The effects of acute and intermittent hypoxia on the expressions of HIF-1α and VEGF in the left and right ventricles of the rabbit heart

Akut ve aralıklı hipoksinin tavşan kalbinin sol ve sağ ventriküllerinde HIF-1α ve VEGF ekspresyonuna etkileri

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ABSTRACT

Objective: Hypoxia-inducible factor-1 alpha (HIF-1α) and vascular endothelial growth factor (VEGF) are involved in signaling mechanisms of cellular responses to hypoxia. These factors have been investigated in tissue samples by simulating different altitudes by changing the percentage of oxygen. We aimed first to evaluate the effect of normobaric, systemic hypoxia (11% O_2) on HIF-1α and VEGF mRNA levels in the heart muscle; secondly, to compare the levels of HIF-1α and VEGF mRNA in the left and right ventricle muscles.

Methods: In this experimental study, 33 New Zealand male rabbits were assigned to control, acute hypoxia (4 hours) and intermittent hypoxia (4 hours/day for 14 days) groups (n=11/group). Total RNA was isolated from right and left ventricles of the heart. The expressions of HIF-1α and VEGF mRNAs were investigated by using Reverse Transcription Polymerase Chain Reaction (RT-PCR) method. The obtained data were compared by using ANOVA and paired t-test.

Results: The results indicated that left ventricle VEGF mRNA expressions in both acute and intermittent hypoxia groups (1.08±0.15 and 1.03±0.19, respectively) were higher than that in the control group (0.88±0.15) (p=0.03). Hypoxia treatments did not significantly alter HIF-1α mRNA in both ventricles (p=0.60 and p=0.51 for left and right ventricles, respectively).

Conclusion: Since systemic hypoxia results in induction of VEGF mRNA up-regulation only in left ventricle, it could be related to its higher metabolic activity and oxygen utilization. Hypoxia induced changes in the expression of HIF-1α mRNA may not be the only determining factor for HIF-1/VEGF pathway induction or the observed VEGF induction could be through other hypoxia sensitive pathways.

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Key words: Acute hypoxia, intermittent hypoxia, expression, heart, HIF-1α, rabbit, VEGF, ventricle

ÖZET

Amaç: Hipoksi ile indüklenen faktör -1 alfa (HIF-1α) ve vasküler endotel büyüme faktörü (VEGF) hipoksiye hücresel yanıtın sinyal mekanizmasında yer alırlar. Bu faktörler, oksijen yüzdesi değiştirilerek ve böylece farklı yükseklikler simüle edilerek doku örneklerinde çalışılmaktadır. Biz, öncelikle normobarik, sistemik hipoksinin (%11 O_2) kalp dokusunda HIF-1α ve VEGF mRNA'sı üzerine etkisini değerlendirmeyi amaçladık. İkinci olarak sol ve sağ ventriküldeki HIF-1α ve VEGF mRNA seviyelerini karşılaştırmayı amaçladık.

Yöntemler: Bu deneysel çalışmada 33 New Zealand erkek tavşan kontrol, akut hipoksi (4 saat) ve aralıklı hipoksi (4 saat/gün, 14 gün) gruplarına ayrıldı (n=11/grup). Bütün RNA, kalbin sağ ve sol ventriküllerinden ayrıştırıldı. HIF-1α ve VEGF mRNA ekspresyonları RT-PCR yöntemi kullanılarak araştırıldı. Elde edilen bulgular ANOVA ve eşleştirilmiş t-testi kullanılarak karşılaştırıldı.

Bulgular: Bulgulara göre sol ventrikül VEGF mRNA ekspresyonları hem akut, hem de aralıklı hipoksi gruplarında kontrol grubundakine göre yüksekti (sırasıyla 1.08±0.15 ve 1.03±0.19) (p=0.03). Hipoksi uygulamaları her iki ventrikülde de HIF-1α mRNA'sını anlamlı ölçüde değiştirmedi (sol ventrikülde p=0.60, sağ ventrikülde p=0.51).

Sonuç: Sistemik hipoksinin yalnızca sol ventrikülde VEGF mRNA up-regülasyonunu tetiklemesi sol ventrikülün yüksek metabolik aktivitesi ve oksijen kullanımı ile ilişkili olabilir. HIF-1α mRNA ekspresyonunda hipoksinin tetiklediği değişimler, HIF-1/VEGF yolağının uyarılmasında tek belirleyici faktör olmayabilir ya da gözlenen VEGF tetiklenmesi hipoksiye duyarlı diğer yolaklar aracılığı ile olabilir. *(Anadolu Kardiyol Derg 2011; 11: 379-85)*

Anahtar kelimeler: Akut hipoksi, aralıklı hipoksi, ekspresyon, kalp, HIF-1α, tavşan, VEGF, ventrikül

