Improved cardiac contractile functions in hypoxia-reoxygenation in rats treated with low concentration Co$_{2}^{+}$

HIROSHI ENDOH, TAKAHO KANEKO, HIRO NAKAMURA, KATSUHIKO DOI, AND EIJI TAKAHASHI

1Department of Physiology, Yamagata University School of Medicine, Yamagata 990-9585; 2Department of Anesthesia, Tokyo Metropolitan Fuchu Hospital, Fuchu, Tokyo 183-0042; and 3Biophysical Chemistry Laboratory, Riken, Wako, Saitama 351-0198, Japan

Received 28 March 2000; accepted in final form 23 June 2000

Endoh, Hiroshi, Takaho Kaneko, Hiro Nakamura, Katsuhiko Doi, and Eiji Takahashi. Improved cardiac contractile functions in hypoxia-reoxygenation in rats treated with low concentration Co$_{2}^{+}$. Am J Physiol Heart Circ Physiol 279: H2713–H2719, 2000.—An intracellular mechanism that senses decreases in tissue oxygen level and stimulates hypoxia-related gene expression has been reported in various cell types including the cardiac cell. The mechanism can also be activated by Co$_{2}^{+}$ in normoxia. Thus we investigated the effects of prior chronic oral CoCl$_2$ on mechanical function of isolated, perfused rat hearts in hypoxia-reoxygenation. In normoxic rats, 43 days of Co$_{2}^{+}$ administration increased hematocrit from 45 ± 0.3% (control, n = 18) to 51 ± 0.6% (n = 19). In hypoxia and reoxygenation, Co$_{2}^{+}$-pretreated hearts exhibited a significantly higher rate-pressure product (267 and 163%, respectively) and coronary flow (127 and 115%, respectively) and lower end-diastolic pressure (72 and 60%, respectively) compared with the control hearts. Although the oral Co$_{2}^{+}$ administration significantly raised myocardial Co$_{2}^{+}$ concentration, it did not affect mitochondrial respiration, tissue glycogen concentration, or myocardial tissue histology. The levels of vascular endothelial growth factor, aldolase-A, and glucose transporter-1 mRNA were significantly elevated in the Co$_{2}^{+}$-treated myocardium. We conclude that cardiac contractile functions would gain hypoxic tolerance when the endogenous cellular oxygen-sensing mechanism is activated.

Several recent studies have revealed three important features of the cellular oxygen-sensing mechanism. First, besides erythropoietin, a variety of genes relating to hypoxia adaptation are induced at low-oxygen tensions, including genes encoding vascular growth factors, glycolytic enzymes, and glucose transporters in various cell types (10, 13). Second, the cellular oxygen-level-sensing mechanism appears to rely on a heme protein, because reagents that modify heme, such as Co$_{2}^{3+}$, can induce the response in the absence of hypoxia, whereas heme synthesis inhibitors were demonstrated to abolish the hypoxic response (15). Third, in contrast to the diversities regarding hypoxia-sensitive genes and cell types, the induction of these genes appears to follow a common intracellular signal transduction pathway that is eventually mediated by a transcriptional factor, hypoxia inducible factor-1 (HIF-1) (13, 30).

In the in vivo heart and cultured cardiac cells, both hypoxia and Co$_{2}^{3+}$ quickly induce mRNA for vascular endothelial growth factor (VEGF) (2, 8, 18, 24, 25, 27), glucose transporter-1 (GLUT-1) (33, 38), and heme oxygenase-1 (8, 23), presumably by the same intracellular mechanism. These findings allow a speculation that the heart could adapt to oxygen deficiencies by activating the endogenous cellular oxygen-sensing mechanism. However, it remains to be elucidated whether activation of such an intracellular mechanism leads to functional improvement of cardiac contraction in hypoxia-reoxygenation in the beating heart.

