Journal of Biomedical Science (2005) 12:601–610
DOI 10.1007/s11373-005-7373-2



# The profile of cardiac cytochrome *c* oxidase (COX) expression in an accelerated cardiac-hypertrophy model

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Received 10 March 2005; accepted 17 May 2005
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*Key words:* cytochrome *c* oxidase (COX), complete coarctation, cardiac hypertrophy, genetically hypertensive rats

**Summary**

The contribution of the mitochondrial components, the main source of energy for the cardiac hypertrophic growth induced by pressure overload, is not well understood. In the present study, complete coarctation of abdominal aorta was used to induce the rapid development of cardiac hypertrophy in rats. One to two days after surgery, we observed significantly higher blood pressure and cardiac hypertrophy, which remained constantly high afterwards. We found an early increased level of cytochrome *c* oxidase (COX) mRNA determined by *in-situ* hybridization and dot blotting assays in the hypertrophied hearts, and a drop to the baseline 20 days after surgery. Similarly, mitochondrial COX protein level and enzyme activity increased and, however, dropped even lower than baseline 20 days following surgery. In addition, in natural hypertension-induced hypertrophic hearts in genetically hypertensive rats, the COX protein was significantly lower than in normotensive rats. Taken together, the lower efficiency of mitochondrial activity in the enlarged hearts of long-term complete coarcted rats or genetically hypertensive rats could be, at least partially, the cause of hypertensive cardiac disease. Additionally, the rapid complete coarctation-induced cardiac hypertrophy was accompanied by a disproportionate COX activity increase, which was suggested to maintain the cardiac energy-producing capacity in overloaded hearts.

## Introduction

The heart has a remarkable capacity to increase its mass in response to an increased work load, such as pressure overload, induced either pathologically or experimentally [1]. In order to be able to face the increase of cardiac muscle contraction for extra work, more energy production is required. The mitochondrial compartment is the main source of energy needed for hypertrophic growth in the myocyte. However, the reports evaluating the mitochondrial compartment behavior during the onset and progression of cardiac hypertrophy are inconsistent [2]. In addition, the investigations on the molecular events involved in mitochondrial biogenesis and function during the hypertrophy process are few. This is surprising since it is well-known that

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insufficient oxidative capacity often contributes to the cardiac failure observed at least in late stage hypertrophic cardiomyopathies [3,4].

Cytochrome *c* oxidase (COX) is located in the inner membrane of mitochondria and is the last component of respiration chain. This enzyme catalyzes the rate-limiting reaction of the mitochondrial oxidative phosphorylation (OXPHOS), which supplies the energy for cardiac contraction [5, 6]. Therefore, COX plays an essential role in maintaining normal cardiac function. Since the effects of this enzyme are important, the reaction rate of the mitochondrial OXPHOS can be altered by a small decrease in this oxidase activity under physiological condition. This tight control of COX suggests that the dysfunction of the enzyme may cause the mitochondrial myopathy [7, 8]. Actually, the number of known mutations of most of the genes encoding the protein subunits of COX in patients with cardiomyopathy has increased [9–11].

Aortic constriction has been used to induce cardiac hypertrophy in many studies. Our previous data showed that the heart was rapidly enlarged within 1–2 days postsurgery in this model by using a complete coarctation of the abdominal aorta in rats to induce a cardiac pressure overload [2,12,13]. In the present study, we investigated the biochemical and molecular events underlying the adaptation of the mitochondrial compartment to meet the increased energy demands of the cardiomyocyte as they underwent this rapid hypertrophic growth. We have used COX as a model protein for investigating the underlying adaptive mechanism. We measured COX activity and examined COX gene expression by determining COX-Vb mRNA, one of the nuclear-encoded subunits of COX enzyme, during cardiac hypertrophy in the model system using complete coarctation of the abdominal aorta from 1 to 20 days. The measurement of cardiac COX protein in spontaneously hypertensive rats (SHR) and stroke prone SHR (SPSHR) with naturally occurring hypertensive cardiac hypertrophy was also included in this study.

## Methods

### *Coarctation of abdominal aorta*

Male Sprague-Dawley rats weighing between 240 and 300 g were used to induce cardiac hypertrophy by complete coarctation of the abdominal aorta between the origins of the renal arteries [14]. After a rat was anesthetized with pentobarbital sodium (45.5 mg/kg), its left side was shaved and a horizontal incision 3 cm in length was made. Using curved hemostats, the superficial fascia were separated and an incision of the same size was made into the abdominal musculature. The viscera were exposed, and adipose tissue was separated from the left kidney which was moved to obtain maximal clearance to the abdominal aorta. The abdominal aorta was isolated and completely coarcted with a ligature (Coats, Dual Duty Plus, carpet thread) fixed between the origins of the renal arteries. The left kidney and adipose tissue were returned to their normal positions, the abdominal musculature was rejoined using coated vicryl sutures (Ethicon, Johnson & Johnson, FS-2 cutting), and the epidermis was rejoined using wound clips. Then, a solution of 1% iodine in 70% ethanol was used to clean the wound. The rats were returned to the animal care room (one per cage) and allowed to recover. All animals were fed standard rat chow and water supplemented with tetracycline (22 mg/kg body weight) to ward off infection. Sham rats were subjected to the same procedure, but no ligature was placed. All animals were handled according to the guidelines of the Taiwan Society for Laboratory Animal Sciences for the Care and Use of Laboratory Animals.

