Cardiac dysfunction in terms of left ventricular mechanical work and energetics in hypothyroid rats

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Running title: Impaired Ca$^{2+}$ handling

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Abstract

We hypothesized that cardiac dysfunction in hypothyroidism is mainly caused by the impairment of Ca\(^{2+}\) handling in excitation-contraction coupling. To prove this hypothesis, we investigated left ventricular (LV) mechanical work and energetics without interference of preload and afterload in the excised, blood-perfused whole heart preparation from hypothyroid rats. We found that LV inotropism and lusitropism were significantly depressed and these depressions were causally related to decreased myocardial oxygen consumption for the Ca\(^{2+}\) handling and for basal metabolism. The oxygen costs of LV contractility for Ca\(^{2+}\) and for dobutamine in the hypothyroid rats did not differ from those in age-matched normal rats. The expression of sarcoplasmic reticulum Ca\(^{2+}\)-ATPase (SERCA2) significantly decreased and that of phospholamban significantly increased. The present results revealed that changes in LV energetics associated with the decreased mechanical work in hypothyroid rats are mainly caused by the impairment of Ca\(^{2+}\) uptake via SERCA2. We conclude that the impairment of Ca\(^{2+}\) uptake plays an important role in the pathogenesis of cardiac dysfunction in hypothyroidism.

Key words: pressure-volume relationship; isomyosin; hypothyroidism; contractility; sarcoplasmic reticulum Ca\(^{2+}\)-ATPase
Introduction

In canine and human hearts, left ventricular myocardial myosin isozyme is V$_3$ in contrast to V$_1$ in normal adult rat hearts (14, 23). It is well known that ATPase activity of V$_1$ myosin isozyme is higher than that of V$_3$ myosin isozyme and that the shortening velocity of V$_1$-dominant myocardium is faster than that of V$_3$-dominant myocardium (15, 35). In the hypothyroid rat (V$_3$-dominant myocardium), the isometric tension of native trabecular preparations substantially decreased (9) and peak developed tension of left ventricular papillary muscle preparations significantly decreased (15), although the isometric tension of glycerinated trabecular preparations hardly changed. We have recently reported that the curvilinearity of the left ventricular end-systolic pressure-volume relationship (ESPVR) decreased in in situ ejecting hearts of hypothyroid rats, indicating a decrease in left ventricular contractility (21). Nevertheless, it is still unknown whether the depressed contractility in in situ ejecting hearts of hypothyroid rats derives from the V$_3$-dominant myocardium, the impairment of calcium (Ca$^{2+}$) handling in excitation-contraction coupling or abnormal pre- and/or afterload conditions due to hypothyroidism.

We hypothesized that cardiac dysfunction in hypothyroidism is mainly caused by the impairment of Ca$^{2+}$ handling in excitation-contraction coupling. To prove this hypothesis, we aimed to investigate cardiac function in terms of the coupling of rat left ventricular mechanical work and energetics without interference of preload and afterload in the excised, cross-circulated whole heart preparations from the hypothyroid rats. This study may lead to a better understanding of the processes that cause cardiac dysfunction in hypothyroidism.

As appropriate indexes for assessing rat left ventricular mechanical work and energetics we adopted PVA$_{mLVV}$ [systolic pressure-volume area, a total mechanical energy per beat at midrange left ventricular volume (mLVV)], the slope (oxygen
cost of PVA) and the VO$_2$ (myocardial oxygen consumption per beat) intercept
(PVA-independent VO$_2$ composed of VO$_2$ for Ca$^{2+}$ handling in excitation-
contraction coupling and for basal metabolism) of the VO$_2$-PVA linear relationship
and the slope (oxygen cost of eEmax) of the linear relation between VO$_2$ for Ca$^{2+}$
handling in excitation-contraction coupling and eEmax (equivalent Emax; an index
for left ventricular contractility), as reported previously  (1, 12, 13, 37, 38).

Methods

The investigation conforms with the Guide for the Care and Use of Laboratory
Animals published by the US National Institutes of Health (NIH Publication No. 85-23,
revised 1996).

Animal preparations

Twenty-four age-matched male crj: Wistar rats (20-21 wks) consisted of two
groups (12 normal rats; body weight, 556 ± 37g vs 12 hypothyroid rats; body
weight, 452 ± 33g). Drinking the water including 8-propylthiouracil (PTU, 0.8
mg/ml) for 4-5 wks made the rat hypothyroidism (15). Decreased T3 and T4 levels
in plasma, which were decreased to 34.2±7.1% and 2.6±0% of normal, respectively,
confirmed hypothyroidism.

Surgical preparation

Experiments were performed on 24 excised, cross-circulated rat heart
preparations from the 12 normal and 12 hypothyroid rats as reported previously (12,
13, 37, 38). In each experiment, two retired breeder male crj: Wistar rats weighing
568 ± 39 g (~32 wks), purchased from Charles River Japan, Inc. (Yokohama, Japan),
were anesthetized with pentobarbital sodium (50 mg/kg, IP) and used as blood
supplier and metabolic supporter rats, respectively. All the rats were heparinized
(1000 U, IV). The beating heart was excised without interruption of coronary
perfusion and supported by cross circulation with the metabolic supporter rat as
previously reported in detail (12). The excised heart was maintained at 37°C.
A thin latex balloon (balloon membrane volume, 0.08 ml) fitted into the left ventricle was connected to a pressure transducer (Life Kit DX-312, Nihon Kohden, Tokyo, Japan) and a 0.5-ml precision glass syringe with fine scales (minimum scale: 0.005 ml). The unstretched balloon volume was below approximately 0.20-0.25 ml. Left ventricular volume was changed and measured by adjusting the intraballoon water volume with the syringe in 0.02-~0.05-ml steps between 0.08 and 0.28 ml. Systolic unstressed volume ($V_0$) was determined by filling the balloon to the level where peak isovolumic pressure and hence PVA (see Data analysis) were zero. The sum of intraballoon water volume and balloon material volume (0.08 ml) was used as an initial estimate of $V_0$. This procedure was repeated during different left ventricular volume-loading runs in control (control vol-run) and during the Ca$^{2+}$ (Ca$^{2+}$ vol-run) or dobutamine infusion at the maximum rate (dobutamine vol-run). $V_0$ was then specifically determined as the volume-axis intercept of the best-fit ESPVR in each vol-run. We obtained the best fit ESPVR and end-diastolic pressure-volume relation (EDPVR) with the two different exponential functions by means of the least squares method (Delta-Graph, DeltaPoint; Montrerey, CA) on a personal computer (1, 32, 38). Correlation coefficients of the best fit ESPVRs and EDPVRs were higher than 0.99.