Considering these factors, we undertook the present study to examine the effects of pretreating rats with a low concentration oral CoCl$_2$ on mechanical function of isolated crystalloid-perfused hearts in hypoxia-reoxygenation.

METHODS

Animals. The authors received approval for the experiment from the Animal Research Committee of the Yamagata University School of Medicine. Six-week-old male Sprague-Dawley rats (~150 g/wt) were purchased from Clea (Japan).

Address for reprint requests and other correspondence: E. Takahashi, Dept. of Physiology, Yamagata Univ. School of Medicine, Yamagata 990-9585, Japan (E-mail: eiji@med.id.yamagata-u.ac.jp).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Pairs of the control and Co2+-treated rats were kept in separate cages for 6–7 wk in our animal facility. The rats had free access to food and water. Rats in the control group drank tap water, whereas rats in the Co2+-group drank water containing 0.01% CoCl2. Body weight and water intake were measured twice a week.

Langendorff perfusion. The rats were anesthetized by intraperitoneal injection with 50 mg/kg pentobarbital sodium (Nembutal, Abbott Laboratories). Blood was then sampled from the tail vein, and hematocrit was measured with the use of the standard microcapillary method. After total heparinization (300 U/kg), the chest was opened, and the heart from the tail vein, and hematocrit was measured with the use of the standard microcapillary method. After total heparinization (300 U/kg), the chest was opened, and the heart was excised and connected to a constant pressure (85 cmH2O) Langendorff perfusion system. The heart was perfused with 95% O2-5% CO2 equilibrated Krebs-Henseleit bicarbonate buffer (KHB) solution containing (in mM) 120 NaCl, 25 NaHCO3, 4.7 KCl, 1.2 MgSO4, 1.2 KH2PO4, 1.2 CaCl2, and 11 glucose at 37°C. A water-filled balloon was inserted into the left ventricle via the left atrium. The left ventricular (LV) pressure was measured with a pressure transducer (model P23 XL, Ohmeda). Myoelectric signal was connected to the heart, and was continuously recorded on a thermal pen recorder (model WR3701, Graphtec). The end-diastolic pressure (EDP) was initially set to 8 mmHg by adjusting the volume of the balloon. In spontaneously beating isolated perfused hearts, perfusion with a hypoxic medium rapidly attenuated LV developed pressure (LVDP) and heart rate (HR), and spontaneous beating became undetectable during almost all the hypoxic period. In these hearts, cardiac contractions recovered close to the control level on reoxygenation. Therefore, using the electrical pacing, the heart was forced to beat as long as possible in hypoxia. A pair of platinum electrodes were placed on the atrium, and electrical pacing was conducted at 300 or 333 beats/min (depending on the spontaneous beating rate before cardiac pacing) with the use of an electric stimulator (model SEN3301, Nihon Kohden). Voltage of the pacing pulse was fixed to 150% of the initial pacing threshold determined during aerobic perfusion. We measured LVDP, EDP, and HR from the tracing of LV pressure. To assess the cardiac work, we calculated rate-pressure product (RPP = LVDP × HR). Coronary flow (CF) was determined by timed collections of coronary effluent that was subsequently normalized to the wet weight of the heart.

After the stabilization period for >1 h, the perfusion solution was switched to 95% N2-5% CO2 equilibrated KHB solution (solution Po2 < 30 mmHg). This hypoxic perfusion was continued for 60 min, followed by a perfusion with 95% O2-5% CO2 equilibrated KHB solution (reoxygenation) for 60 min. Electrical pacing was discontinued during the reoxygenation period.

Tissue Co2+ concentration. The hearts were excised from anesthetized and heparinized rats. The coronary artery was immediately perfused with cold saline to wash out blood. Small pieces of myocardial tissue (5–10 mg) were then sampled from the LV free wall, hydrolyzed, and quantitated by the fluorometry at 340 nm (model UV260, Shimadzu).