### *Femoral arterial pressure measurement*

The blood pressure of coarcted and sham animals was measured at 1, 2, 3, 5, 7, 10 and 20 days postsurgery. The animals not subjected to the above surgery were measured as 0 day controls. After catheterization of the femoral artery of animals, blood pressure monitor (Recorder 2200S, GOULD, U.S.A.) was used to measure both the systolic and diastolic pressure by connecting the femoral artery with a sensor. The blood pressure was measured and recorded continuously [15].

### *Heart isolation*

Once the blood pressure measurements were taken, the animals were sacrificed by cervical transaction. The thoracic cavity was opened, and the heart was removed. After removing excess adipose and connective tissue from the heart, it was drained of blood

and weighed. The ratios of the total heart weight to body weight were calculated. The isolated heart was washed in cold (0–4° C) SNTE buffer (0.20 M sucrose, 0.13 NaCl, 1 mM Tris–HCl, pH 7.4 [5], and 1 mM EGTA neutralized with Tris to pH 7.4), and the entire left ventricle (LV) was excised from the heart. The LV was separated into four parts, and stored at −70° C for further analysis.

*Preparation of heart mitochondria*

One part of LV was minced with scissors, added with SNTE buffer (1.5 ml per g tissue) and homogenized at ice temperature with a Model PT 10/35 Polytron homogenizer for 2 cycles of 10 s each. The homogenate was centrifuged at $500 \times g$ for 10 min in a Beckman J20.1 centrifuge. Then, the pellet was discarded, and the supernatant was further centrifuged at $8000 \times g$ for 15 min. The mitochondrial pellet was resuspended with SNTE buffer. The mitochondrial protein was measured [16], and prepared for COX enzyme activity assay.

*Cytochrome c oxidase activity*

Cytochrome *c* (horse heart, Sigma Chemical Co. no. C-7752, Type III, prepared without using TCA) was reduced with dithionite as described [17] in 50 mM phosphate ($K_2HPO_4$–$KH_2PO_4$) buffer at pH 7.0 [18]. Reduced cytochrome *c* was made fresh for each experiment and stored in an air-tight container on ice for the assays. COX activity was measured spectrophotometrically by following the oxidation of reduced cytochrome *c* at 550 nm [18] in 50 mM phosphate buffer (pH 7.0) containing 0.5% Tween 80 [19]. Rates are expressed as μmol cytochrome *c* oxidized per min per mg mitochondrial protein.

*Protein extraction and Western blot analysis*

The second part of LV was minced with scissors, homogenized with lysis buffer (50 mM Tris (pH 7.5), 0.5 M NaCl, 1.0 mM EDTA (pH 7.5), 10% glycerol, 1 mM BME, 1% IGEPAL-630 and proteinase inhibitor cocktail tablet (Roche)) and spun down 12,000 rpm for 30 min. Then, the supernatant was collected and stored at −70° C for further Western blotting. Proteins from animal heart extracts were quantitated by the Bradford protein assay [16] and then separated in 10% gradient sodiumdodecyl sulfate-polyacrylamide gel electrophoresis. The samples were electrophoresed at 140 V for 3.5 h and equilibrated for 15 min in 25 mM Tris–HCl, pH 8.3, containing 192 mM glycine and 20% (v/v) methanol. Electrophoresed proteins were transferred to nitrocellulose paper (Amersham, Hybond-C Extra Supported, 0.45 Micro) at 100 mA for 14 h. Nitrocellulose papers were incubated at room temperature for 2 h in blocking buffer containing 100 mM Tris–HCl, pH7.5, 0.9% (w/v) NaCl, 0.1% (v/v) fetal bovine serum. Antibody of COX-Vb (Santa Cruz Biotechnology) was diluted in antibody binding buffer containing 100 mM Tris–HCl, pH7.5, 0.9%(w/v) NaCl, 0.1% (v/v) Tween 20 and 1%(v/v) fetal bovine serum. Incubations were performed at room temperature for 3.5 h. The immunoblot was washed three times in 50 ml blotting buffer for 10 min and then immersed in the second antibody solution containing alkaline phosphatase goat anti-rabbit IgG (Promega) for 1 h and diluted 1000-fold in binding buffer. The filters were then washed in blotting buffer for 10 min three times. Color development was presented in a 20 ml mixture consisting of 7 mg nitro blue tetrazolium, 5 mg 5-bromo-4-chloro-3-indolyl-phosphate, 100 mM NaCl and 5 mM $MgCl_2$ in 100 mM Tris–HCl, pH 9.5. The immunoblot with antibody against α-tubulin which was performed with the same procedure was used as an internal control.