The left ventricular epicardial electrocardiogram was recorded and the mean heart rate was constantly maintained at 300 beats/min in normal rats and 262 beats/min in hypothyroid rats by electrical pacing of the right atrium (Table 1). Although the EDPVR at 300-beats/min pacing showed an upward shift due to incomplete relaxation, 262-beats/min pacing caused complete relaxation in the hypothyroid rats. Exceptionally, to analyze a pressure-time curve, the mean pacing rate was maintained at 264 ± 13 beats/min in normal (n=10) and 264 ± 9 beats/min in hypothyroid rats (n=10). The systemic arterial blood pressure of the supporter rat served as coronary perfusion pressure (100-130 mmHg). Arterial pH, Po$_2$, and Pco$_2$
of the supporter rat were maintained within their physiological ranges with supplemental oxygen and sodium bicarbonate.

**Oxygen consumption**

Myocardial oxygen consumption was obtained as the product of coronary flow and coronary arteriovenous oxygen content difference. The measurements of coronary flow and arteriovenous oxygen content difference have been previously reported in detail (12). VO$_2$ was obtained as myocardial oxygen consumption divided by heart rate. As shown previously (1,12,13, 37, 38), the VO$_2$-PVA relationship was linear in the rat left ventricle. Its slope represents the oxygen cost of PVA. Its VO$_2$ intercept represents PVA-independent VO$_2$ and is mainly composed of VO$_2$ for Ca$^{2+}$ handling in the excitation-contraction coupling and for basal metabolism (12). The total Ca$^{2+}$ handling VO$_2$ is mainly consumed for Ca$^{2+}$ uptake via the sarcoplasmic reticulum (SR) Ca$^{2+}$-ATPase (SERCA2) (13, 36). The right ventricle was kept collapsed by continuous hydrostatic drainage of the coronary venous return, so that the right ventricular PVA and hence PVA-dependent VO$_2$ were assumed to be negligible (12, 37). The right ventricular component of PVA-independent VO$_2$ was calculated by multiplying biventricular PVA-independent VO$_2$ in each contractile state with the ratio of right ventricular weight divided by the sum of right and left ventricular weights. The right ventricular PVA-independent VO$_2$ was subtracted from the total VO$_2$ to yield left ventricular VO$_2$.

The left ventricle including the septum and the right ventricle were weighed to normalize left ventricular volume. They were 1.07 ± 0.08 and 0.29 ± 0.06 g in normal rats (n=12) and 0.85 ± 0.10 and 0.21 ± 0.03 g in hypothyroid rats (n=12). The left and right ventricular weights were significantly different between the two groups.

**Experimental protocol**

Left ventricular pressure, VO$_2$ and PVA data during isovolumic contractions were obtained at five to six different volumes (mean volume range: 0.17 ± 0.02
ml/g) (vol-run) in each normal and hypothyroid rat heart. Left ventricular volume was increased in steps up to an end-diastolic pressure of 10 mmHg in a control vol-run. The “control vol-run” was performed without any inotropic interventions. After the control vol-run, a Ca^{2+}-induced (Ca^{2+} ino-run; n=6) or a dobutamine-induced different inotropic run (dobutamine ino-run; n=6) was performed at mLVV [0.16 ml= water volume infused into the balloon (=0.08 ml) plus \( V_0 (=0.08 \text{ ml}) \)] in normal and hypothyroid rats (n=12 each). Mean values for mLVV and \( V_0 \) (normalized for 1g) were 0.16 (± 0.01) and 0.076 (±0.005) ml/g in normal rats (n=12), and 0.19 (±0.02) and 0.094 (±0.011) ml/g in hypothyroid rats (n=12). These values were significantly different between the two rat groups due to the significant difference in left ventricular weight (Table 1). The infusion rates of 1% CaCl_2 solution and 66 µM of dobutamine were increased in steps up to 10-20 ml/hr (n=6) and up to 4-10 ml/hr (n=6) in the two rat groups (n=12 each). The “Ca^{2+} vol-run” or “dobutamine vol-run” was performed during Ca^{2+} or dobutamine infusion at the maximum rate. The measured blood Ca^{2+} concentration reached 3.1-8.1 mM. The calculated blood dobutamine concentration reached 1.1-2.4 µM under a coronary flow rate of 3-4 ml/min. In the normal rats, mean \( V_0 \) was 0.075 ± 0.004 ml/g in control vol-run and 0.075 ± 0.004 ml/g in Ca^{2+} vol-run (n=6), and 0.076 ± 0.006 ml/g in control vol-run and 0.076 ± 0.006 ml/g in dobutamine vol-run (n=6). There were no significant differences in mean \( V_0 \) values between control and Ca^{2+} vol-runs and between control and dobutamine vol-runs. In the hypothyroid rats, mean \( V_0 \) was 0.093 ± 0.011 ml/g in control vol-run and 0.092 ± 0.010 ml/g in Ca^{2+} vol-run (n=6), and 0.094 ± 0.011 ml/g in control vol-run and 0.095 ± 0.010 ml/g in dobutamine vol-run (n=6). There were no significant differences in mean \( V_0 \) values between control and Ca^{2+} vol-runs and between control and dobutamine vol-runs. Each \( V_0 \) value of control, Ca^{2+} and dobutamine vol-runs in the normal and hypothyroid rats is summarized in Table 2.
Cardiac arrest was induced by infusing KCl (1 M) into the coronary perfusion tubing at a constant rate in the two rat groups (n=7 each out of 12 rats), which was adjusted to abolish electrical excitation under monitoring ventricular electrocardiograms but not to generate any KCl-induced constriction of coronary vessels. VO$_2$ and PVA data were obtained by minimal volume loading to avoid volume-loading effects on VO$_2$ data.

