BIOCHEMICAL PHARMACOLOGY 75 (2008) 900–906

available at www.sciencedirect.com

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journal homepage: www.elsevier.com/locate/biochempharm

Reduced expression of thyroid hormone receptors and beta-adrenergic receptors in human failing cardiomyocytes

Pietro Amedeo Modesti [a,*], *Matilde Marchetta* [a], *Tania Gamberi* [a], *Gianluca Lucchese* [b], *Massimo Maccherini* [b], *Mario Chiavarelli* [b], *Alessandra Modesti* [a]

[a] *Department of Critical Care Medicine, University of Florence, Viale Morgagni 85, 50134 Florence, Italy*
[b] *Department of Cardiothoracic Surgery, University of Siena, Siena, Italy*

ARTICLE INFO

Article history:
Received 10 August 2007
Accepted 9 October 2007

Keywords:
Heart failure
Cardiomyopathy
3,5,3′-Triiodo-L-thyronine
Myocytes

ABSTRACT

An altered thyroid hormone profile has been reported in patients with congestive heart failure. However, information regarding the status of thyroid hormone receptors in human failing cardiomyocytes is lacking. Therefore the expression of thyroid hormone and beta-adrenergic receptors was investigated in human ventricular cardiomyocytes isolated from patients with end-stage heart failure (FM, $n = 12$), or from tentative donors (C, $n = 4$). The expression of thyroid (TRalpha1, and TRbeta1) and beta-adrenergic receptors (ARB1 and ARB2) was measured at both the gene, and at the protein level.

In FM the reduced mRNA expression of ARB1 ($p < 0.05$, −37%) and ARB2 ($p < 0.05$, −42%) was associated with a reduction of the messenger for TRalpha1 ($p < 0.05$, −85%) and TRalpha2 ($p < 0.05$, −73%). These findings were confirmed at the protein level for ARB1, ARB2 and TRalpha1.

These data reveal that in human heart failure the reduction of beta-adrenergic receptors is associated with reduced expression of both TRalpha1 and TRalpha2 isoforms of thyroid hormone receptors.

1. Introduction

Many of the clinical manifestations of thyroid diseases are mediated by changes in cardiovascular hemodynamics [1]. These changes, in particular heart rate, are known to improve in response to treatment with beta-adrenergic receptor antagonists [2]. 3,5,3′-Triiodo-L-thyronine (T3) was indeed reported to affect cardiac function by enhancing the expression of genes involved in the regulation of beta-adrenergic signalling, such as sarcoplasmic reticulum Ca^{2+} ATPase [3], and of the beta-adrenergic receptor itself [4], in addition to its effects on the expression of other cardiac specific genes [3–7]. This aspect is particularly important in the treatment of thyroid storm disease where the use of beta-adrenergic antagonism is a priority. On the other hand, human studies have shown that the progression to failure is characterized not only by a significant reduction of adrenergic receptors [8], but also by cardiac changes in phenotype and gene expression similar to those described for hypothyroidism [9–11]. A low triiodiothyronine (T3) syndrome, characterized by low circulating levels of the biologically active form of T3 in the presence of normal thyrotropin (TSH) and of thyroxine was indeed reported to occur in approximately 30% of patients with advanced heart failure [12]. However, a reduced expression of cardiac genes stimulated by thyroid hormone was observed also in explanted hearts removed from patients who were clinically and chemically euthyroid [9,13,14]. Therefore a potential alteration of cardiac T3 signal transduction in failing heart was hypothesized so that several studies investigated the expression of receptors for thyroid hormone

* *Corresponding author*. Tel.: +39 055 7949376; fax: +39 055 7949376.
E-mail address: pamodesti@unifi.it (P.A. Modesti).
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doi:10.1016/j.bcp.2007.10.011

in homogenated human failing hearts. However, those studies gave conflicting results because either a decrease [15,16] or an increase [17] in the expression of the physiologically active alpha1 isoform of T3 receptor was found. Most importantly, notwithstanding the recognized effects of T3 on cardiac contractility, investigations assessing the T3 receptor population on cardiac contractile cells in human heart failure are lacking. Therefore the aims of the present study were to investigate the expression of TRs and beta-adrenergic receptors in ventricular cardiomyocytes isolated from human failing hearts.

2. Materials and methods

2.1. Subjects investigated

Hearts were obtained from 12 patients ($n = 12$, aged 58 ± 2 years) with end-stage dilated cardiomyopathy (DCM) scheduled to undergo cardiac transplantation. Diagnosis of dilated cardiomyopathy was based on clinical and echocardiographic examination and coronary angiography. Subjects with arterial hypertension, history of ischemic heart disease or myocardial infarction, and echocardiographic evidence of valve or congenital heart disease were not considered for the study. Exclusion criteria for patients and controls were also the history of thyroid disease and therapy with amiodarone, thyroid hormone and use of dopamine during a period of 4 weeks preceding the study. Hearts obtained from four putative organ donors ($n = 4$, aged 54 ± 5 years) with no histories or signs of heart disease, whose hearts could not be transplanted because of non-cardiac reasons (non-failing hearts, NF) served as controls. Characteristics of subjects investigated are reported in Table 1. Serum TSH, free T_4 (fT_4), and free T_3 (fT_3) levels were determined by means of commercial kits (Abbott, Chicago, USA).