*RNA extraction*

The third part of LV was used to extract total RNA using the Ultraspec RNA Isolation System (Biotecx Laboratories, Inc.) according to directions supplied by the manufacturer. Each heart was thoroughly homogenized (1 ml Ultraspec reagent per 100 mg tissue) with a Polytron homogenizer. The RNA precipitate was washed twice by gentle vortexing with 70% ethanol, collected by centrifugation at $12{,}000 \times g$, dried under vacuum for 5–10 min, dissolved in 50–100 μl of diethylpyrocarbonate-treated water, and incubated for 10-15 min at 55–60° C.

*Measurement of heart mRNA levels by dot blotting*

RNA dot blotting was used for the hybridization and detection of mRNAs according to the method described in our previous study [20]. The

corresponding digoxigenin (DIG)-labeled antisense RNA probes were prepared from pGEM-7Zf(+) containing a 500-bp cDNA encoding COX subunit Vb insert (supplied by Dr. Dennis E. Buetow, Univ. of Illinois, IL, USA, and originally form Dr. Avadhani, Univ. of Pennsylvania, PA, USA).

*In-situ hybridization of COX-Vb mRNA and quantitation*

The fourth part of LV was used to detect the COX-Vb mRNA in tissues, using DIG-labeled antisense RNA probes described above. The procedures of *in-situ* hybridization used in this study were described previously [13].

*Statistical analysis*

Statistical differences were analyzed by one-way analysis of variance (ANOVA). Fisher's Least Significant Difference test was used to determine differences. $p < 0.05$ was considered statistically significant. Data are expressed as the mean ± standard error (SE).

## Results

*Hypertensive cardiac hypertrophy in pressure-overloaded rats*

Blood pressure measurements of rats subjected to complete coarctation clearly demonstrated the hypertensive effects of the aortic coarctation (Figure 1). On day 1, 7 and 20 postsurgery, the arterial systolic blood pressure of sham animals was 137 ± 5, 148 ± 3 and 152 ± 6 mm Hg, respectively, while that of the coarcted animals was 172 ± 6, 220 ± 10 and 200 ± 10 mm Hg, respectively. Similarly, the arterial diastolic blood pressure of sham animals was 100 ± 5, 110 ± 5 and 115 ± 5 mm Hg, while that of the coarcted animals was 128 ± 5, 154 ± 6 and 148 ± 9 mm Hg on day 1, 7 and 20 postsurgery, respectively.

The development of cardic hypertrophy after complete aortic coarctation is illustrated in Table 1. Heart weights of sham animals remained unchanged throughout the postsurgery period. In contrast, by day 2 after coarctation, hearts of coarcted rats were significantly enlarged and remained so through the rest of the observation period of 20 days. The heart weight-to-body weight ratios were increased significantly from day 2 through 20 postsurgery in the coarcted animals compared to the 0-day controls. In sham animals, these ratios remained unchanged compared to the 0-day controls during the same postsurgery period.

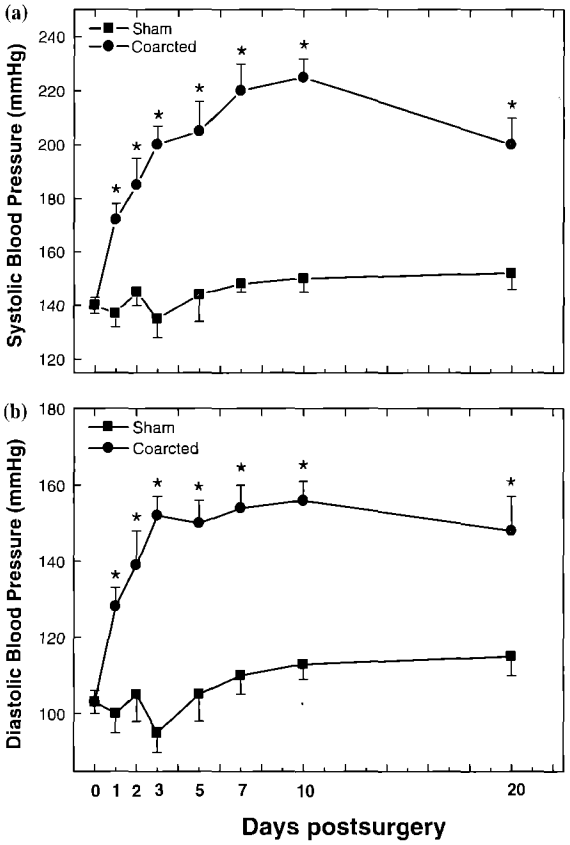



*Figure 1.* Arterial systolic and diastolic blood pressures of sham-operated and coarcted rats following surgery. Zero day values represent baseline data from animals sacrificed on the day of surgery. Values are mean ± SE. Numbers of animals measured at each data point are given in Table 1. $*p < 0.001$ represents the comparison to zero day sham animals.