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Introduction

Mammals have developed several protective mechanisms against hypoxia because oxygen has crucial function in energy production as a terminal electron acceptor in mitochondrial respiratory chain. One of these mechanisms involves hypoxia inducible factor-1 (HIF-1) pathway. HIF-1 induces the transcription of more than one hundred enzymes and proteins including vascular endothelial growth factor (VEGF). VEGF is an angiogenic factor, which plays a role in cellular response to hypoxia (1, 2). The effects of hypoxia on the expression of HIF-1, which is the hypoxia-sensitive protein subunit of HIF-1, and the expression of VEGF in umbilical vein endothelial cells, leukocytes, plasma and different kinds of tissues, were investigated previously (3-5). Either the hypoxia conditions or the findings of these studies varied. For example, in a study by Vogt et al. (5), intermittent normobaric hypoxia corresponding to the height of 3850 meter caused an increase in HIF-1α mRNA in the human skeletal muscle. However, Lundby et al. (6) could not show an increase in mRNAs of HIF-1α and VEGF and capillarization in skeletal muscle of humans staying at 4100 meter for 2 and 8 weeks.

In animal models, the responses of several tissues to systemic hypoxia have been evaluated by decreasing the percentage of oxygen in breathing air and thereby simulating the different altitudes. There was limited number of studies related to the heart tissue. Furthermore, the hypoxia protocols used in these studies were different (7-9). It has been shown in an immunohistochemical study that, the exposure of intermittent hypoxia 12h/day, for 12 days, did not increase the expression of HIF-1α protein in the rat heart (8). Birot et al. (7) observed that VEGF mRNA expression increased in left and right ventricle of the rat heart in the first and 8th days of hypobaric hypoxia, respectively with no increase in the VEGF protein level.

Considering the contradictory findings and different methodological approaches in the literature, we aimed to investigate the effects of acute and intermittent, systemic normobaric hypoxia on the mRNA expressions of HIF-1α and VEGF in the rabbit heart in the present study. Such studies on signaling mechanisms of intermittent hypoxia may help to better understand pathophysiological responses during intermittent hypoxic diseases such as sleep apnea, which is associated with increased risk of cardiovascular diseases. We hypothesized that systemic hypoxia increases the expressions of HIF-1α and VEGF mRNAs in the heart. In addition, since the oxygen demand of the left ventricle is more than that of the right ventricle (10), the response of left ventricle to hypoxia can be more significant. The duration and percentage of hypoxia applications used in other studies were different comparing to the present study. In addition, as of our knowledge, there is no study on the rabbit heart tissue in terms of investigating the expressions of HIF-1α and VEGF in response to the hypoxic conditions applied in this study.

We aimed first to evaluate the effect of normobaric, systemic hypoxia (11% O_2) on HIF-1α and VEGF mRNA levels in the heart muscle; secondly, to compare the levels of HIF-1α and VEGF mRNA in the left and right ventricle muscles.

Methods

Animals

Adult male New Zealand rabbits (weighing 2017±17 g) were included in this experimental study. The animals were housed for ten days before the experiments in a proper laboratory space reserved for experimental animals. Water and rabbit food were provided both in their cage and in the hypoxia chamber. A 12-hour light/dark cycle was provided using automated lighting system. All animal experiments were conducted under the guidelines on human use and care of laboratory animals for biomedical research published by National Institutes of Health (NIH) (No. 85-23, revised 1996) and were in conformation with the Declaration of Helsinki. The Ethics Committee of Ankara University approved the experimental protocol (No: 15-2002/260).

Experimental groups

Thirty three rabbits were randomly divided into three groups of eleven rabbits; control (C), acute hypoxia (AH) and intermittent hypoxia (IH) in this experimental study. Group C had no intervention but their tissues were extracted following ten day-adaptation period. Group AH was applied acute hypoxia for 4 hours. Tissues were collected at the following hour. Group IH was exposed to hypoxia for 4 hours/day, 14 days. Tissues were extracted on 15th day.

Hypoxia treatment and tissue collection

All hypoxia regimens were performed in a normobaric chamber connected to a tank containing an admixture of 89% nitrogen and 11% oxygen, which corresponds to an altitude of 5000 meter. Intra-chamber O_2 and CO_2 levels were monitored continuously by an analyzer. The chamber was ventilated with the same admixture when CO_2 was over 0.03%. Following the hypoxia treatment, the rabbit was anesthetized intramuscularly with Xylazine HCl (10 mg/kg, im) and Ketamine (50 mg/kg, im). The heart tissue was extracted. Left and right ventricles were separated and the excess blood was washed in saline. Tissue samples were immediately shocked with liquid nitrogen and stored in –80° until the next experiment.

Molecular studies

Total RNA isolation

Total RNA samples of left and right ventricles were isolated using a commercial isolation kit for fibrous tissues (RNAeasy® Fibrous Tissue, Qiagen). Briefly; following mechanochemical tissue homogenization and proteinase incubation, RNA samples were eluted in spin columns with several centrifuging and washing steps. The concentration of total RNA was measured at

260 nm. The ratios of 260/280 and 260/230 were considered for the purity and quality of the RNA and the extractions were repeated until 1.8-2 values were achieved. All total RNA samples were run on 1% agarose gels to check their integrity.