The protocol of this study complies with the principles of the Helsinki declaration and was approved the review committee of our Institution and by MIUR (Ministero Italiano Università e Ricerca, project 2003, no. 2003063257). All patients gave their informed written consent to participate and to have their hearts used for the study.

2.2. Cardiac tissue and myocyte isolation

Heart was placed in ice-cold oxygenated physiological salt solution immediately after removal. One gram transmural specimens was taken within 10 min of explantation from the central portion of left ventricular free wall, immediately frozen in liquid nitrogen, and stored at −80 °C until use. The heart was then immediately transported to the laboratory where myocytes were isolated with enzymatic digestion method as previously described [18,19]. In details a coronary artery branch was cannulated and perfused for 10–15 min with a low calcium buffer (Basic Buffer, BB). Basic Buffer was composed by Jocklic buffer (Sigma M0518) supplemented with 0.3 g/L glutamine (Sigma G6201), 1.25 g/L taurine (Sigma T0625), 2.9 mmol/L HEPES (Sigma H3375), 20 U/L insulin, 10 mL/L penicillin–streptomycin (Sigma P0781, 5000 U/mL penicillin and 5 mg/mL streptomycin), and 7.5 μmol/L $CaCl_2$, pH 7.4. The Basic Buffer was previously leaked through filters by 0.2 μm pore. Perfusion was then switched to collagenase solution for 20–25 min. Collagenase solution was composed by collagenase type II (Sigma C6885, 100 U/mL, 20 mL/min) in Basic Buffer. The collagenase-perfused tissue was then minced and shaken for approximately 20 min in Basic Buffer. The suspension was then filtered through a sterile gauze to separate cells from tissue mass. The suspension was allowed to sediment for 10 min to separate dead cells from those alive that sediment more fastly. Supernatant was aspirated off up to 15 mL. Basic Buffer was then added up to 30 mL and the procedure was repeated once. Pellets were then resuspended and smears were made. Rod shaped, trypan blue excluding cells constituted nearly 70% of all myocytes. Nonmyocytes accounted for less than 2% of the cells in all groups.

Table 1 – Clinical characteristics of subjects investigated

	NF	DCM
Age (years)	54 ± 5	58 ± 2
Sex (M/F)	3/1	9/3
Body surface area (m^2)	1.8 ± 0.1	2.0 ± 0.2
New York Heart Association class (III/IV)	–	6/0
Left ventricular end diastolic diameter index (mm/m^2)	26 ± 4	44 ± 7*
Left ventricular mass index (g/m^2)	100 ± 15	270 ± 50*
Ejection fraction (%)	60 ± 3	26 ± 4*
Cardiac index ($L/(min\ m^2)$)	–	1.8 ± 0.3
Mean pulmonary artery pressure (mmHg)	–	29 ± 14
Left ventricular end diastolic pressure (mmHg)	–	16 ± 3
Pulmonary capillary wedge pressure (mmHg)	–	19 ± 11
End systolic stress ($kdyn/cm^2$)	–	100 ± 20
End diastolic stress ($kdyn/cm^2$)	–	22 ± 5
Serum-free triiodothyronine (fT3) (pg/mL)	3.1 ± 0.2	2.5 ± 0.3*
Serum-free thyroxine (fT4) (mg/L)	17.5 ± 3.5	16.0 ± 3.0
Thyroid-stimulating hormone (TSH) (μU/mL)	1.45 ± 0.5	1.30 ± 0.8

*$p < 0.05$ vs. NF.

2.3. Reverse transcriptase-polymerase chain reaction

RT-PCR experiments were performed both on cardiac samples and on isolated myocytes. Total amount of RNA was extracted with FastRNA Pro Green Kit (Q-Biogen) and then reverse-transcribed using TaqMAN Reverse Transcription Reagents kit (Applied Biosystem) according to the manufacturer's instructions. The resulting cDNA was then amplified using specific primers for each isoform of the receptors (Table 2) [17,20,21] with GAPDH used as internal standard. Briefly, PCRs were performed using 50 ng of cDNA, 200 μmol/L dNTP, Taq buffer 1×, Taq polymerase 1 U and 50 pmol of each primer, for 40 PCR cycles. Other PCR parameters are reported in Table 2. To ensure that different amounts of PCRs on myocardial biopsies were not due to markedly different mRNA starting concentrations, PCR analysis for GAPDH was performed on serial twofold dilutions of cDNA for each sample. The last dilution