RT-PCR. The hearts were excised from anesthetized and heparinized rats. The coronary artery was immediately perfused with cold saline, and the ventricle was isolated, frozen, and stored at −80°C. The total mRNAs were isolated with the use of QuickPrep mRNA Purification kit (Pharmacia Biotech). The concentration of the mRNAs were determined by spectrophotometry at 260 nm. The first strand cDNAs were synthesized in 20-µl reaction solutions from 500 ng of total mRNAs using reverse transcriptase with oligodT primer (GIBCO-BRL). The primers for PCR were designed on the basis of the published mRNA sequences. To avoid amplification of the genomic DNA, each pair of the primers was derived from the coding regions intervened with introns. The sense and antisense primers, respectively, were composed of 5′-CGCATCTGCAATGATGTC-3′ and 5′-TCTTACCACTCACAGGGCTTTCTG-3′ for ribosomal protein L28 mRNA, 5′-ACCATGAACTTCTGCTCTG-3′ and 5′-TCTATGCTGACGTCAGGCACAC-3′ for VEGF mRNA, 5′-GGCAAGTCAGTCTGATGTCG-3′ and 5′-GGCAACTTCTGCTGACGTCACAG-3′ for GLUT-1 mRNA. L28 was used as a standard of the constitutive expression. All PCRs were performed in a final volume of 20 µl containing dNTPs (0.2 mM), Taq polymerase (1 U), the primers (1 µM), and cDNAs (0.5 µl). The PCR products (5 µl) were separated by a 3% Nusieve GTG agarose gel electrophoresis and visualized by Fas-3 CCD camera (Toyobo) after ethidium bromide staining. All of the PCR products were confirmed by DNA sequencing.

Statistics. Data are represented as means ± SE. The differences between Co2+-treated and control rats for hemat...
ocrit, body weight, heart weight, cellular oxygen consumption, and myocardial glycogen concentration were judged by the Student’s unpaired t-test. Differences between Co2-1-treated and control rats for cardiac mechanical functions were assessed by a two-way ANOVA followed by Scheffé’s F-test. For all statistical tests, P < 0.05 was considered significant.

RESULTS

CoCl2 effects on hematocrit. Nineteen rats were treated with oral CoCl2 for 43 days on average, whereas 18 rats were kept for the same time period as the control group. Net intake of CoCl2, calculated from water intake per day and CoCl2 concentration in water (0.01%), slightly increased from ~4 to ~5 mg/day at the end of CoCl2 administration period, whereas CoCl2 intake normalized to the body weight significantly decreased from ~20 mg·kg⁻¹·day⁻¹ (start) to ~10 mg·kg⁻¹·day⁻¹ (end) due to approximately threefold increase in the body weight. Table 1 summarizes changes in hematocrit, body weight, and heart weight at the end of the Co2-1 administration period. Chronic oral administration of CoCl2 significantly increased hematocrit without any effect on body weight or heart weight.

Cardiac contractile functions. All of the functional parameters measured during initial normoxic perfusion (baseline values) were similar in control and Co2-1 groups: LVDP, 76 ± 3 vs. 75 ± 3 mmHg; HR, 307 ± 6 vs. 309 ± 3 vs. 309 ± 3 beats/min; RPP, 23.4 ± 0.9 × 10³ mmHg/min vs. 23.1 ± 1.0 × 10³; EDP, 9 ± 1 vs. 8 ± 1 mmHg; and CF, 12 ± 1 vs. 11 ± 1 ml·min⁻¹·g⁻¹ wet for control and Co2-1-pretreated hearts, respectively. Hereafter, cardiac contractile function data are represented as a percentage of respective baseline values.