Reverse transcription polymerase chain reaction (RT-PCR)

2µg of total RNA per sample was converted to total cDNA by reverse transcriptase using commercially available reverse transcription (RT) kit (RevertAid™ First Strand cDNA Synthesis Kit, Fermentas, Life Sciences, EU). RT products were amplified with PCR using rabbit HIF-1α, VEGF and 18S rRNA (house-keeping gene) specific primers (11). The gene regions corresponding to these primers were double-checked from "NCBI, Entrez Nucleotide Database (http://www.ncbi.nlm.nih.gov/sites/entrez?db=Nucleotide&itool=toolbar)" and the base-pair counts of PCR products were calculated. In addition, optimal PCR conditions were adjusted according to the base sequences (Table 1).

Agarose gel electrophoresis and mRNA analysis

PCR products (10µl) were run on 2% agarose gel with etidium bromide at 100 volts for 1 hour. The mRNA bands in the gel were visualized under UV and transferred to a computer by a digital camera attached to it. The sizes of the sample's bands were compared to the bands of a DNA marker (PhiX174 DNA/BsuRI {HaeIII} Marker, 9) with known standard base pairs to determine if the obtained cDNA bands were corresponding to the specific genes. The band density was measured using a software program (Image J 1.38X, Wayne Rasband, NIH, USA). The relative contents of the VEGF and HIF-1α mRNAs were calculated as proportion of the density of 18S rRNA for each sample. All measurements were triplicated.

Statistical analysis

The statistical analysis was performed by using SPSS 13.0 program (SPSS Inc. and Lead Tech. Inc., Chicago, USA). The values were presented as mean±SD. The results from three experimental groups were compared by using parametric ANOVA test for two different genes separately. When p value to be equal or smaller than 0.05, the relation between each two groups was controlled by Bonferroni's post-hoc test. Paired t-test was used to evaluate the findings from left and right ventricles of the heart of the same animal.

Results

The mRNA expression of HIF-1α in the heart tissue

Left ventricle: The expression of HIF-1α mRNA in left ventricle showed a tendency to an increase in both the AH and IH groups comparing to the control group. However, there was no statistical difference (Table 2 and Fig. 1A).

Right ventricle: The expression of HIF-1α mRNA in right ventricle was not different between the experimental groups (Table 2 and Fig. 1B).

Ventricle difference: HIF-1α mRNA expression of the right ventricle was significantly higher than that of the left ventricle in the control group with paired t test (p= 0.02), (Table 2 and Fig. 1C). The expression of HIF-1α mRNA was not statistically different between ventricles in hypoxia treated groups.

The mRNA expression of VEGF in the heart tissue

Left ventricle: The expression of VEGF mRNA increased in the left ventricle of the AH and IH groups comparing to the control group. This change was statistically significant with ANOVA test (p=0.03), (Table 3 and Fig. 2A).

Right ventricle: The expression of VEGF mRNA in the right ventricle was not different among the all experimental groups (Table 3 and Fig. 2B).

Ventricle difference: VEGF mRNA expression of the right ventricle was also higher than that of the left ventricle in the control group, but the difference was not statistically significant (p>0.05). In addition, the expression of VEGF mRNA was not statistically different between ventricles in hypoxia treated groups (p>0.05).

Discussion

The positive results of the present study include; 1. Acute and intermittent, systemic, normobaric hypoxia exposure significantly increased the mRNA expression of VEGF in the left ventricle of rabbit heart, compared to normoxic control hearts. The degree of the effect of intermittent hypoxia was less than the effect of acute hypoxia. 2. The baseline HIF-1α mRNA expression in the right ventricle was higher than that in the left ventricle.

Hypoxic injury of the heart is caused by the imbalance between cardiac oxygen supply and demand. The oxygen sup-

Table 1. The PCR conditions and base sequences of primers for HIF-1α, VEGF and 18S rRNA

	Primer sequence	Base count	PCR program (30 cycle)
HIF-1α	f: 5'-CCACAGGACAGTACAGGATG - 3' r: 5'-TCAAGTCGTGCTGAATAATACC - 3'	150 bp	94˚C (3')/94˚C (30'') - 57˚C (30'') - 72˚C (1')/72˚C (5')
VEGF	f: 5'-CGAGACCTTGGTGGACATC - 3' r: 5'-CTGCATGGTGACGTTGAAC - 3'	151 bp	94˚C (3')/94˚C (30'') - 54˚C (30'') - 72˚C (1')/72˚C (5')
18S rRNA (control)	f: 5'-CGGCGACGACCCATTCGAAC - 3' r: 5'-GAATCGAACCCTGATTCCCCGTC-3'	99 bp	94˚C (3')/94˚C (30'') - 64˚C (30'') - 72˚C (1')/72˚C (5')