In every vol-run, ino-run, and KCl arrest-run, a steady state, where left ventricular pressure, coronary arteriovenous O$_2$ content difference and coronary flow were stable, was reached 3 min after changing left ventricular volume or after KCl-induced arrest, or 6 min after changing the infusion rate of Ca$^{2+}$ or dobutamine. In each steady state, data were sampled at 500 Hz for 2 sec simultaneously, and the sampling was usually repeated 3 times at intervals of 0.5-1 min.

Data analysis

We attempted to fit experimentally obtained left ventricular pressure-volume data to the two different exponential equations to obtain ESPVRs and EDPVRs and thus determined PVA by subtracting the area under the EDPVR from the area under the ESPVR (1, 32, 38).

Based on our previous proposal (1, 32, 37, 38), we obtained control ESPVR and EDPVR, end-systolic pressure at mLVV (ESP$_{mLVV}$) and end-diastolic pressure at mLVV (EDP$_{mLVV}$), and PVA$_{mLVV}$ to assess left ventricular mechanical work and energetics in the two groups.

Oxygen cost of left ventricular contractility

We obtained the specific best-fit curves for the observed ESP$_{mLVV}$ and ESP (0 mmHg) at specifically determined $V_0$ with the best-fit ESPVR functions obtained by the control vol-runs and maximum dobutamine or Ca$^{2+}$ vol-run and for the observed EDP$_{mLVV}$ and EDP (0 mmHg) at specifically determined $V_0$ with the best-fit EDPVR function obtained by the control vol-runs and maximum dobutamine or
Ca\textsuperscript{2+} vol-run by the least squares method and calculated PVA\textsubscript{mLVV} during dobutamine or Ca\textsuperscript{2+} infusion on a personal computer (1, 32, 37, 38). The parallelism of the VO\textsubscript{2}-PVA linear relation during dobutamine (1) or Ca\textsuperscript{2+} infusion (12, 32, 37) has been confirmed in normal rat hearts. In the present study, we confirmed the parallelism of the VO\textsubscript{2}-PVA linear relation, i.e., there were no significant differences in the slopes of the VO\textsubscript{2}-PVA linear relations before and during dobutamine infusion (see Fig. 3B & D) or before and during Ca\textsuperscript{2+} infusion in each normal and hypothyroid rat heart. Each slope of the VO\textsubscript{2}-PVA linear relations of the normal and hypothyroid rats in control, Ca\textsuperscript{2+} and dobutamine vol-runs is summarized in Table 3. In only one normal heart, the slope showed a significant difference between control and dobutamine vol-run. There were no significant differences in the mean slopes of the VO\textsubscript{2}-PVA linear relations in the normal rat hearts (n=12) between control [(1.16 ± 0.08) x 10\textsuperscript{-2} \textmu{}lO\textsubscript{2}•mmHg\textsuperscript{-1}•ml\textsuperscript{-1}] and Ca\textsuperscript{2+} vol-runs [(1.19 ± 0.11) x 10\textsuperscript{-2} \textmu{}lO\textsubscript{2}•mmHg\textsuperscript{-1}•ml\textsuperscript{-1}] and between control [(1.14 ± 0.12) x 10\textsuperscript{-2} \textmu{}lO\textsubscript{2}•mmHg\textsuperscript{-1}•ml\textsuperscript{-1}] and dobutamine vol-runs [(1.00 ± 0.19) x 10\textsuperscript{-2} \textmu{}lO\textsubscript{2}•mmHg\textsuperscript{-1}•ml\textsuperscript{-1}]. There were no significant differences in the mean slopes of the VO\textsubscript{2}-PVA linear relations in the hypothyroid rat hearts (n=12) between control [(1.25 ± 0.18) x 10\textsuperscript{-2} \textmu{}lO\textsubscript{2}•mmHg\textsuperscript{-1}•ml\textsuperscript{-1}] and Ca\textsuperscript{2+} vol-runs [(1.10± 0.17) x 10\textsuperscript{-2} \textmu{}lO\textsubscript{2}•mmHg\textsuperscript{-1}•ml\textsuperscript{-1}] and between control [(1.34 ± 0.40) x 10\textsuperscript{-2} \textmu{}lO\textsubscript{2}•mmHg\textsuperscript{-1}•ml\textsuperscript{-1}] and dobutamine vol-runs [(1.26 ± 0.30) x 10\textsuperscript{-2} \textmu{}lO\textsubscript{2}•mmHg\textsuperscript{-1}•ml\textsuperscript{-1}]. Based on the parallelism, the lines including all VO\textsubscript{2}-PVA data obtained during dobutamine or Ca\textsuperscript{2+} infusion in steps at the mLVV, were drawn in parallel to the control VO\textsubscript{2}-PVA relation line, as described previously (12, 32, 37, 38). The gradually increased VO\textsubscript{2}-intercept values (PVA-independent VO\textsubscript{2} values) of the lines proportional to the enhanced left ventricular contractility induced by dobutamine or Ca\textsuperscript{2+}, were obtained by this procedure.

Our recently proposed index for the left ventricular contractility, equivalent E\textsubscript{max} (eE\textsubscript{max}) was defined as an ESP-V ratio of the specific virtual triangular
PVA_{mlLVV} that is energetically equivalent to the real PVA_{mlLVV} \cite{1, 32, 37, 38}. E_{Emax} is calculated by 2PVA_{mlLVV}/\text{ESV}_{mlLVV}^{2}. Mean end-systolic volume (\text{ESV}=\text{mlLVV-V}_0) at mean mLVV (0.16 \pm 0.01 \text{ ml/g}) (\text{ESV}_{0.16}) was 0.076 \pm 0.005 \text{ ml/g in normal rats (n=12)}, and mean ESV at mean mLVV (0.19 \pm 0.02 \text{ ml/g}) (\text{ESV}_{0.19}) was 0.10 \pm 0.01 \text{ ml/g in hypothyroid rats (n=12)}. Mean values of ESV_{mlLVV} were not significantly different between normal and hypothyroid rats. The oxygen cost of left ventricular contractility was the slope of the relationship between PVA-independent VO_2 and E_{Emax}, indicating changes in VO_2 consumed for Ca^{2+} handling in excitation-contraction coupling per unit change in the contractility under the constant basal metabolism \cite{1, 32, 37, 38}.