Figure 1 compares changes in LVDP between control and Co2-1-pretreated hearts. During hypoxic perfusion, LVDP showed biphasic suppressions and remained low during reoxygenation. LVDP of the Co2-1 group heart was significantly higher than that of the control group heart at the end of hypoxic perfusion (196% at 65–70 min) and during reoxygenation (156% at 125–130 min). Figure 2 represents changes in HR. Severe bradycardia occurred immediately after the onset of hypoxic perfusion in both groups. However, HR gradually restored toward the baseline in the rest of hypoxic perfusion period. It is worth noting that severe arrhythmia developed and the heart stopped beating during hypoxic perfusion in 5 of 18 control hearts, whereas all of the hearts in the Co2-1 group sustained rhythmic contractions. Cardiac contractile work assessed by RPP most clearly indicates better tolerance of the Co2-1 group heart to hypoxia (Fig. 3); although significantly downregulated, RPP of the Co2-1 group heart was more than two times higher than the control heart throughout hypoxic period (267% at 65–70 min) and at almost all the measuring points in reoxygen-

Table 1. Effects of chronic oral CoCl2 administration on hematocrit and body and heart weights

<table>
<thead>
<tr>
<th></th>
<th>Age, days</th>
<th>Hematocrit, %</th>
<th>Body Weight, g</th>
<th>Heart Weight, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>90 ± 0.9</td>
<td>45 ± 0.3</td>
<td>485 ± 8</td>
<td>1.3 ± 0.03</td>
</tr>
<tr>
<td>Co2-1</td>
<td>91 ± 0.9</td>
<td>51 ± 0.6*</td>
<td>473 ± 7</td>
<td>1.3 ± 0.03</td>
</tr>
</tbody>
</table>

Values are means ± SE. Average duration of CoCl2 administration was 43 days. Numbers of measurement for control and Co2-1 groups were 18 and 19, respectively. *P < 0.001 vs. control.

Fig. 1. Changes in left ventricular developed pressure (LVDP) during normoxic (open bar) and hypoxic (solid bar) perfusions. *P < 0.05 vs. control. Data are expressed as means ± SE.

Fig. 2. Changes in heart rate (HR) during normoxic (open bar) and hypoxic (solid bar) perfusions. *P < 0.05 vs. control. Data are expressed as means ± SE.

Fig. 3. Changes in rate pressure product (RPP) during normoxic (open bar) and hypoxic (solid bar) perfusions. *P < 0.05 vs. control. Data are expressed as means ± SE.
Data are expressed as means ± SE in the Co2 pretreated group during reoxygenation period were significantly lower than those in the control group. Peak EDP during hypoxic period and average EDP during hypoxic and reoxygenation periods were higher in the control group compared to the Co2 pretreated group. The EDP in the control group was significantly higher at the end of hypoxia (127% at 65–70 min) and during reoxygenation period (118% at 125–130 min), suggesting higher cellular ATP level. Figure 4 represents changes in EDP.

**Fig. 4.** Changes in end-diastolic pressure (EDP) during normoxic (open bar) and hypoxic (solid bar) perfusions. *P < 0.05 vs. control. Data are expressed as means ± SE.

Better cardiac energy state in the Co2+ group heart can be inferred from EDP (Fig. 4). Although there were substantial rises in EDP during hypoxic and reoxygenation periods, peak EDP during hypoxic period and average EDP during reoxygenation period were significantly lower in the Co2+ pretreated heart (72% at 65–70 min and 60% at 125–130 min), suggesting higher cellular ATP level. Figure 5 represents changes in CF. Hypoxic perfusion caused immediate vasodilatation, but CF gradually decreased during hypoxia, presumably due to hypercontracture that was reflected by the elevation of EDP. CF in the Co2+ group heart was significantly higher than that of the control (127% at 65–70 min) and during reoxygenation period (118% at 125–130 min).

**Fig. 5.** Changes in coronary flow (CF) during normoxic (open bar) and hypoxic (solid bar) perfusions. *P < 0.05 vs. control. Data are expressed as means ± SE.

**Tissue Co2+ concentration.** In two more rats given CoCl2 for 45 and 42 days, the myocardial tissue Co2+ concentrations were 0.57 and 0.63 μg/g wet, respectively, and hematocrit increased to 52 and 50%, respectively. The body weight of the rats was the same (480 g).

