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INDUCTION OF THYROID HORMONE-DEGRADING DEIODINASE IN CARDIAC HYPERTROPHY AND FAILURE

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The similarities between the changes in cardiac gene expression in pathological ventricular hypertrophy and hypothyroidism suggest a role of impaired cardiac thyroid hormone (TH) action in the development of contractile dysfunction during chronic cardiac pressure overload. Here we studied the possible involvement of altered cardiac TH metabolism using a rat model of right-ventricular (RV) hypertrophy induced by pressure-overload. Pathological RV hypertrophy was indicated by decreased mRNA levels of sarcoplasmic reticulum (SR) Ca^{2+}–ATPase type 2a (SERCA2a) and myosin heavy chain α (MHCα), and increased levels of MHCβ mRNA. Enzyme activity of type III deiodinase (D3), which converts T_4 and T_3 to the inactive compounds rT_3 and 3,3'-T_2, respectively, was identified in ventricular tissue. This activity was stimulated up to five fold in hypertrophic RV, but remained unaltered in the non-hypertrophic left ventricle (LV). A low level of type I deiodinase activity was also detected, which decreased significantly in both RV and LV. Stimulation of RV D3 activity was significantly higher in those animals in which hypertrophy progressed to heart failure, compared to animals that developed compensatory hypertrophy. The induction of a cardiac TH-degrading deiodinase may be expected to result in reduced cellular levels of T_3 and thereby contribute to a local hypothyroid state in the hypertrophic and, particularly, in the failing ventricle.

Pathological ventricular hypertrophy caused by chronic overload of the heart is characterized by changes in expression levels of contractile proteins and enzymes involved in intracellular Ca^{2+} regulation. The latter effects are a primary cause of the systolic and diastolic dysfunction seen in pathological hypertrophy. Progressive deterioration of Ca^{2+} homeostasis is thought to be critical in the transition from compensatory hypertrophy to heart failure (1). For many key enzymes the observed changes are similar to those induced by hypothyroidism, e.g., a shift to slower contractile proteins (MHCα to MHCβ, particularly in rodents), repression of SERCA2a and the SR Ca^{2+}-release channel (ryanodine receptor) and up-regulation of the sodium-calcium exchanger and phospholamban (2). An impairment of TH signaling has been suggested as a factor in re-directing gene expression. This is supported by the recent findings of diminished expression of nuclear TH receptors (TR's) in hypertrophic rat hearts (3). Altered conversion or degradation of TH in the cardiomyocyte could be another mechanism resulting in reduced local biological activity of TH in pathological hypertrophy. Here we analyzed the activities of the three known iodothyronine deiodinases as a possible factor in altering the tissue thyroid state of the hypertrophic and failing heart. We show for the first time that D3 activity is present in ventricular tissue, and that this TH-degrading activity is upregulated in pathological hypertrophy.

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Materials and Methods

Animals

Animals were treated according to the national guidelines and with permission of the Animal Experimental Committee of the VU Medical Center Amsterdam, The Netherlands. Male Wistar rats, weighing 170-190 g (Harlan, Zeist, The Netherlands) were housed individually (250 cm^2/animal) and received food and water *ad libitum*. Animals were randomly assigned to the treated (n=30) or control (n=20) group, and given a single subcutaneous injection of either monocrotaline (MCT) (40 mg/kg) or saline. Animals were euthanized with an overdose of halothane after four weeks.

Analyses

Ventricular tissue homogenates were prepared and enzyme activities of deiodinase subtypes D1, D2 and D3 were performed as described previously (4).

Total RNA was isolated using the RNAzol B method (Campro Scientific, Veenendaal, The Netherlands). Serial dilutions of RNA were applied to nylon membranes (Hybond N^+, Amersham) using a vacuum slot-blot system (BioRad, Veenendaal, The Netherlands). Blots were hybridized with [α^{32}P]dCTP-labeled cDNA probes for SERCA2a, MHCα, MHCβ and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Membranes were exposed to phosphor imager screens, scanned and analyzed with ImageQuant software (Molecular Dynamics).

