission of nerve influx, triggering the contraction of atrial muscle.

Hyperthiroidism is associated with an abnormal metabolism of Ca+2 .

Our experiments of incubation of heart fragments from treated rabbits with different doses of thyroxine, with $^{45}Ca$ have pointed out a decrease in $^{45}Ca$ uptake dose dependent versus controls.

Table 3. $^{45}Ca$ uptake in heart of old Controls and Thyroxin treated rabbits

| **Heart** | **Age** | **Control** | **Thyroxine treated rabbits** | | |
|---|---|---|---|---|---|
| | | | 0.250ug | 0.50ug | 1.0ug |
| | 24 | 320 | 270 | 235 | 178 |

The reduction in the uptake of $^{45}Ca$ is inversely proportional with thyroxine dose administrated and it can be correlated with the fact that hypertiroidism induced with hormone excess leads to an increase in calcium in rat heart in such a way that all binding sites available for $Ca^{2+}$ are already engaged by the existing $Ca^{2+}$ from cardiac tissue.

Table 4. The uptake of $^{45}Ca$ (dpm/g) in young and old rabbits treated with hydrocortisone

| **Heart** | **Age** | **Control** | **Thyroxine treated rabbits** | | |
|---|---|---|---|---|---|
| | | | 0.150mg | 0.625mg | 1.250mg |
| | 8 | 2.6 | 2.2 | 1.7 | 2.7 |
| | 24 | 1.6 | 2.6 | 2.9 | 4.8 |

Table 5. The uptake of $^{45}Ca$ (dpm/g) in young and old rabbits treated with hydrocortisone

| **SKELETAL MUSCLE** | **Age** | **Control** | **Thyroxine treated rabbits** | | |
|---|---|---|---|---|---|
| | | | 0.150mg | 0.625mg | 1.250mg |
| | 8 | 5.5 | 4.4 | 5.1 | 2.5 |
| | 24 | 4.1 | 5.0 | 3.7 | 3.9 |

The administration of thyroxine in the rabbit produces cardiac hypertrophy in vivo by increasing protein synthetic rates to a greater degree than degradative rates, resulting in the net accumulation of cardiac protein (Parmacek et al., 1986). Under the treatment with thyroxine in excess, there is a reduction in $^{3}H$ tryptophane uptake in rabbits heart versus controls,accounting for a decrease in protein synthesis. The lowest uptake has been recorded for 0.50 mg thyroxine dose. Our results support the findings of previous investigators regarding the effect of thyroxine administration on protein synthesis during the development of cardiac hypertrophy (Morgan et al., 1987). Our data have pointed out an age dependent reduction in $^{45}Ca$ uptake in heart of rabbits belonging to Control group in comparison with age matched.

In young rabbits treated with treated with 0.625 mg Hydrocortisone a reduction in the uptake of 45Ca was recorded, while in old rabbits there is a progressive uptake in $^{45}Ca$ as a function of administrated dose. That means that with aging,

and in conditions of hormone excess a lot of disturbances in heart metabolism occurred expressed by an increase in the affinity of cellular receptors for $^{45}Ca$ with important consequences at the functional level. Concerning skeletal muscle, both in young and old rabbits treated with hydrocortisone there is a decline in $^{45}Ca$ uptake.

Corticosteroids foster hypertension not only by enhancing renal sodium reabsorption but also by augmenting vascular tone. Corticosteroids augment vascular tone by potentiating the actions of vasoconstrictor hormones and by direct actions on VSMCs that are independent of vasoconstrictor hormone.

## CONCLUSIONS

Investigation of striated and cardiac muscle tissue biology have pointed out that hormones in excess administrated in rabbits lead to an increase in protein metabolism as well as in membrane permeability.

In conditions of hydrocortisone administrated in excess, there is an increase in cell receptors affinity for $^{45}Ca$ with important consequences on the functional level, while in young rabbits treated in excess with hydrocortisone, there is a decline in $^{45}Ca$ uptake.

Under the treatment with thyroxine in excess, there is a reduction in $^{3}H$ tryptophane uptake in rabbits heart versus controls,accounting for a decrease in protein synthesis. The lowest uptake has been recorded for 0.50 mg thyroxine dose.

In skeletal muscle, on the contrary, there is an increase in the uptake of $^{3}H$ tryptophane in thyroxin treated rabbits versus controls.

There is an age dependent modification in affinity of cell receptors for $^{45}Ca$ in rabbits treated with excess of corticosteroids.

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