\text{Delta E}_{Emax}/Ca^{2+} or \text{delta E}_{Emax}/dobutamine was calculated as the ratio of increases in left ventricular contractility for Ca^{2+} or dobutamine vs. the concentrations of Ca^{2+} or dobutamine, respectively.}

\text{Analyses of one beat left ventricular pressure-time curve by hybrid logistic function and logistic function}

To evaluate the left ventricular systolic and diastolic functions, we analyzed the contraction rate (+dP/dt_{max}), relaxation rate (-dP/dt_{max}) and their ratio (+dP/dt_{max}) / (-dP/dt_{max}) from a best-fit function to one beat left ventricular pressure-time curve at mLVV during contraction and relaxation with our proposed “hybrid logistic function” \cite{24} in normal (n=10) and hypothyroid rats (n=10). To evaluate left ventricular end-diastolic relaxation rate or lusitropism, we analyzed the “logistic” time constant (T_L) and the conventional “exponential” time constant (T_E) from respective best-fit functions to the one beat left ventricular pressure-time curve at mLVV during relaxation with our proposed “logistic function” \cite{25} and the monoexponential function in normal (n=10) and hypothyroid rats (n=10). Both time constants are decreased as the heart rate increases, and thus were compared under the same pacing rate condition of 264 beats/min.
Membrane proteins from the left ventricular myocardium of each heart were isolated as described previously by Yoshida et al (40). The frozen hearts were homogenized and centrifuged at 1,000 x g for 10 min. The supernatants were centrifuged at 100,000 x g for 60 min at 4°C. The 100,000g pellets were cellular membrane fractions and used for immunoblotting of SERCA2 and phospholamban.

Membrane proteins (20-25 µg/lane) were separated on SDS-polyacrylamide gels (10% for SERCA2 and 15% for phospholamban) in a minigel apparatus (Mini-PROTEAN II, Bio-Rad), and transferred to polyvinylidene difluoride membranes. The membranes were blocked (4% Block Ace, Dainippon Pharmaceutical Co., Osaka) and then incubated with anti-SERCA2 antibody (1:1000 dilution, Affinity Bio Reagents) or anti-phospholamban antibody (1:2000 dilution, Upstate Biotechnology). The antigens were detected by the luminescence method (ECL Western blotting detection kit, Amersham) with peroxidase-linked anti-mouse IgG (1:2000 dilution). After immunoblotting, the film was scanned with a scanner, and the intensity of the bands was calculated by NIH image analysis.

The left ventricular myocardium from each heart was frozen and stored at –70°C. Myosin was extracted from 50 mg of the left ventricular myocardium and three myosin isozymes (V₁, V₂ and V₃) were separated by 3.7% polyacrylamide gel electrophoresis in the presence of pyrophosphate according to a slightly modified procedure (21) of Hoh et al (14).

Statistics

Analysis of covariance (ANCOVA) was applied to compare the two regression lines of left ventricular VO₂ on PVA in each heart in control vol-run and dobutamine or Ca²⁺ vol-runs. Comparisons of paired and unpaired individual values
were performed by paired and unpaired t-test, respectively. Multiple comparisons were performed by ANOVA and Bonferroni’s posthoc analysis. A value of $P < 0.05$ was considered statistically significant. All data are expressed as the mean ± S.D.

**Results**

**Body and heart weight**

The mean body weight of the hypothyroid rats was significantly ($P < 0.005$) lighter by 18.7% than that of the age-matched control rats. The mean left ventricular weight was also significantly ($P < 0.005$) lighter by 20.6% and the mean right ventricular weight was significantly ($P < 0.005$) lighter by 27.6% than that of the age-matched control rats, but neither the ratio of left ventricular weight to left and right ventricular weights nor the ratio of left ventricular weight to body weight in hypothyroid rats differed from those of the normal rats.

**Figure 1** shows a representative set of left ventricular pressure-time curves at mLVV in each heart paced by 261 beats/min of a normal (A) and a hypothyroid rat (B). Left ventricular ESP$_{mlLVV}$ was smaller in a hypothyroid rat than in a normal rat. Each left ventricular contraction and relaxation rate was slower in a hypothyroid rat than in a normal rat. The mean contraction ($b$, $+dP/dt_{max}$) and relaxation rate ($i$, $-dP/dt_{max}$) were significantly slower (**Figure 2A**) and $b/i$ ($+dP/dt_{max}/-dP/dt_{max}$) was significantly larger in the hypothyroid rats than in the normal rats ($2.32 ± 0.38$ vs. $1.80 ± 0.21$). Mean $T_L$ and $T_E$ were significantly larger in the hypothyroid rats, indicating that the end-diastolic relaxation rate was slower in the hypothyroid rats (**Figure 2B**).

**Figure 3, A and C** show a representative set of left ventricular curved ESPVRs and EDPVRs, and end-systolic pressure data at mLVV (ESP$_{mlLVV}$) of a normal and a hypothyroid rat in control conditions and during dobutamine infusions. We have already confirmed that left ventricular ESPVR and EDPVR did not significantly change in the hearts paced by 240 and 300 beats/min (32) and therefore we paced the hearts in normal and hypothyroid rats at different rates in the present study.
The control ESPVR (cESPVR) in the hypothyroid rat (C) was obviously lower than that in the normal rat (A). The control EDPVR (cEDPVR) in the hypothyroid rat (C) was slightly higher than that in the normal rat (A) at the larger LVV, but the end-diastolic pressure (EDP) at mLVV (EDP_{mlLVV}) at 300 beats/min-pacing in the normal rat (A) and EDP_{mlLVV} at 262 beats/min-pacing in the hypothyroid rat (C) were almost zero. The mean ESP_{mlLVV} was significantly smaller and the mean EDP_{mlLVV} was significantly larger in the hypothyroid rats than in the normal rats, so that PVA_{mlLVV} was significantly smaller in the hypothyroid rats than in the normal rats (Table 1). The mean ESV_{mlLVV} was similar in the two groups, so that eEmax (=2PVA_{mlLVV}/ESV_{mlLVV}^2) was significantly smaller in the hypothyroid rats than in the normal rats (Table 1).