*COX-Vb gene expression*

COX-Vb gene expression was measured as levels of COX-Vb mRNA using *in-situ* hybridization (Figure 2) and dot blotting (Figure 3) with an antisense COX-Vb mRNA DIG-labeled probe in hearts from rats. The level of hybridized COX-Vb mRNA in heart sections of coarcted animals significantly increased and reached a peak at day

*Table 1.* Heart weight and heart weight to body weight ratios of sham-operated and coarcted rats.

| Day postsurgery | H, g | | H:B, mg:g | |
|---|---|---|---|---|
| | Sham | Coarcted | Sham | Coarcted |
| 0 | 0.96±0.02 ($n$ = 8) | – | 3.63±0.08 ($n$ = 8) | – |
| 1 | 0.92±0.03 ($n$ = 7) | 0.97±0.02 ($n$ = 7) | 3.48±0.08 ($n$ = 7) | 3.64±0.14 ($n$ = 7) |
| 2 | 0.94±0.02 ($n$ = 7) | 1.07±0.03* ($n$ = 6) | 3.53±0.07 ($n$ = 7) | 4.31±0.19* ($n$ = 6) |
| 3 | 0.95±0.03 ($n$ = 7) | 1.13±0.03** ($n$ = 6) | 3.47±0.07 ($n$ = 7) | 4.56±0.13** ($n$ = 6) |
| 5 | 0.93±0.01 ($n$ = 7) | 1.11±0.02** ($n$ = 6) | 3.51±0.08 ($n$ = 7) | 4.98±0.18** ($n$ = 6) |
| 7 | 1.00±0.07 ($n$ = 7) | 1.12±0.04* ($n$ = 4) | 3.44±0.08 ($n$ = 7) | 4.63±0.30*** ($n$ = 4) |
| 10 | 1.04±0.05 ($n$ = 7) | 1.16±0.03* ($n$ = 4) | 3.42±0.09 ($n$ = 7) | 4.83±0.31*** ($n$ = 4) |
| 20 | 1.10±0.04 ($n$ = 7) | 1.20±0.03* ($n$ = 3) | 3.40±0.12 ($n$ = 7) | 4.99±0.20** ($n$ = 3) |

Data are mean±SE. H and H:B are the heart weights (g) and heart weight to body weight ratios (mg:g), respectively, following surgery; $n$, number of animals. Zero day values represent baseline data from animals sacrificed on the day of surgery. *$p<0.05$, **$p<0.025$, ***$p<0.001$ compared to the sham animals.

5. Then, the level declined and returned to the baseline at day 20 postsurgery (Figure 2a and 2c). In the sham animals, LV COX-Vb mRNA did not change during the 20-day postsurgery period (Figure 2c). The representative images of COX-Vb gene expression in *in-situ* hybridization at day 7 are shown in Figure 2b. No hybridization was detectable in control experiments that used a sense probe or lacked a probe. The dot blotting of hybridized COX-Vb mRNA is shown in Figure 3a. Levels of GAPDH mRNA were used as a loading control. The density of each COX mRNA dot was measured and divided by the density of the corresponding GAPDH mRNA dot. Data were quantified by densitometry, Figure 3b. Clearly, the levels of COX-Vb mRNA are significantly higher in the coarcted animals compared to the sham animals at each day postsurgery, and decreased to basal level at day 20 postsurgery.

*Changes of COX-Vb protein level and enzyme activity*

The levels of COX activity and subunit Vb protein were measured by enzyme kinetic assay and western blotting, respectively. The effects of the stress of the surgery were apparent in coarcted animals. A general increase in cardiac COX enzyme activity in coarcted animals after day 2 postsurgery was observed (Table 2). In all cases, COX activity per mg mitochondnal protein of coarcted hearts was significantly higher than that of sham hearts by day 10 with an exception at day 20 which was significantly lower. Cardiac COX-specific activity in coarcted animals was significantly different from that of shams. Compared with 0-day protein level, the COX-Vb protein levels at day 2, 3, 5 and 7 were significantly increased. Conversely, the COX protein level at day-20 is significantly lower than that at 0-day control (Figure 4).