**Histological studies.** Four more rats were subjected to the histological studies. Two rats were treated with oral CoCl2 for 47 days, and their hematocrit levels rose to 52% and 54%, respectively. Neither light nor electron microscopic study indicated abnormalities, including inflammation, myocardial hypertrophy, degeneration, and loss of myofilaments, or interstitial fibrosis, similar to the control hearts (n = 2).

**Cellular oxygen consumption.** Cobalt may directly interfere with oxidation of fatty acids and pyruvate (36). This might affect cellular energy metabolism in hypoxia-reoxygenation. We examined the metabolic effect of chronic oral CoCl2 administration in two ways; mitochondrial respiration and tissue glycogen concentration. Fourteen more rats (seven control and seven Co2+ treated) were subjected to the measurement of cellular oxygen consumption. At 1 mM extracellular Ca2+, oxygen consumption of quiescent cardiac cells isolated from the Co2+ group heart was 146 ± 9 nmol O2·min⁻¹·10⁶ rod cells⁻¹ (n = 7) at 37°C, which was comparable with that of the control group heart (130 ± 12 nmol O2·min⁻¹·10⁶ rod cells⁻¹, n = 7). When the mitochondrial respiration was maximally stimulated by 1 μM CCCP, oxygen consumption of control cardiomyocytes increased to 790 ± 65 nmol O2·min⁻¹·10⁶ rod cells⁻¹, whereas that of Co2+-pretreated rat heart cells was 783 ± 97 nmol O2·min⁻¹·10⁶ rod cells⁻¹, again without a significant difference. Values for hematocrit of control and Co2+-treated rats of this particular experiment were 44% (SE = 0, n = 7) and 50 ± 0.5% (n = 7), respectively.

**Tissue glycogen concentration.** Suppression of glycolytic metabolism by Co2+, if any, may raise tissue glycogen concentration (28). Fourteen more rats (seven control and seven Co2+ treated) were subjected to this study. Tissue glycogen concentration was the same in the control (26.0 ± 3.1 μmol glucose/g wet, n = 7) and the Co2+-treated rat hearts (31.4 ± 3.1 μmol glucose/g wet, n = 7, p = 0.24). The hematocrit values of the control and Co2+-treated rats of this particular experiment were 45 ± 0.5 and 52 ± 0.7%, respectively.

**RT-PCR.** Eight (four control and four Co2+ treated) rat hearts were used. Figure 6 represents semiquantitative assessment of VEGF, aldolase-A, and GLUT-1 mRNAs. On days 7 and 14 of oral CoCl2 administration, significant inductions of VEGF, aldolase-A, and GLUT-1 mRNAs were observed, whereas levels of these mRNAs were comparable to the control heart on day 30.

**Fig. 6.** RT-PCR analysis of vascular endothelial growth factor (VEGF), aldolase-A, glucose transporter-1 (GLUT-1), and ribosomal protein L28 mRNAs. cnt, Control; Co, Co2+-treated hearts.
DISCUSSION

Activation of cellular oxygen-sensing mechanism in vivo by a low-concentration chronic oral CoCl₂. Erythropoiesis is known to be greatly stimulated by tissue hypoxia caused by hypoxemia, anemia, or hemorrhage (21). Glycoprotein hormone erythropoietin is the primary regulator of erythropoiesis. It has been known for a long time that cobalt increases erythropoietin production in vitro (12) and in vivo (20) in normoxia. Cobalt was once given to human patients for the treatment of anemia (6), although the mechanism for enhanced erythropoietin production by Co²⁺ had been unknown.