HIF-1α - hypoxia inducible factor-1, PCR - polymerase chain reaction, VEGF - vascular endothelial growth factor

Table 2. The expression of HIF-1α mRNA in the heart ventricles of the experimental groups

HIF-1α	Left ventricle	Right ventricle
Control	0.50±0.13	0.63±0.11**
Acute hypoxia	0.56±0.21	0.64±0.17
Intermittent hypoxia	0.58±0.24	0.70±0.15
F*	0.53	0.68
p*	0.60	0.51

The values are the band volume ratios of HIF-1α mRNA to 18S rRNA
The values are mean±SD with n=11/group
*ANOVA
**- paired t-test - p<0.05 left ventricle vs. right ventricle in control group
HIF-1α - hypoxia inducible factor-1

Table 3. The expression of VEGF mRNA in the heart ventricles of the experimental groups

VEGF	Left ventricle	Right ventricle
Control	0.88±0.15	1.06±0.33
Acute hypoxia	1.08±0.15**	0.97±0.27
Intermittent hypoxia	1.03±0.19**	0.93±0.20
F *	4.17	0.57
p*	0.03	0.57

The values are the band volume ratios of VEGF mRNA to 18S rRNA.
The values are mean±SD with n=11/group
*ANOVA, posthoc Bonferroni test
**p<0.05 vs. control Bonferroni post-hoc test in the left ventricle
VEGF - vascular endothelial growth factor