*Impairments of cardiac COX-Vb protein in the rat models of genetic hypertension*

Relative to coarctation-induced pressure-over loaded rats, the cardiac COX-Vb protein level by Western blotting was examined in naturally hypertensive rats, spontaneously hypertensive rats (SHR) and stroke prone SHR (SPSHR), using normotensive Wistar rats (WKY) as a control group, and results were shown in Figure 5. Using β-actin as a loading control, cardiac COX-Vb protein levels of both SHR and SPSHR at age of 4-week, 6-week or 12-week-old were apparently lower than that of WKY. This indicates that the COX-Vb of hearts was impaired in genetically hypertensive rats.

## Discussion

Cardiac hypertrophy resulting from chronic pressure overload was rapidly induced in the present model system in which complete coarctation of the rat abdominal aorta was done between the origins of the renal artery. In this model system, systolic blood pressure was found to increase significantly one day following surgery. It reached a peak and remained fairly constantly above 200 mm Hg after

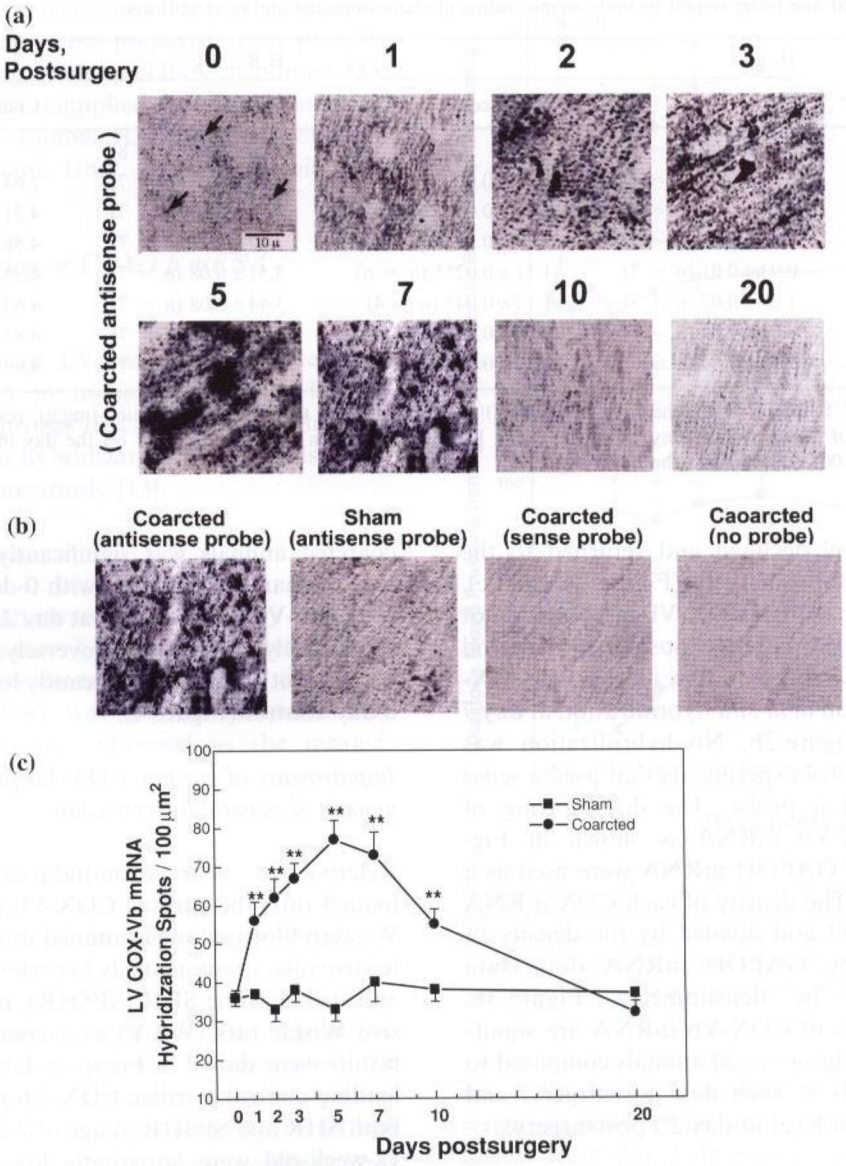



*Figure 2. In-situ* hybridization of LV COX-Vb mRNA. (a) Hybridization of antisense COX-Vb mRNA to LVs of sham-operated and coarcted rats at 0, 1, 2, 3, 5, 7, 10 and 20 days postsurgery. Arrows point to spots of immuno-detected hybridized antisense COX-Vb mRNA. (b) Hybridization with a digoxigenin (DIG)-labeled antisense COX-Vb mRNA probe in the LVs from sham-operated and coarcted animals at day 7 postsurgery. Control hybridizations with a sense DIG-labeled COX-Vb mRNA probe and with no probe are also shown, (essentially no hybridization is detected as expected). (c) Quantitation of *in-situ* hybridization of COX-Vb mRNA in the LVs from sham-operated and coarcted rats following surgery. The 0-day value represents baseline data from animals sacrificed on the day of surgery. Hybridized spots were counted in 100 $\mu m^2$ sections. Values are expressed as mean ± SE (3 animals per value). Data were analyzed by ANOVA and significant differences between coarcted vs. sham animals on each day postsurgery. $^{**}p < 0.01$.