Left ventricular ESP_{mlLVV} gradually increased with an increase in the infusion rate of dobutamine in both normal and hypothyroid rats. cESPVR shifted upward (dESPVR) and cEDPVR shifted downward (dEDPVR) during dobutamine infusion in the hypothyroid rat (C); the effects of dobutamine on ESP and EDP appeared to be rather more potent than those in the normal rat (A), but, dESPVR and dEDPVR in the hypothyroid rat (C) corresponded to those in the normal rat (A).

Figure 3, B and D show the demonstrative linear VO_2-PVA relations in the control vol-runs (cVO_2-PVA) and composite linear VO_2-PVA relations (comp.VO_2-PVA) during the dobutamine ino-runs in the same set of normal and hypothyroid rats. Since the VO_2-PVA relations and comp.VO_2-PVA relations were not significantly different at 240 and 300 beats/min (32), we used different pacing rates in normal and hypothyroid rats.

The slopes of the VO_2-PVA relations were similar in the normal and hypothyroid rats, but the VO_2 intercept of the relation was smaller in the hypothyroid rats (D). The VO_2-PVA data points at mLVV during the dobutamine ino-run shifted right-upwardly from the control VO_2-PVA data points. The effect of dobutamine on VO_2-PVA data
appeared to be rather more potent in the hypothyroid rat (D). But the VO$_2$-PVA relation in the dobutamine vol-run (dVO$_2$-PVA) in the hypothyroid rat (D) corresponded to that in the normal rat (B). The slopes did not differ between cVO$_2$-PVA and dVO$_2$-PVA in the normal and hypothyroid rats. The effects of Ca$^{2+}$ infusion on ESP and EDP, ESPVR and EDPVR, and VO$_2$-PVA relations were similar to those of dobutamine (data not shown).

**Figure 4, A and B** summarize the mean slopes of and the mean VO$_2$ intercepts of the VO$_2$-PVA relations in the normal and hypothyroid rats (n=12 each). The slopes were similar in the two groups, whereas the VO$_2$ intercepts in the hypothyroid rats were significantly (P<0.05) smaller than those in the normal rats. The VO$_2$ intercept was composed of VO$_2$ for Ca$^{2+}$ handling in excitation-contraction coupling and for basal metabolism. **Figure 4, C and D** summarize the data for the components of the VO$_2$ intercept in the two groups (n=7 in each out of the 12 rats). The mean VO$_2$ for Ca$^{2+}$ handling in excitation-contraction coupling (C) and for basal metabolism (D) in the hypothyroid rats were significantly smaller than those in the normal rats (decreased by 75.5 ± 5.7% and 50.9 ± 10.4% of the normal rats, respectively).

As the slope of the plot of VO$_2$ for Ca$^{2+}$ handling in excitation-contraction coupling on eEmax, we obtained the oxygen costs of left ventricular contractility in responses to Ca$^{2+}$ (n=6) and dobutamine (n=6). Surprisingly, the oxygen costs of eEmax for dobutamine and for Ca$^{2+}$ were not significantly different in the hypothyroid rats (n=12) from those in the normal rats (n=12) (**Figure 5**). Furthermore, the mean ratios of increases in left ventricular contractility in responses to the concentrations of Ca$^{2+}$ (delta eEmax/Ca$^{2+}$; 400 ± 170 vs. 450 ± 100 mmHg/ml*g*mM$^{-1}$) and dobutamine (delta eEmax/Dob; 660 ± 280 vs. 720 ± 190 mmHg/ml*g*mM$^{-1}$) did not show significant differences between the normal (the former) and the hypothyroid rats (the latter). These results indicate that the changes in Ca$^{2+}$ handling VO$_2$ in excitation-contraction coupling in responses to Ca$^{2+}$ and dobutamine in the hypothyroid rats also do not differ
from those in the normal rats.

The \( \text{Ca}^{2+} \) handling \( \text{VO}_2 \) in excitation-contraction coupling is mainly consumed by SR \( \text{Ca}^{2+} \)-ATPase (SERCA2)(13, 36). SERCA2 and phospholamban regulate the \( \text{Ca}^{2+} \) uptake at SR. The protein level of phospholamban was significantly higher and that of SERCA2 was significantly lower in the hypothyroid rats (n=12) than in the normal rats (n=10) (Figure 6), so that the ratio of phospholamban vs. SERCA2 was markedly higher in the hypothyroid rats than in the normal rats.

Myosin isozyme in left ventricular myocardium was \( V_3 \) (95.1 ± 2.0% of total myosin isozyme) in the hypothyroid rats (n=8), whereas it was \( V_1 \) (89.5 ± 9% of the total) in the normal rats (n=3).

Discussion

Although the body weight and the left and right ventricular weights of the hypothyroid rats were significantly lighter than those in the age-matched control rat, the ratio of left ventricular weight to body weight was unchanged in the hypothyroid rats. In a previous report (15), the body weight, heart weight and ratio of left ventricular weight to body weight were significantly decreased in 9-10 wk old rats after a 3-wk period of treatment with addition of PTU to the drinking water compared to those in the age-matched control rats. Furthermore, another report (22) has also shown a decreased ratio of heart weight to body weight in about 12-14 wk old hypothyroid rats.

The present results indicate that a 4-5-wk period of treatment with the addition of PTU to the drinking water leads to an impairment of normal growth, lighter body weight, smaller heart weight and an unchanged ratio of left ventricular weight to body weight in 20-21 wk old hypothyroid rats compared to those in the age-matched control rats. The discrepancy between the previous reports (15, 22) and ours seems to be due to the difference in age (9-14 wks vs. 20-21 wks). It seems likely that younger rats are susceptible to an impairment of increase in heart
weight.

In the present hypothyroid rats, we found left ventricular systolic and diastolic dysfunctions. It is well known that thyroid hormone affects the SERCA2 activity through the expression of both SERCA2 and phospholamban (4, 5, 16, 18, 19, 20, 30, 31). We hypothesized that the left ventricular systolic and diastolic dysfunctions in hypothyroid rats are related to the severe impairment of Ca$^{2+}$ uptake by SERCA2 in excitation-contraction coupling. We actually found higher protein levels of phospholamban and lower protein levels of SERCA2 in the hypothyroid rats. Furthermore, we found a marked decrease in total Ca$^{2+}$ handling VO$_2$ in excitation-contraction coupling, which is primarily consumed by SERCA2 (13,36). Therefore, the present results proved our hypothesis that the severe impairment of the SERCA2 activity caused systolic and diastolic dysfunctions in the hypothyroid rats.