Recently, Goldberg et al. (14) demonstrated in the human hepatoma cell lines, Hep3B and HepG2, that production of erythropoietin mRNA was stimulated 6- to 12-fold in response to Co²⁺ in the absence of hypoxia. It was subsequently demonstrated that carbon monoxide blocked erythropoietin production in hypoxia, and inhibition of heme synthesis abolished the erythropoietin response to CoCl₂. These lines of evidence suggest an involvement of a heme protein in the intracellular signal transduction. Goldberg et al. (15) postulated that Co²⁺ might substitute for Fe²⁺ of the putative intracellular heme protein oxygen sensor, thus inhibiting binding of molecular oxygen to the heme, and fix the oxygen sensor in the deoxy form.

Expression of erythropoietin gene is regulated by a transcriptional factor HIF-1 that binds to the enhancer region of the erythropoietin gene (10, 29, 30). Therefore, the alternative hypothesis for activation of the oxygen-sensing mechanism by Co²⁺ is that Co²⁺ might stabilize HIF-1 by interfering with the action of oxygen in producing reactive oxygen species (the redox reaction) (1, 4, 9, 16).

In the present study, in accordance with previous observations in animals (20) and humans (6), chronic oral administration of CoCl₂ induced polycythemia without an effect on body or heart weight. Although we did not measure the blood erythropoietin level, these findings strongly suggest an in vivo activation of the cellular oxygen-sensing mechanism.

Excess amounts of Co²⁺ is cardiotoxic in humans and experimental animals, known as the cobalt cardiomyopathy (28). The mechanism appears to involve suppression of the citric acid cycle, which is reflected by depression of mitochondrial oxygen uptake (36). Therefore, optimization of Co²⁺ administration protocol is of critical importance in this study. In fact, the rats lost considerable weight if they were given water containing >0.02% CoCl₂. One rat that drank 0.01% CoCl₂ water for 45 days developed pericardial effusion, a cardiac lesion characteristic to the cobalt cardiomyopathy (28). However, all the Co²⁺-treated rats participated in the cardiac function study showed normal weight gain and normal wet weight of the heart (Table 1). Separate histological studies excluded possibility of degeneration, hypertrophy, or inflammatory changes in the myocardium. Furthermore, oxygen uptake of isolated quiescent single cardiomyocytes, even when maximally stimulated, was normal, suggesting that metabolic substrates utilization was not disturbed. Concentration of tissue Co²⁺ in the present Co²⁺-treated rat hearts was ~0.6 µg/g wet. The value is significantly higher compared with normal human hearts (0.01~0.04 µg/g dry, Ref. 5), but it appears to be significantly lower than that found in the cobalt cardiomyopathy (0.7~5.5 µg/g dry, average 3.3 µg/g dry, Ref. 5). Collectively, we conclude that the present method of cobalt administration was adequate to activate the cellular oxygen-sensing mechanism without significant damage to myocardium.

Improvement of cardiac mechanical function during hypoxia-reoxygenation in Co²⁺-treated rat hearts. In hypoxia and reoxygenation, crystalloid-perfused hearts isolated from the Co²⁺-pretreated rats showed superior contractile performance compared with control hearts. Thus the Co²⁺-pretreated rat heart can better adapt to reduced oxygen supply. In the perfused heart preparation, EDP and RPP reflect cellular ATP level and energy consumption by cardiac contraction, respectively. The present findings that the heart isolated from the Co²⁺-pretreated rat exhibited a lower increase in EDP despite higher RPP in hypoxia-reoxygenation may imply that ATP production in the Co²⁺-pretreated rat heart was better maintained in hypoxia than the control heart. Several effects of Co²⁺ may potentially account for the observed hypoxic tolerance.

Because excess Co²⁺ interferes with oxidation of pyruvate to acetyl CoA (36), nonutilized pyruvate may proceed to glycogen, thus increasing cellular glycogen stores (28). In the face of severe suppression of oxidative ATP production in hypoxia, the already-increased glycogen stores could continuously supply some amounts of ATP by anaerobic glycolysis so that the cellular energy state would not be severely impaired. Nevertheless, in the present study, an increase in myocardial tissue glycogen in Co²⁺-treated rats was insignificant to allow for such an account.