Plasma T_4, freeT_4 (fT_4), T_3 and TSH levels were determined using specific RIA's as described before (5).

Data are presented as means ± SEM. One-way analysis of variance was performed followed by Bonferroni comparison for post hoc analysis using Prism 3.0 software (GraphPad). Differences were considered significant at $p<0.05$.

Results and Discussion

MCT-induced right ventricular hypertrophy and cardiac gene expression

A single dose of MCT induces pulmonary vasculitis resulting in chronic pulmonary hypertension, affecting only the right side of the heart (6). The MCT-treated rat is consequently used as a model for pressure-overload induced RV hypertrophy and ventricular failure (7). In agreement with earlier studies (8,9) we found that approximately half of the animals developed RV hypertrophy (HYP) without signs of heart failure (n=16), whereas in the remaining animals (n=14) the RV hypertrophy progressed to severe congestive heart failure (CHF). The animals in the latter group started losing weight around day 15 and showed pleural effusion and ascites at the time of sacrifice. The degree of RV hypertrophy was significantly higher in the CHF group compared to the HYP group, as indicated by the ratio of RV over LV+septum weight. Compared to controls this parameter was 2.5-fold higher in the CHF group and 2-fold higher in the HYP group (Table 1). The wet/dry ratio of either ventricle was the same in all groups. There was no indication of LV hypertrophy in MCT-treated rats (data not shown). As indicators of the re-direction of gene expression in pathological hypertrophy we determined the RV mRNA levels for the

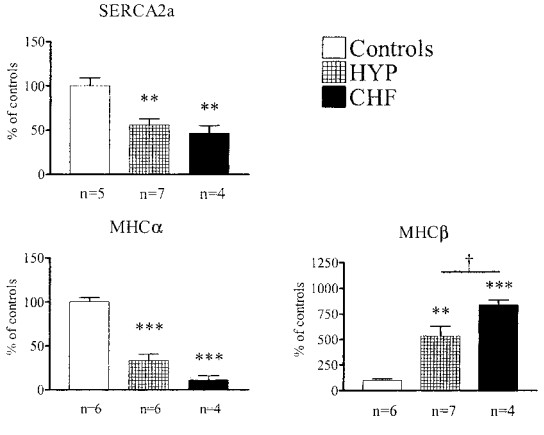

Figure 1: SERCA2a and MHC isoform mRNA levels in right ventricles of treated and untreated rats. Data are means ± SEM and expressed as % of controls relative to GAPDH levels. ** = $p< 0.01$, *** = $p< 0.001$ versus controls, † = $p< 0.01$ HYP versus CHF.

Table 1: Body weight (BW), ratio of RV over LV plus septum wet weight and plasma thyroid hormone values of all groups.

	Controls (n=20)	HYP (n=16)	CHF (n=14)
BW (g)	359 ± 5.5	325 ± 5.4 *	234 ± 6.5*, ††
Ratio	0.21 ± 0.00	0.41 ± 0.02 *	0.50 ± 0.01*, ††
T_4	51.3 ± 2.8	39.8 ± 4.1	27.0 ± 3.4 *, †
fT_4	28.5 ± 0.9	21.1 ± 3.5	10.5 ± 2.2 *, †
T_3	2.12 ± 0.09	2.02 ± 0.07	1.19 ± 0.14 *, ††
TSH	1.73 ± 0.20	1.57 ± 0.15	2.18 ± 0.26

Values are means ± SEM, n = number of animals per group. T_4 and T_3 in nmol/L, fT_4 in pmol/L and TSH in ng/ml. * = $p<0.001$ versus controls, † = $p< 0.05$ and †† = $p<0.001$ versus HYP.

MHC isoforms and SERCA2a. Figure 1 shows that the characteristic shift from the fast MHCα isoform to the slower MHCβ isoform was intermediate in the HYP group, but almost complete in the CHF group. Expression of SERCA2a mRNA was decreased by approximately 50% in both HYP and CHF rats. Similar results concerning the degree of RV hypertrophy and SERCA2a mRNA levels have been reported for rats in which either compensatory hypertrophy or heart failure was induced by ligation of the pulmonary artery (10). There was no decrease in SERCA2a mRNA in LV of these animals and even though there was a shift in MHC isoforms in the CHF group, it was much less pronounced than in RV (Fig.2).