Curved ESPVR, linear VO$_2$-PVA relationship and oxygen cost of PVA

Marked differences in ESP$_{ml,VV}$ or PVA$_{ml,VV}$ were found between normal and hypothyroid rats whereas each mean slope of the VO$_2$-PVA relation (O$_2$ cost of PVA) in the two groups is almost the same as that previously reported (12,13, 32, 37, 38). In hyperthyroid rabbits, where myosin isozyme is V$_1$, the increased O$_2$ cost of PVA is observed (11), although in hyperthyroid dogs, where myosin isozyme remains V$_3$, the unchanged O$_2$ cost of PVA is observed (34). From these findings, it has been predicted that the O$_2$ cost of PVA is related to types of myosin isozyme. Present results, however, indicate that there exists no correlation between O$_2$ cost of PVA and types of myosin isozyme in the normal and the hypothyroid rats. Unchanged O$_2$ cost of PVA in the hypothyroid rats indicates that the efficiency of chemomechanical energy transduction (the inverse of O$_2$ cost of PVA) remained unchanged despite the different types of myosin isozyme.

PVA-independent VO$_2$
The mean VO$_2$ intercept of the VO$_2$-PVA relation (PVA-independent VO$_2$) in the hypothyroid rats was significantly smaller than that in the normal rats. The PVA-independent VO$_2$ corresponds primarily to the VO$_2$ for total Ca$^{2+}$ handling in the excitation-contraction coupling and for basal metabolism (12, 13, 37, 39). Both the VO$_2$ for total Ca$^{2+}$ handling in the excitation-contraction coupling and for basal metabolism were significantly smaller in the hypothyroid rats. The decrease in VO$_2$ for total Ca$^{2+}$ handling reflects a decrease in the uptake activity of SERCA2 on a beat-to-beat basis as mentioned above.

Thyroid hormone changes the expression of mitochondrially encoded respiratory genes (29). In the hypothyroid state, mitochondrial respiratory function may be depressed by the depressed gene expression and further mitochondrial cytochrome oxidase activity is depressed (28). Therefore, it seems likely that hypothyroidism leads to a depressed mitochondrial respiration. This speculation is supported by the present result showing the noticeably smaller basal metabolism in the left ventricle of the hypothyroid rats.

**Oxygen cost of left ventricular contractility, eEmax**

Dobutamine is known to stimulate β-adrenergic receptors and thereby to activate protein kinase A (27), resulting in enhanced Ca$^{2+}$ handling in the excitation-contraction coupling, whereas Ca$^{2+}$ directly enhances the Ca$^{2+}$ handling by increasing Ca$^{2+}$ influx. Although the mechanism of enhancing the Ca$^{2+}$ handling is different, the O$_2$ costs of left ventricular contractility (changes in total Ca$^{2+}$ handling VO$_2$ in the excitation-contraction coupling against unit change in left ventricular contractility) for Ca$^{2+}$ and for dobutamine were similar in the normal and the hypothyroid rats. In hypothyroid rat hearts, the decrease in the myocardial β-receptor population and thus the reduced responsiveness to a β-adrenergic agonist has been reported (8). In contrast, the greater apparent Ca$^{2+}$ sensitivity to contractile activation has been also reported in the papillary muscle of the
hypothyroid rat heart (10). Consequently, the net change between the reduced responsiveness to dobutamine and the greater Ca$^{2+}$ sensitivity to contractile activation may lead to the present result showing similar O$_2$ costs of left ventricular contractility for dobutamine in the normal and the hypothyroid rats.

On the other hand, the greater apparent Ca$^{2+}$ sensitivity to contractile activation in hypothyroid rats (10) would result in a smaller O$_2$ cost of left ventricular contractility for Ca$^{2+}$. The O$_2$ cost of left ventricular contractility for Ca$^{2+}$ in the present hypothyroid rats, however, was similar to that in the normal rats. This result suggests no occurrence of greater apparent Ca$^{2+}$ sensitivity to contractile activation in the hypothyroid rats. Consequently, the above explanation for the unchanged O$_2$ cost of left ventricular contractility for dobutamine also seems to be unlikely.

In the hypothyroid rats, the pacing of the heart at 300 beats/min failed but the pacing at 262 beats/min succeeded. Although this reflects the dysfunction of SERCA2, the decreased pacing rate may lead to unchanged O$_2$ costs of left ventricular contractility for Ca$^{2+}$ and for dobutamine on a beat-to-beat basis in the hypothyroid rats.

In developing rat hearts, hypothyroidism decreases the function of SERCA2 and increases the function of Na$^+$/Ca$^{2+}$ exchanger (6,17, 30). Banijiamali reported that in normal adult rats, 81% of the Ca$^{2+}$ released from the SR was re-circulated by SERCA2; Ca$^{2+}$ handling relies largely on SR (3). If the present hypothyroidism had decreased the function of left ventricular SERCA2 and increased the function of left ventricular Na$^+$/Ca$^{2+}$ exchanger, the O$_2$ cost of left ventricular contractility might have increased (26). But the unchanged O$_2$ cost of left ventricular contractility in the present hypothyroid rats indicates no change in the relative functions of SERCA2 and Na$^+$/Ca$^{2+}$ exchanger. This speculation is supported by a recent study showing no increase in left ventricular Na$^+$/Ca$^{2+}$ exchanger protein in
hypothyroid rats compared to that in normal rats (33). The decreased total Ca\(^{2+}\) handling VO\(_2\) in the excitation-contraction coupling in the present hypothyroid rats suggests a decreased in the total amount of Ca\(^{2+}\) handled in the excitation-contraction coupling with no change in the re-circulation fraction of Ca\(^{2+}\) caused by SR.

**Left ventricular systolic and diastolic dysfunctions in hypothyroid rats**

In the excised hearts of the hypothyroid rats, at the pacing rate (262 beats/min) lower than normal (300 beats/min), we found significant slowing of the left ventricular contraction and relaxation rates. In *in situ* hearts, the preload and afterload, and hormonal and neuronal influences could interfere with left ventricular function. In contrast, in the present cross-circulated (blood-perfused) excised hearts, left ventricular function can be characterized by the myocardium per se. Therefore, true left ventricular systolic and diastolic dysfunctions seem to be caused in the present hypothyroid rats.