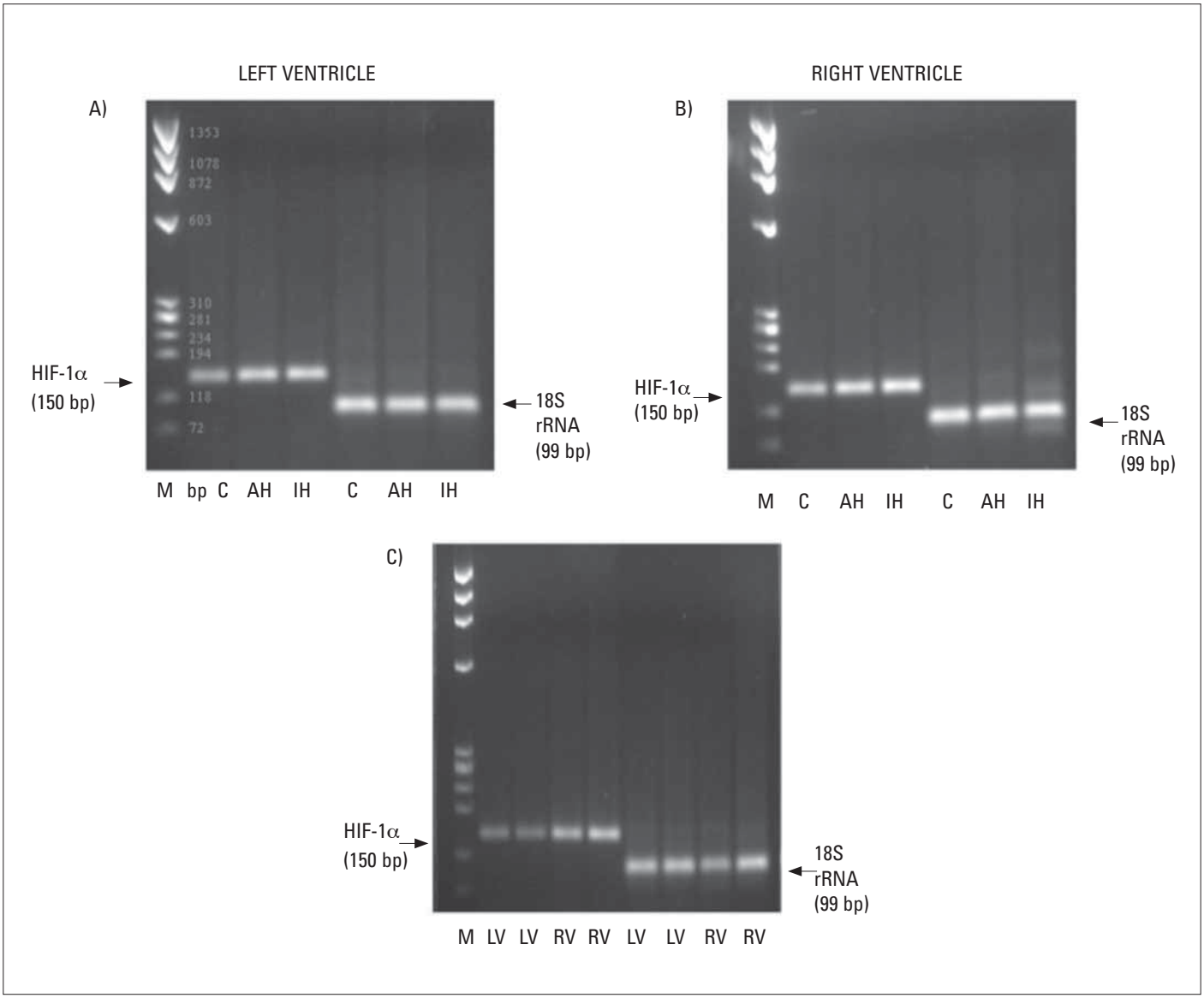

Figure 1. Samples of 2% agarose gel showing HIF-1α mRNA expressions in (A) the left ventricle and (B) the right ventricle of the hearts removed from the three experimental groups. (C) A sample of 2% agarose gel illustrating the expression of HIF-1α mRNA in the left and right ventricles of the control group

AH - acute hypoxia, bp - base pair, C - control, HIF-1α - hypoxia inducible factor-1, IH - intermittent hypoxia, LV - left ventricle, M - marker, RV - right ventricle

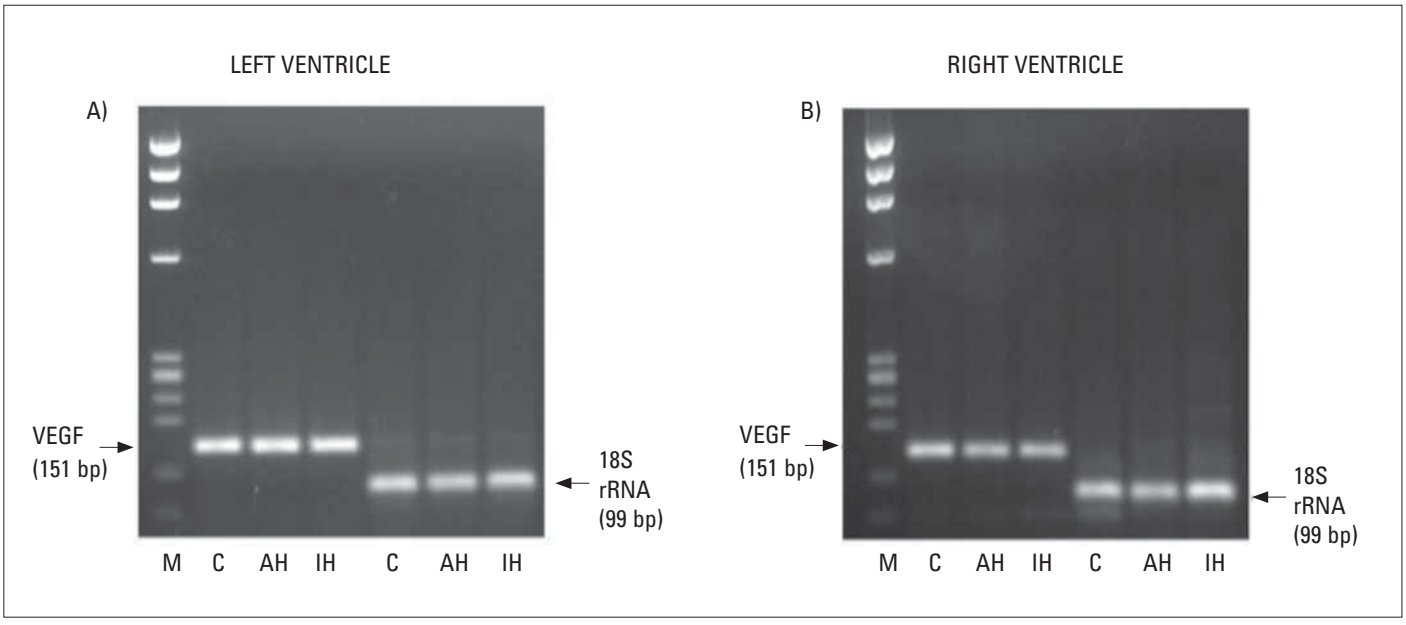

Figure 2. Samples of 2% agarose gel showing VEGF mRNA expressions in (A) the left ventricle and (B) the right ventricle of the hearts removed from the three experimental groups

AH - acute hypoxia, C - control, IH - intermittent hypoxia, VEGF - vascular endothelial growth factor, M - marker

port comes from myocardial blood perfusion, blood oxygen carrying capacity, and partial oxygen pressure (12). Coronary angiogenesis, one of the heart's response ways to hypoxia, reported to be induced by acute and/or chronic hypoxia with the involvement of HIF-1α/VEGF signaling mechanism. For instance, in a clinical study, the investigators demonstrated that the myocardial perfusion of the patients with coronary heart disease increased by intermittent hypobaric hypoxia exposure (13). Furthermore, in a recent study on the patients with coronary heart disease, the expression of HIF-1α protein in leukocytes was found to be enhanced and the authors suggested that this pathway involves in ischemia-induced coronary collateralization (14).