If erythropoiesis as observed in the present study occurred secondary to activation of the cellular oxygen-sensing mechanism by oral CoCl₂, it is natural to postulate that not only erythropoietin gene expression in the kidney but also other hypoxia-sensitive genes in other organs would be upregulated, because hypoxia should have activated the common intracellular mediator HIF-1 that operates at physiological oxygen concentrations (19, 22, 35, 39). In the heart, we therefore expected that activation of the oxygen-sensing mechanism by Co²⁺ would stimulate expression of genes that are important for hypoxic adaptations. Such molecular adaptation to hypoxia might eventually lead to functional improvement of cardiac contractile activity during hypoxia and reoxygenation.

The present study demonstrates in vivo induction of VEGF mRNA in cardiac tissue (Fig. 6) as shown in the previous studies (24, 25, 27). VEGF is a potent angiogenic factor and upregulation of VEGF may facilitate tissue perfusion and oxygenation in hypoxia, an important adaptation to hypoxia. It has been reported that Co²⁺ induces various glycolytic enzymes (7, 11, 26, 31)
along with glucose transporters (38). The present study also demonstrated in vivo inductions of aldolase-A and GLUT-1 mRNAs in Co\(^{2+}\)-treated cardiac tissue (Fig. 6). These findings suggest that the reserve for glycolytic energy production was higher in Co\(^{2+}\)-pretreated rat hearts. It is then presumable that the augmented glycolytic ATP flux would decelerate loss of high-energy phosphate pools in hypoxia.

In the cobalt-treated rats, hematocrit started to rise from day 30 and reached a plateau after day 40. Cardiac mechanical functions were subsequently determined on day 43, on average. Increases in levels of VEGF, GLUT-1, and aldolase-A mRNAs preceded the increase in hematocrit; they were detectable as early as on days 7 and 14 and leveled off on day 30. Because time course of the regulatory changes in genes and proteins induced by hypoxia and/or cobalt has not been precisely defined in vivo, it is difficult to definitely relate these genetic changes to the observed changes in cardiac contractility. However, previous reports suggest that in vivo effects of activation of hypoxia-related genes may persist for a prolonged period time. Harik et al. (17) demonstrated that exposure of rats to hypobaric hypoxia for 3 wk significantly increased GLUT-1 in cerebral microvessels, whereas it remained elevated even 3 wk after normoxic recovery at which GLUT-1 mRNA was returned to the control level. Similarly, Xie et al. (37) observed strong immunohistochemical stainings against basic fibroblast growth factor and VEGF near capillaries of rat hearts that underwent a transient ischemia (3 min) 30 days previously. Moreover, a half-life of 20 days has been reported for rat muscle aldolase (32). Together, it is possible that, among many hypoxia-related genes, at least three genes examined in the present study may well contribute to the hypoxic resistance of cardiac contractile functions determined after 43 days (average) of oral cobalt administration.

The heart can adapt to reduced oxygen supply in various ways, including coronary vasodilation, down-regulation of cellular oxygen demands, ischemic preconditioning, stimulation of anaerobic glycolysis (Pasteur effect), neovascularization, and increased myocardial myoglobin concentration. In addition, activation of the endogenous oxygen-sensing mechanism and subsequent induction of genes that are beneficial for hypoxic adaptation should be an effective strategy of the heart to adapt to hypoxia.

We thank Dr. Mitsunori Yamakawa at the Pathology I, Yamagata University School of Medicine, for conducting the histological studies.

REFERENCES

14. Goldberg MA, Glass GA, Cunningham JM, and Bunn HF. The regulated expression of erythropoietin by two human hepa
19. Huang LE, Gu J, Schau M, and Bunn HF. Regulation of hypoxia-inducible factor 1α is mediated by an O2-dependent degradation domain via the ubiquitin-proteasome pathway. Proc Natl Acad Sci USA 95: 7987–7992, 1998.