In the present hypothyroid rats, the left ventricular myosin isozyme V\(_1\) was transformed to V\(_3\) as suggested by Lompre et al (23). This transformation might be causally related to significant slowing of the left ventricular contraction and relaxation rates (9,15). But in the same-type heart preparations of the type II diabetic rats as the present one, where myosin isozyme was V\(_3\) just as in hypothyroid rats, only diastolic dysfunction is found under the decreased pacing rate without any change in the VO\(_2\) intercept of the VO\(_2\)-PVA relation and O\(_2\) cost of PVA (1). These findings indicate that the hypothyroid and the type II diabetic rats quite differ in the left ventricular mechanical work and energetic characterization, though their myosin isozymes were the same V\(_3\). Therefore, it seems unlikely that the only transformation of myosin isozyme into V\(_3\) is causally related to the left ventricular systolic and diastolic dysfunctions in the present hypothyroid rats, although in the long-term hypothyroidism the possibility for the
contribution of the $V_3$-dominant myocardium or abnormal pre- and/or afterload conditions due to the hypothyroidism cannot be excluded.

In agreement with our hypothesis, we found not only systolic and diastolic dysfunctions and a decrease in VO$_2$ for Ca$^{2+}$ handling in excitation-contraction coupling in physiological studies but also depressed expression of SERCA2 and enhanced expression of phospholamban in the hypothyroid rats, as previously reported (16, 18, 19, 20, 30). Furthermore, it has been reported that in hypothyroid mice, decreases in SERCA2A gene expression are accompanied by prolonged contraction and relaxation of papillary muscles (5). It seems likely that the dysfunction of SERCA2 leads to a primary diastolic dysfunction and the decreased Ca$^{2+}$-release resulting from the decrease in Ca$^{2+}$ stored in SR leads to a secondary systolic dysfunction. On the other hand, the possibility of final impairment of ryanodine receptor function cannot be excluded, because a lower level of expression of ryanodine receptor mRNA has been reported in 16-wk hypothyroid rabbits (2).

Recently, it is reported that in the failing rat heart model made by aortic banding, the ratio of SERCA2A to phospholamban decreased associated with worsening metabolism, i.e., decreased ratio of phosphocreatine to ATP (7). Similar to this failing heart model, in the present hypothyroid rat heart, the ratio of SERCA2A to phospholamban decreased, but this was not associated with worsening metabolism, i.e., unchanged O$_2$ costs of PVA and eEmax. The present study revealed, in terms of the coupling of left ventricular mechanical work and energetics, that left ventricular systolic and diastolic functions in the present hypothyroid rats are impaired by the depressed Ca$^{2+}$ uptake function by SERCA2.

We conclude that the impaired Ca$^{2+}$ uptake function by SERCA2 plays an important role in the pathogenesis of cardiac systolic and diastolic dysfunctions in the hypothyroidism. Overexpression of SERCA2A in the failing rat heart made by aortic banding improved cardiac function associated with an improvement of
phosphocreatine to ATP ratio, the energy potential (7). Furthermore, in hypothyroid mice, overexpression of SERCA2A also improves both contraction and relaxation of the papillary muscle (5). Therefore, overexpression of SERCA2A would be a promising strategy to rescue various cardiac dysfunctions including the present one.
REFERENCES


17. **Kaasik A, Minajeva A, Paju K, Eimre M, and Seppet EK.** Thyroid hormones


Ca\(^{2+}\) extrusion activated by partial inhibition of sarcoplasmic reticulum Ca\(^{2+}\)-ATPase 

27. Opie LH. Chap.7 Receptors and signal transduction. In: *The Heart. Physiology, 
p.173-207.

28. Paradies G, Petrosillo G, and Ruggiero FM. Cardiolipin-dependent decrease of 
cytochrome c oxidase activity in heart mitochondria from hypothyroid rats. *Biochim 

29. Pillar TM, and Seitz HJ. Thyroid hormone and gene expression in the regulation 

30. Reed TD, Babu GJ, Ji Y, Zilberman A, Heyen MV, Wuytack F, and 
Periasamay M. The expression of SR calcium transport ATPase and the Na\(^+\)/Ca\(^{2+}\) 
exchanger are antithetically regulated during mouse cardiac development and in 

31. Rohrer D and Dillmann WH. Thyroid hormone markedly increases the mRNA 
coding for sarcoplasmic reticulum Ca\(^{2+}\)-ATPase in the rat heart. *J Biol Chem* 263: 

32. Sakata S, Ohga Y, Abe T, Tabayashi N, Kobayashi S, Tsuji T, Kohzuki H, 
Misawa H, Taniguchi S, and Takaki M. No dependency of a new index for 
oxygen cost of left ventricular contractility on heart rates in the blood-perfused 

33. Shenoy R, Klein I, and Ojamaa K. Differential regulation of SR calcium 
transporters by thyroid hormone in rat atria and ventricles. *Am J Physiol Heart Circ 

34. Suga H, Tanaka N, Ohgoshi Y, Saeki Y, Nakanishi T, Futaki S, Yaku H, and 
Goto Y. Hyperthyroid dog left ventricle has the same oxygen consumption versus


Legends to Figures

Figure 1
Left ventricular pressure-time curves in a normal (A) and hypothyroid rat (B) under 261 beats/min-pacing at midrange left ventricular volume (mLVV), where 0.08 ml of water was infused into the balloon.

Figure 2
Mean best-fit parameter b, corresponding to +dP/dt_{max} and best-fit parameter i, corresponding to -dP/dt_{max} (A) in P(t) = a/[1+exp\{-4(b/a)(t-c)\}] - h/[1+exp\{-4(i/h)(t-j)\}] to left ventricular pressure-time curves at mLVV in normal and hypothyroid rats (A).
Mean logistic and exponential time constants in normal and hypothyroid rats (B). *, P<0.05 vs. values in normal rats.