In the present study, HIF-1α expression increased in the left ventricle of the heart with acute and intermittent hypoxia as it was hypothesized, but the difference did not reach a statistical significance. As an example to a positive result, it has been shown that short cycled, mild intermittent hypoxia induced HIF-1α pathway in rabbit heart (15). However, in another study, although intermittent hypoxia induced the nuclear translocation of HIF-1α protein with following increase in VEGF protein, the myocardial expression of HIF-1α protein was not found to be increased (8). This result was similar to our finding showing that the increase of VEGF mRNA expression was not associated with an increase in HIF-1α mRNA expression in the left ventricle. Immunohistological methods can be used to further explain the hypoxia-induced changes in cellular behavior of HIF-1α protein in the future studies.

The difficulty of showing the changes in HIF-1α could be overcome using other methods such as Real-time PCR with directly transferring PCR products to more quantitative values. We could consider this as our limitation in this study. Nevertheless RT-PCR and agarose gel electrophoresis are well known, useable applications. In addition, we carefully performed all experiments in standard conditions. All density measurements were studied three times. In a previous study, the alterations in the type of muscle fibers, capillarization and the mRNA expressions of HIF-1α and VEGF in the skeletal muscle samples taken from human subjects who were elevated to 4100 meter up to sea level for 2 and 8 weeks, were investigated (6). There were no changes of these expressions with acclimatization, although sensitive methods such as "fluorescence-based real-time PCR" and visual methods such as "ATPase histochemistry analysis" were used (6).

One other reason for observing no change but trend in HIF-1α mRNA with hypoxia can be thought as the degree of hypoxia. Mild hypoxic hypoxia (11% O_2) may not be sufficient to induce HIF-1α mRNA in cardiac tissue. However, in the present study, we observed an increase of VEGF mRNA expression in the same tissue samples. Furthermore, Tokyol et al. (16) demonstrated the hypoxic tissue injury histopathologically associated with up-regulation of the stress proteins such as HSP70 in the livers of these same animals subjected to same hypoxic experiments. This indicates that the degree of applied hypoxia is enough to create stress response in cells.

The role of VEGF in cellular response to hypoxia is a well known mechanism. VEGF involves in pulmonary and cerebral edema in acute mountain sickness. For example, it was shown that VEGF mRNA expression in lung tissue increased as a result of acute (4 h at 8000 m) and intermittent (4 h/day for 2 weeks at 5000 m) hypoxia exposure. The VEGF response to acute hypoxia was more evident and associated with fluid leakage from the lungs. As the duration of acclimatization of rats get longer, the expression level of VEGF and the fluid leakage from the lungs

decreased (17). In the present study, the up-regulation pattern of VEGF mRNA in rabbit heart was similar to this previous finding. AH group showed more increase in VEGF mRNA expression than the IH group. This can be explained by the effect of acclimatization.

HIF-1α is a transcription factor for VEGF and other angiogenetic factors (2). It is expected that any change in VEGF should follow the activation of HIF-1α protein. In response to hypoxia, HIF-1α protein is stabilized and translocated to the nucleus where it binds HIF-1β forming HIF-1α, active transcription factor. Thus, an initial increase in mRNA is not necessary for HIF-1α to become active and promote transcription of the VEGF gene. The present study does not rule out HIF-mediated activation of VEGF as there are no data in regard to HIF-1α protein or HIF-1α DNA-binding capacity. The lack of the data on the protein expressions of HIF1α and VEGF is another limitation for our study. Therefore this study does not prove that HIF-1α is not involved in the increase in VEGF. Alternatively, other agents may intermediate VEGF signaling. Very recently, a new transcriptional factor named PGC-1α (peroxisome-proliferator-activated receptor-gamma coactivator-1α) was discovered. It was demonstrated that PGC-1α was induced by an insufficiency of oxygen and nutrients and PGC-1α increased both VEGF expression and vascularization in skeletal muscle (18). This interaction is a potential candidate for next step studies in cardiac hypoxia.

Normal energy metabolism in the left ventricle differs from that in the right ventricle. Another aim of the present study was to investigate the heart tissue by separating it into left and right ventricles because the metabolic activity and oxygen utilization of the left ventricle is more than the right ventricle (10). This enhanced oxidative capacity of the left ventricle was found to be diminished during hypoxic adaptation (19). Oxidation of different carbon substrates, including pyruvate, palmitoyl-L-carnitine, and glutamate, was compromised in the left ventricle within 24-hour of hypoxic exposure. By contrast, substrate oxidation in the right ventricle was unaffected by chronic hypoxia. As a result, hypoxia resulted in a reduced capacity to synthesize ATP via oxidative phosphorylation in the left, but not in the right ventricle (19). Furthermore, the right ventricle gives specific adaptive responses to chronic hypoxia such as hypertrophy (20). We found that the change of VEGF mRNA with hypoxia was evident only in the left ventricle. This shows the sensitivity of the left ventricle to hypoxia. Birot et al. (7) demonstrated that VEGF mRNA expression was enhanced at the first day of hypoxia in the left ventricle and at the 18th day of hypoxia in the right ventricle. However they failed to show following protein expression of VEGF (7). This is comparable to our findings if we don't consider the hypoxia duration as well. We saw higher HIF-1α mRNA levels in the right ventricles of all experimental groups comparing to the left ventricles. Only control groups were statistically significant. The reason of these baseline values is unknown and the trend in other groups might be related to this baseline elevation.