3 days. Blood pressures of sham controls remained around 140 mm Hg during the same postsurgical period. In the coarcted animals, cardiac hypertrophy was observed at one and two days after the surgery. These results support the view that the model system using complete coarctation in rats was a more accelerated model of inducing cardiac hypertrophy than in the other rat models, for example, those that are partially coarcted [2,12] or treated with excess thyroid hormone [12]. To

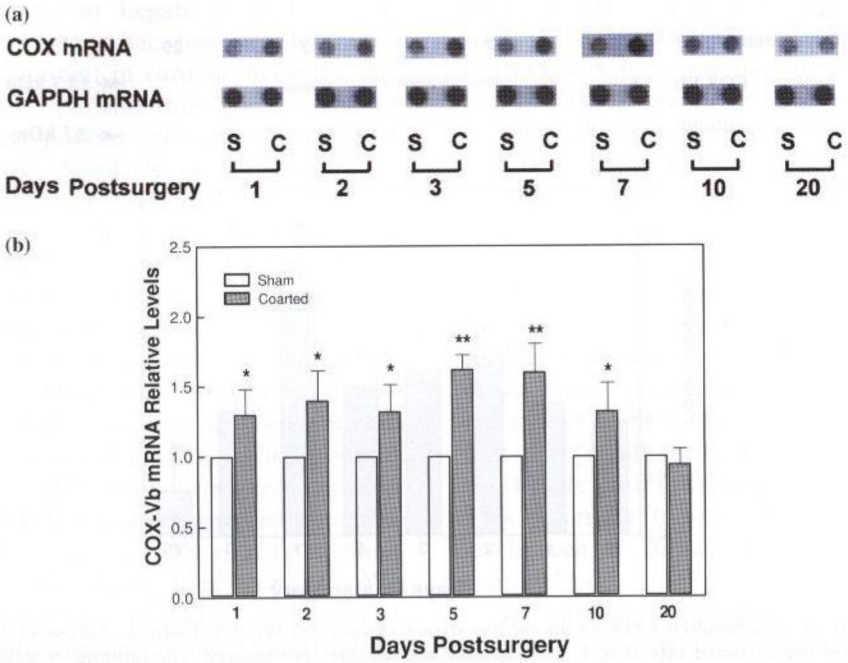



*Figure 3.* Gene expression for COX-Vb and GAPDH (loading control) mRNA in the hearts of sham-operated and coarcted rats at day 1, 2, 3, 5, 7, 10 and 20 postsurgery. (a) COX-Vb probe labeled with DIG on UTP was hybridized to 80 ng mRNA from sham (S) and coarcted (C) animals. COX-Vb and GAPDH mRNA levels were determined from the dot blots by densitometry. No hybridization occurred with yeast tRNA which was used as a negative control (data not shown). (b) Graph showed the level of COX-Vb mRNA relative to the GAPDH mRNA level in the hearts of sham and coarcted rats at day 1 day 1, 2, 3, 5, 7, 10 and 20 postsurgery ($n = 3$ in all cases). The level of COX-Vb mRNA in sham hearts was standardized to 1.0. $*p < 0.05$ and $**p < 0.01$ when compared to the sham rats.

maintain normal tissue function in response to extra workload during cardiac hypertrophy, the occurrence of cellular enlargement must be synchronized with proportional increases in the components of cellular organelles. In order to provide the energy needed for the hypertrophic growth and the functional maintenance of the enlarged cardiac myocytes, increases in the mitochondrial components can be expected.

*Table 2.* COX-Vb enzyme activity in mitochondria of coarcted (CR) and sham (S) rats on day 1 through 20 postsurgery.

| Days postsurgery | Activity | |
|---|---|---|
| | S | CR |
| 1 | 0.31 ± 0.06 ($n = 7$) | 0.43 ± 0.11 ($n = 7$) |
| 2 | 0.30 ± 0.05 ($n = 7$) | 0.44 ± 0.06* ($n = 6$) |
| 3 | 0.30 ± 0.03 ($n = 7$) | 0.43 ± 0.05* ($n = 6$) |
| 5 | 0.33 ± 0.02 ($n = 7$) | 0.47 ± 0.02* ($n = 6$) |
| 7 | 0.33 ± 0.05 ($n = 7$) | 0.49 ± 0.04* ($n = 4$) |
| 10 | 0.35 ± 0.03 ($n = 7$) | 0.47 ± 0.03* ($n = 4$) |
| 20 | 0.36 ± 0.09 ($n = 7$) | 0.24 ± 0.10** ($n = 3$) |

Activity values are μmoles oxidized cytochrome *c*/min/mg mitochondrial protein (mean ± SE); *n*, number of animals. $*p < 0.05$ represents significant increase when compared to the sham animals. $**p < 0.05$ represents significant decrease when compared to the sham animals.