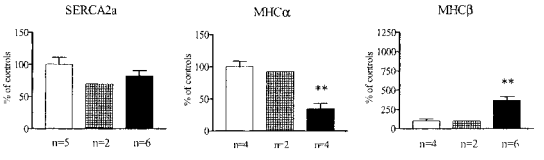

Figure 2: SERCA2a and MHC isoform mRNA levels in left ventricles of treated and untreated rats. Data are means ± SEM and expressed as % of controls relative to GAPDH levels. ** = $p< 0.01$ versus controls. See Fig.1 for column legend.

Plasma thyroid hormone levels

Plasma TH levels were measured at the time of sacrifice. Plasma T_4, fT_4, T_3 and TSH in the HYP group were not different from control values indicating that the observed changes in T_3-responsive genes in compensatory hypertrophic RV are not due to systemic hypothyroidism. The CHF group showed a significant 40-60% reduction of plasma T_4, fT_4 and T_3 with normal TSH levels (Table 1). Such changes are typical of serious, non-thyroidal illness, including chronic heart failure (2). Although Ojamaa et al. recently showed that normalizing plasma T_3 levels in a rat model of LV hypertrophy following myocardial infarction restored the mRNA level

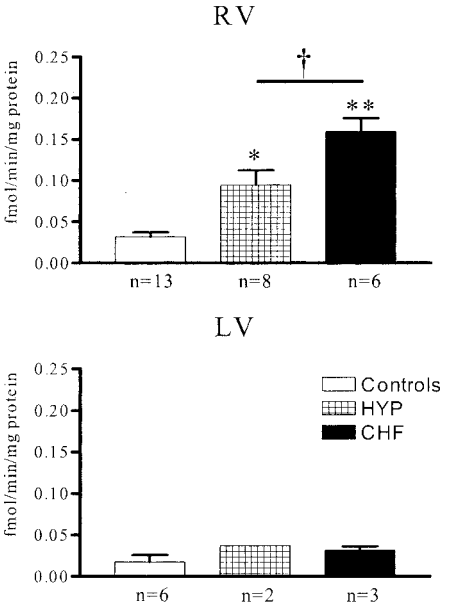

Figure 3: Myocardial D3 activity in right and left ventricular homogenates. Data are means ± SEM. ** = p< 0.001 and * = p< 0.01versus controls, † = p< 0.01 HYP versus CHF.

of MHCβ, those of MHCα and SERCA2a were unaffected (11). Furthermore, using a rat model of pressure-overload induced LV hypertrophy, Kinugawa et al. reported a nearly complete shift in MHC isoform expression and a 50% reduction of SERCA2a mRNA in the absence of changes in plasma TH levels (3). These and our results, therefore, suggest that development of the phenotype of pathological hypertrophy, at least with respect to the MHC and SERCA2a genes, is largely independent of changes in plasma TH levels. However, an additional effect of reduced plasma TH levels in the CHF group on these and other T_3-responsive genes may be expected in view of the LV data in Fig.2.

Expression of deiodinases

Apart from systemic levels, the intracellular availability of TH depends on cellular uptake rates of TH and on the intracellular metabolism of TH. Little is known about the cardiac expression of deiodinases and their possible role in determining TH levels in the heart. Low levels of D1 activity have been reported for cardiomyocytes from neonatal rats (12) and D2 mRNA has been found in the human heart (13), but enzyme activity has not been reported. Previous kinetic studies of cardiac iodothyronines in rats indicated no significant local T_3 production from T_4 (14), suggesting negligible activity of D1 or D2 and/or negligible cardiac uptake of T_4. The latter is supported by the recent observation that overexpression of D2 in cardiomyocytes of transgenic mice does not result in a considerable increase in cardiac T_3 levels (15). The significance of D1 or D2 activity and local cardiac T_3 production therefore seems limited and we directed our attention first to type III deiodinase, which converts T_3 and T_4 to the inactive compounds 3,3'-T_2 and rT_3, respectively (16). Many fetal tissues, as well as the placenta, express high levels of D3 activity, but in adult animals substantial levels of this enzyme have so far only been reported for the brain (16,17,18). Figure 3 shows the D3 activity that was readily detectable in ventricular homogenates, with similar levels in RV and LV from control rats. The activity level increased 3-fold in hypertrophic RV and more than 5-fold in failing RV with no change in D3 activity in the non-hypertrophic LV of the same animals. Given this marked effect on D3 activity we determined the activity levels of the other deiodinases in these homogenates.