Figure 3
Left ventricular pressure-volume data and VO_2-PVA data in a normal (A and B) and hypothyroid rat (C and D). Left ventricular pressure-volume data during different volume loading runs between 0.08 and 0.23 ml/g of a normal rat (A) and between 0.09 and 0.25 ml/g of a hypothyroid rat (C) in control (large solid circles) and under dobutamine (Dob) infusion (small solid circles) and left ventricular pressure-volume (A and C) and VO_2-PVA data at mLVV (open circles) during dobutamine infusion in a normal and a hypothyroid rat (B and D).
ESPVR, curved end-systolic pressure-volume relationships in control (cESPVR; correlation coefficient, R=0.999) and under Dob (dESPVR; R=0.999). EDPVR, end-diastolic pressure-volume relationships in control (cEDPVR; R=0.991) and under Dob (dEDPVR; R=0.999). PVA, systolic pressure-volume area at mLVV (striped area in A). Left ventricular control linear relations between VO_2 (myocardial oxygen consumption per beat) and PVA (systolic pressure-volume area) in control (cVO_2-PVA) and under Dob (dVO_2-PVA), and the composite linear relations between VO_2 and PVA (comp.VO_2-PVA) during Dob-induced different inotropism runs (Dob...
ino-runs) in a normal (B) and hypothyroid rat (D). No significant differences in the slopes were found between cVO$_2$-PVA and dVO$_2$-PVA in a normal (B) and hypothyroid rat (D) by ANCOVA. Thus, the thinnest VO$_2$-PVA lines parallel to the cVO$_2$-PVA relationship line were drawn to obtain PVA-independent VO$_2$ (B and D).

Figure 4

The mean slope (A) and VO$_2$ intercept (B) of the linear VO$_2$-PVA relationship and VO$_2$ consumed in excitation-contraction (E-C) coupling (C) and basal metabolic VO$_2$ (D) in normal (n=12) and hypothyroid rats (n=12). *, P<0.05 vs. values in normal rats.

Figure 5

Mean oxygen costs of left ventricular contractility for Ca$^{2+}$ and dobutamine in normal (n=12) and hypothyroid rats (n=12).

Figure 6

Immunoblottings of phospholamban (PLB) and SERCA2.

Immunoblotting of cardiac sarcoplasmic reticulum ATPase (SERCA2 = 100 kD) and phospholamban (PLB pentamer = 22 kD) in normal (N) and hypothyroid (H) rats (n=2 each)(upper panels). Protein levels of phospholamban were high and those of SERCA2 were low in hypothyroid rats. Lower graph: summarized data of PLB and SERCA2 expression and the ratio of PLB to SERCA2 expression in normal (N) (n=10) and hypothyroid (H) rats (n=12). *, P<0.05 vs. values in normal rats (unpaired t-test).
Fig. 2

A

\[ \text{mmHg/msec} \]

\begin{align*}
+{\text{dP/dt}}_{\text{max}} &: \quad 2.0 \pm 0.5^* \\
-{\text{dP/dt}}_{\text{max}} &: \quad 1.5 \pm 0.5^*
\end{align*}

(Best-fit parameter \( b \))

(Best-fit parameter \( i \))

B

\begin{align*}
\text{Logistic time constant} &: \quad 20 \pm 10^* \\
\text{Exponential time constant} &: \quad 30 \pm 10^*
\end{align*}

\( n=10 \)

Normal

Hypothyroid
Oxygen cost of contractility

\[
\left(10^{-4} \, \mu l \, O_2 \cdot ml \cdot beat^{-1} \cdot mmHg^{-1} \cdot g^{-2}\right)
\]

- **Ca\(^{2+}\)**
  - Normal
  - Hypothyroid

- **Dobutamine**
  - Normal
  - Hypothyroid

Fig. 5
Table 1. Variables of left ventricular mechanical work.

<table>
<thead>
<tr>
<th>Inotropism and Lusitropism</th>
<th>Normal (n=12)</th>
<th>Hypothyroid (n=12)</th>
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</thead>
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<td>mLVV (ml/g)</td>
<td>0.16 ± 0.01</td>
<td>0.19 ± 0.02**</td>
</tr>
<tr>
<td>Vo (ml/g)</td>
<td>0.076 ± 0.005</td>
<td>0.094 ± 0.011**</td>
</tr>
<tr>
<td>ESPmax (mmHg)</td>
<td>172.1±19.6</td>
<td>148.2 ± 20.9*</td>
</tr>
<tr>
<td>EDPmax (mmHg)</td>
<td>9.8 ± 3.9</td>
<td>21.9 ± 11.1**</td>
</tr>
<tr>
<td>ESPmLVV (mmHg)</td>
<td>129.5 ± 23.6</td>
<td>82.6 ± 15.1**</td>
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<tr>
<td>EDPmLVV (mmHg)</td>
<td>0.4 ± 0.7</td>
<td>7.3 ± 8.4*</td>
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<tr>
<td>ESVmLVV (ml/g)</td>
<td>0.08 ± 0.01</td>
<td>0.10 ± 0.01</td>
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<tr>
<td>PVAmLVV (mmHg·ml·beat⁻¹·g⁻¹)</td>
<td>6.1 ± 1.6</td>
<td>4.3 ± 1.0*</td>
</tr>
<tr>
<td>eEmaxmLVV (mmHg·ml⁻¹·g)</td>
<td>1963.4 ± 705.2</td>
<td>902.3 ± 201.6**</td>
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<tr>
<td>Pacing rate (beats/min)</td>
<td>300 ± 0</td>
<td>262 ± 16**</td>
</tr>
</tbody>
</table>

Values are means ± SD. mLVV: midrange LV volume (see text). Vo: volume intercepts of best-fit ESPVRs (see text). ESPmax: observed maximum end-systolic pressure. EDPmax: observed maximum end-diastolic pressure. ESPmLVV: end-systolic pressure at mLVV. EDPmLVV: end-diastolic pressure at mLVV. ESVmLVV: mLVV-Vo. PVAmLVV; systolic pressure-volume area at mLVV (see text). eEmaxmLVV: equivalent Emax at mLVV (see text). *: P<0.05 and **: P<0.001 vs normal.
Table 2. Specifically determined Vo (ml/g)

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* P < 0.05 vs corresponding mean Vo values in normal rat hearts
Table 3. Slopes of VO₂-PVA relations (x10⁻² μl O₂ • mmHg⁻¹ • ml⁻¹)

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