COX is an enzyme containing thirteen different subunits. Among them, the three largest polypeptides (I, II and III subunits) are synthesized within mitochondria under the control of mitochondria DNA (mtDNA). The other ten smaller subunits (IV, Va, Vb, VIa, VIb, VIc, VIIa, VIIb, VIIc and VIII) are encoded by nuclear DNA (nDNA) and synthesized in cytosol [6].In the present study, COX-Vb mRNA detected by *in-situ* hybridization and dot blotting increased after day 1 following the surgery, reached a peak at day 5 or 7 and decreased to baseline by day 20. The results of the COX protein and activity demonstrated similar alteration patterns, and the values on the 20th day even dramatically dropped below the baseline. Indeed, by 20 days postsurgery, the mortality of completely coarcted rats at this time point was

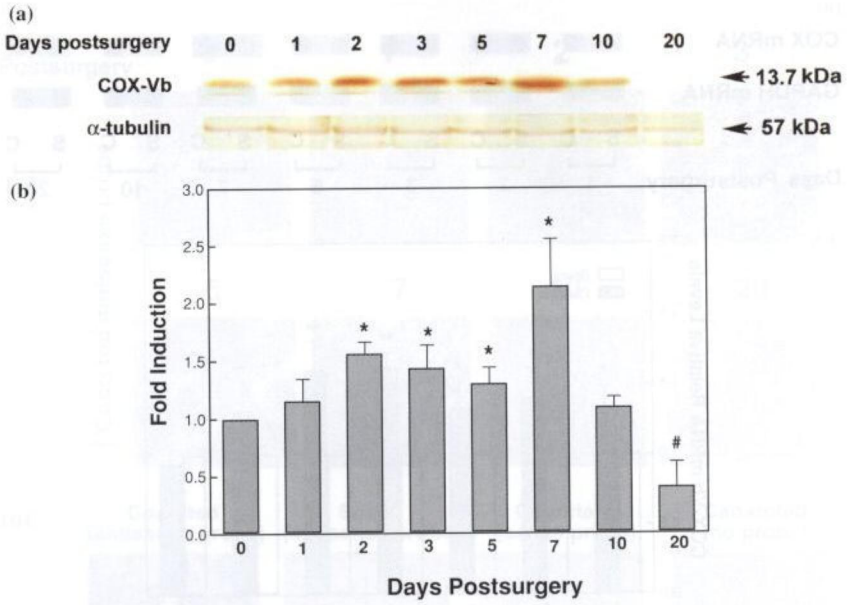



*Figure 4.* Activations of mitochondrial COX-Vb in cardiac tissues of rats. (a) Western blotting analysis of COX-Vb proteins in LVs of sham-operated and coarcted rats at 0, 1, 2, 3, 5, 7, 10 and 20 days postsurgery. The blotting of α-tubulin was used as a loading control. (b) Signal intensity of western blotting was quantitated using a PhosphoImager. The averaged result ± SD of three independent experiments is shown. The 0-day value represents baseline data from animals sacrificed on the day of surgery. *Represents a significant increase compared with the control group with $p < 0.05$. #Represents a significant decrease compared with the control group with $p < 0.05$.

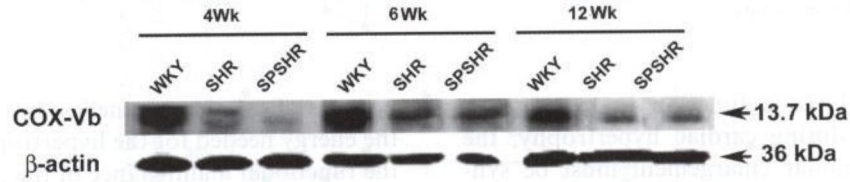



*Figure 5.* COX-Vb level in the left ventricle of hypertensive rats at different ages as determined by western blotting. Spontaneously hypertensive rats (SHR) and stroke prone SHR (SPSHR) were sacrificed at different ages as indicated. Lysates were prepared from hearts, and equal amounts of protein were separated by SDS/PAGE and immunoblotting with anti-COX-Vb antibody. β-actin was used as a loading control. WKY, Wistar rats as a normotensive control; Wk, week.

around 50% (data not shown). Our findings indicate that COX may be an essential adaptive factor of hypertensive cardiac hypertrophy in early onset and even prolonged pathological conditions.