High altitude studies have been continuously conducted with the purpose of decreasing the harmful effects of hypoxia down to minimum while putting forward the beneficial effects of hypoxia. Intermittent hypoxia has pronounced effects on cross protection from several pathological events (21, 22) and on improvement in exercise performance of athletes (23, 24). Another clinical advantage that has been searched in hypoxia studies is the protective role of hypoxia treatment in the heart. Intermittent hypoxic adaptation has protective effect on myocardial ischemic injury (22, 25). Although same impact could be seen in chronic continuous hypoxia, adverse effects such as pulmonary hypertension and right ventricular hypertrophy are more marked (26). At the cellular level, Xi et al. (22) proved that iNOS plays the trigger as well as mediator roles in intermittent hypoxia-induced delayed cardioprotection in adult mice. Belaidi et al. (27) also confirmed the causative role of iNOS in cardioprotection induced by sub-acute intermittent hypoxia. Furthermore, they demonstrated for the first time that HIF-1α is the primary transcription factor responsible for the myocardial iNOS gene up-regulation following preconditioning by systemic intermittent hypoxia. The HIF-1α dependence identified in this study is also partially supportive for an earlier study by Cai et al. (25), who demonstrated the loss of delayed cardioprotection induced by acute intermittent systemic hypoxia (5 cycles of 6 min of 6% hypoxia and 6 min of normoxia) in the heterozygous HIF-1α knockout mice.

Study limitations

We used the semi-quantitative reverse transcription PCR method for analysis of mRNA expression. Since this method requires a delicate personal effort for standardization, real time PCR is the more secure method. However, other studies in the literature have been using the semi-quantitative method (28). On the other hand, the results of the study could be supported by the protein expressions of HIF-1α and VEGF. These two limitations were discussed in detail above.

Conclusion

The results of present study add valuable information to existing literature by describing the effects of systemic acute and intermittent hypoxia on HIF-1α mRNA and VEGF mRNA expressions in the left and right ventricles of rabbit hearts. Our aim was to investigate the cellular effects of the systemic hypoxia in the heart. The prominent finding was an up-regulation of VEGF in the left ventricular muscle with acute and intermittent hypoxia exposure. Determining the optimal conditions for hypoxia applications by eliciting the signaling mechanisms of the effects of hypoxia on the heart is important. Because the HIF-1α/VEGF pathway have been implicated in the development of collateral circulation in the myocardium and cardioprotection in ischemia/reperfusion injury, and intermittent hypoxia protocols can eventually be used therapeutically, the data from this study is useful for the clinical translation of the basic findings.

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Conflict of interest: None declared.