We failed to detect significant D2 activity in any of our samples but D1 activity was present, albeit at low levels (the specific activity in control ventricles was approximately 0.1% of that in the livers of these rats). In contrast to D3, the D1 activity was suppressed in the HYP and the CHF group (Fig.4). The similar reduction observed for RV and LV suggests a systemic effect on D1 activity in MCT-treated rats, rather than the hypertrophy-specific effect observed for the D3 activity. Gene expression of both D1 and D3 is positively regulated by T_3 (16) and it cannot be ruled out that the low plasma TH levels contribute to the down-regulation of D1 activity in the CHF group. However, the reduction of D1 activity in the HYP group, where plasma TH levels were normal, suggests a different mode of regulation. Given the already low D1 activity and the considerations presented in the previous paragraph, it is unlikely that the observed effect on this deiodinase will be of significance for cardiac TH metabolism.

The RV D3 activity in the CHF group is about 10% of that in the brain of these animals. Whether this is sufficient to affect cardiac T_3 levels remains to be established, but preliminary analyses of RV tissue T_3 content indicate significant reductions of 30% in the HYP group and more than 50% reduction in the CHF group (M-J. Obregon and W.S. Simonides, unpublished

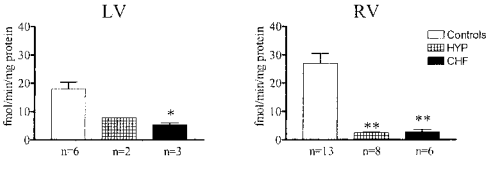

Figure 4: Myocardial D1 activity in right and left ventricular homogenates. Data are means ± SEM. * = p< 0.05, ** = p< 0.001 versus controls. Note that the absolute activities of D1 and D3 can not be compared because of the different assay conditions for both enzymes.

observation). The HYP data are of particular relevance since plasma TH levels are still normal in these animals. D3 is expressed in the fetal stage of different tissues (18) and in non-thyroidal illness (17), and in a recent study we also detected significant D3 activity in the fetal human heart, but very little activity in the adult human heart (R. Hume and T.J. Visser, unpublished observation). The induction or increased expression of various genes typical of the fetal stage is a characteristic aspect of pathological hypertrophy (19), and the enhanced RV-specific expression of D3 in our model of pressure-overload hypertrophy and failure may be part of such a growth program. Nonetheless, since our experiments were performed in whole tissue homogenates we can not exclude the possibility that cells other than cardiomyocytes contribute to the measured activity. In two experiments we assessed ventricular D3 mRNA levels in the three groups by RT-PCR and the results indicated strong upregulation of this message in RV only, in parallel with the observed D3 enzyme activity (data not shown). This suggests that the observed regulation of deiodinase activity is at least in part pre-translational.

Our findings do not negate the importance of possible defects in the T_3 signal transduction system, such as the recently reported down-regulation of TR's in chronic LV-pressure overload in rats (3). However, whether this is enough to create a local hypothyroid-like condition affecting T_3-responsive genes is not certain, since the full effect of hypothyroidism depends on the presence of TR's, which actively repress transcription of TH-responsive genes in the absence of hormone. As also noted in Ref. 3, the bio-availability of T_3 is, therefore, an essential factor determining the thyroid state of the cardiomyocyte. The present data suggest that increased D3 activity in pathological hypertrophy may lead to a reduction of local intracellular T_3 and as such contribute to a hypothyroid condition in the affected myocardium.

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