A review of the relevant literature [2], however, reveals discrepancies regarding the behavior of the mitochondrial components during the onset and progression of cardiac hypertrophy even when induced by the same stimulus. Some studies of cardiac hypertrophy induced by partial aortic coarctation found an increase in mitochondrial content, i.e., mitochondrial biogenesis, but this increase has not always been found in other studies [2]. Similarly, the mitochondrial responses to renovascular hypertension-induced cardiac hypertrophy are also variable. Two reports, however, indicate changes in COX activity and the levels of mRNAs encoding COX subunits remain constant per mg total heart protein during adaptive cardiac growth induced by partial aortic coarctation [2,12]. It has been suggested that mitochondrial biogenesis is balanced with the increase of ventricular weight for 7 days [12] or even up to 28 days [2] following partial aortic coarctation. On the other hand, in the present accelerated model of cardiac hypertrophy, COX-specific activity was increased by 30–47% during the period of 1–10 days postsurgery. This result correlates with the

ultrastructural study of Legato et al. [21] who showed that during cardiac hypertrophy mitochondrial DNA content in cardiac myocytes was increased and the mitochondria became smaller and more densely packed with cristae and inner membrane proteins. Similarly, in our study, since the COX activity was calculated by per gram mitochondrial protein, the increased COX activity more likely resulted from increased COX content per mitochondrion than from increased number of mitochondria. The imposition of a more severe aortic constriction in the present study than in the other studies, combined with a resulting greater pressure overload may lead to disproportionate changes in mitochondrial activity. In addition, an increased level of mRNA for the nuclear-encoded subunit Vb of COX was also observed, and this met the parallel changes of COX activity reaching a maximum as the heart hypertrophied. We suggest that the synthesis of COX is regulated at the level of transcription in the hypertrophied heart. Similar behavior of other nuclear-encoded subunits composed of the enzyme must be predictable [12]. Because there was a rapid increase of COX protein and mRNA, COX may be considered an important adaptive factor of hypertrophic hearts. The drop in cardiac COX by 20 days postsurgery in treated rats might indicate the failure of this increase of COX to balance the cardiac hypertrophy after long term coarctation, resulting in heart failure. These findings are also consistent with Lin's study [22], which demonstrated abnormal cardiac mitochondria, reduced COX enzyme activity and reduced mtDNA level in pigs with hypertrophic cardiomyopathy.

Cardiac hypertrophy found in SHR and SPSHR, other models of genetic hypertension, showed an apparent reduction of COX protein level even at ages as young as 4 weeks old. Interestingly, Tokoro et al. demonstrated a decreased antioxidant ability due to lower cardiac superoxide dismuatse (SOD) activity in SPSHR [23]. In addition, due to the lack of protection by histone, several mtDNA encoded subunits of COX genes are particularly susceptible to oxidative damage, leading to mutation occurrence [24]. Therefore, the electrophoretic band of deleted mtDNA analyzed by restriction fragment length polymorphisms (RFLP) was found in myocardiums of SPSHR [23]. These observations provide an explanation for the reduction of COX protein levels in SHR and SPSHR. Although Wanagat's study [25] demonstrated that the abnormal activity of COX was age-associated, we observed a reduction of COX protein occurring at very young age in genetically hypertensive rats. We believe genetic defects resulting in COX dysfunction at young age in SHR and SPSHR cannot be ruled out. The average life span of hypertensive rats, SHR and SPSHR, is around 8 months to one and half years, which is shorter than that of the normal rats that have an average life span of 2.5 years, and the cardiac COX protein in SHR and SPSHR was down-regulated early. Thus, the failure to have an increase of COX to balance the cardiac hypertrophy may result in heart failure and lead to a shorter life span in these genetic hypertensive rats. This is similar to the decrease in COX protein levels seen at 20 days postsurgery of complete-coarcted rats.

In summary, the rapid cardiac hypertrophy induced by complete coarctation of the abdominal aorta in rats is accompanied by a disproportionate increase in mitochondrial energy-producing activity, a result shown here for the first time. Additionally, insufficient oxidative capacity contributes to the cardiac failure frequently observed in hypertrophic cardiomyopathies induced by a variety of conditions [19]. In our study, the dysfunction of cardiac COX in SHR, SPSHR and completely coarcted rats long term (20 days) after surgery suggests that COX might be the key factor for the cardiac oxidative ability during cardiac hypertrophy. The rapid cardiac hypertrophy that develops in the present model provides a unique system to study the molecular basis underlying the adaptation of the oxidative system in cells in response to the demands of hypertrophic growth. Future work will attempt to identify the mechanism of the increment of COX, using the same complete coarctation model we used in this study.

**Acknowledgements**

We thank the National Science Council (grant NSC 89-2320-B-053) of Taiwan, ROC for financial support. This work could not be finished without generous gifts of COX-Vb plasmid and the guidance from Dennis E. Buetow and the help from Sam N. Pate at University of Illinois, USA.

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