References

1. Semenza GL. Hypoxia-inducible factor 1: oxygen homeostasis and disease pathophysiology. Trends Mol Med 2001; 7: 345-50.
2. Forsythe JA, Jiang BH, Iyer NV, Agani F, Leung SW, Koos RD, et al. Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. Mol Cell Biol 1996; 16: 4604-13.
3. Chavez JC, Agani F, Pichiule P, LaManna JC. Expression of hypoxia-inducible factor-1alpha in the brain of rats during chronic hypoxia. J Appl Physiol 2000; 89: 1937-42.
4. Lemus-Varela ML, Flores-Soto ME, Cervantes-Munguía R, Torres-Mendoza BM, Gudiño-Cabrera G, Chaparro-Huerta V, et al. Expression of HIF-1α, VEGF and EPO in peripheral blood from patients with two cardiac abnormalities associated with hypoxia. Clin Biochem 2010; 43: 234-9.
5. Vogt M, Puntschart A, Geiser J, Zuleger C, Billeter R, Hoppeler H. Molecular adaptations in human skeletal muscle to endurance training under simulated hypoxic conditions. J Appl Physiol 2001; 91: 173-82.
6. Lundby C, Pilegaard H, Andersen JL, van Hall G, Sander M, Calbet JA. Acclimatization to 4100 m does not change capillary density or mRNA expression of potential angiogenesis regulatory factors in human skeletal muscle. J Exp Biol 2004; 207: 3865-71.
7. Birot OJ, Peinnequin A, Simler N, van Cuyck-Gandre H, Hamel R, Bigard XA. Vascular endothelial growth factor expression in heart of rats exposed to hypobaric hypoxia: differential response between mRNA and protein. J Cell Physiol 2004; 200: 107-15.
8. Cataldi A, Bianchi G, Rapino C, Sabatini N, Centurione L, Di Giulio C, et al. Molecular and morphological modifications occurring in rat heart exposed to intermittent hypoxia: role for protein kinase C alpha. Exp Gerontol 2004; 39: 395-405.
9. Forkel J, Chen X, Wandinger S, Keser F, Duschin A, Schwanke U, et al. Responses of chronically hypoxic rat hearts to ischemia: KATP channel blockade does not abolish increased RV tolerance to ischemia. Am J Physiol Heart Circ Physiol 2004; 286: H545-51.
10. Goh K, Dallam RD. Oxygen consumption of the auricles, right and left ventricles of the normal, hypothyroid rat heart. Am J Physiol 1957; 188: 514-8.
11. Patel TH, Kimura H, Weiss CR, Semenza GL, Hofmann LV. Constitutively active HIF-1α improves perfusion and arterial remodeling in an endovascular model of limb ischemia. Cardiovasc Res 2005; 68: 144-54.
12. Ardehali A, Ports TA. Myocardial oxygen supply and demand. Chest 1990; 98: 699-705.
13. del Pilar Valle M, García-Godos F, Woolcott OO, Marticorena JM, Rodríguez V, Gutiérrez I, et al. Improvement of myocardial perfusion in coronary patients after intermittent hypobaric hypoxia. J Nucl Cardiol 2006; 13: 69-74.
14. Chen SM, Li YG, Zhang HX, Zhang GH, Long JR, Tan CJ, et al. Hypoxia-inducible factor-1alpha induces the coronary collaterals for coronary artery disease. Coron Artery Dis 2008; 19: 173-9.
15. Ning XH, Chen SH, Buroker NE, Xu CS, Li FR, Li SP, et al. Short-cycle hypoxia in the intact heart: hypoxia-inducible factor 1α signaling and the relationship to injury threshold. Am J Physiol Heart Circ Physiol 2007; 292: H333-41.
16. Tokyol C, Karaorman G, Başţuğ M. Effects of acute and adaptive hypoxia on heat shock protein expression in hepatic tissue. High Alt Med Biol 2005; 6: 247-55.
17. Yang X, Xie Y, Zhang D. Research on the relationship between expression of VEGF and high altitude pulmonary edema. Zhonghua Yi Xue Za Zhi 2000; 80: 931-5.
18. Arany Z, Foo SY, Ma Y, Ruas JL, Bommi-Reddy A, Girnun G, et al. HIF-independent regulation of VEGF and angiogenesis by the transcriptional coactivator PGC-1alpha. Nature 2008; 451: 1008-12.
19. Rumsey WL, Abbott B, Bertelsen D, Mallamaci M, Hagan K, Nelson D, et al. Adaptation to hypoxia alters energy metabolism in rat heart. Am J Physiol 1999; 276: H71-80.
20. Penaloza D, Arias-Stella J. The heart and pulmonary circulation at high altitudes: healthy highlanders and chronic mountain sickness. Circulation 2007; 115: 1132-46.
21. Meerson FZ, Ustinova EE, Manukhina EB. Prevention of cardiac arrhythmias by adaptation to hypoxia: regulatory mechanisms and cardiotropic effect. Biomed Biochim Acta 1989; 48: S83-8.
22. Xi L, Tekin D, Gürsoy E, Salloum F, Levasseur JE, Kukreja RC. Evidence that NOS2 acts as a trigger and mediator of late preconditioning induced by acute systemic hypoxia. Am J Physiol Heart Circ Physiol 2002; 283: H5-H12.
23. Fulco CS, Rock PB, Cymerman A. Improving athletic performance: is altitude residence or altitude training helpful? Aviat Space Environ Med 2000; 71: 162-71.
24. Beidleman BA, Muza SR, Fulco CS, Cymerman A, Ditzler DT, Stulz D, et al. Intermittent altitude exposures improve muscular performance at 4.300 m. J Appl Physiol 2003; 95: 1824-32.
25. Cai Z, Manalo DJ, Wei G, Rodriguez ER, Fox-Talbot K, Lu H, et al. Hearts from rodents exposed to intermittent hypoxia or erythropoietin are protected against ischemia-reperfusion injury. Circulation 2003; 108: 79-85.
26. Ostadal B, Ostadalova I, Dhalla NS. Development of cardiac sensitivity to oxygen deficiency: comparative and ontogenetic aspects. Physiol Rev 1999; 79: 635-59.
27. Belaidi E, Beguin PC, Levy P, Ribuot C, Godin-Ribuot D. Prevention of HIF-1 activation and iNOS gene targeting by low-dose cadmium results in loss of myocardial hypoxic preconditioning in the rat. Am J Physiol Heart Circ Physiol 2008; 294: H901-8.
28. Sasso FC, Carbonara O, Persico E, D'Ambrosio R, Coppola L, Nasti R, et al. Increased vascular endothelial growth factor mRNA expression in the heart of streptozotocin-induced diabetic rats. Metabolism 2003; 52: 675-8.