Enhancement of glycolysis in cardiomyocytes elevates endothelin-1 expression through the transcriptional factor hypoxia-inducible factor-1α

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ABSTRACT

We investigated whether the type of energy metabolism directly affects cardiac gene expression. During development, the heart switches from glycolysis to fatty acid β-oxidation in vivo, as demonstrated by the developmental switching of the major isofrom of myosin heavy chain (MHC) from β to α. However, the β-MHC isoform predominates in monocrotaline-induced pulmonary hypertension, a model of right ventricular hypertrophy in vivo. Cultured cardiomyocytes showed a predominance of β-MHC expression over that of α-MHC, the same pattern as in the hypertrophied heart, suggesting that the in vitro condition itself causes the energy metabolism of cardiomyocytes to be switched to glycolysis. Electrical stimulation of cultured cardiomyocytes decreased the expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and hypoxia-inducible factor-1α (HIF-1α), but not that of peroxisome-proliferator-activated receptor-γ co-activator, suggesting that electrical stimulation suppresses the glycolytic system. Furthermore, a higher oxygen content (50%) decreased drastically the expression of GAPDH, HIF-1α and endothelin-1 (ET-1), and increased [1H]palmitate uptake. These findings indicate that the intrinsic energy metabolic system in cultured cardiomyocytes in vitro is predominantly glycolysis, and that the gene expression of cardiac ET-1 parallels the state of the glycolytic system. An antisense oligonucleotide against HIF-1α greatly decreased the gene expression of ET-1 and GAPDH, suggesting that cardiac ET-1 gene expression is regulated by cardiac energy metabolism through HIF-1α. In conclusion, it is suggested that the pattern of gene expression of ET-1 reflects the level of the glycolytic system in cardiomyocytes, and that enhanced glycolysis regulates the cardiac gene expression of ET-1 via HIF-1α.

INTRODUCTION

Many studies have been performed to investigate the mechanisms involved in the pathogenesis of heart failure. Although many cardiac diseases can cause heart failure (e.g. valvular diseases, ischaemic heart diseases and cardiomyopathy), several biochemical disturbances have been revealed to commonly occur in the failing heart. Among these, we have focused on cardiac energy metabolism, which is impaired in the failing heart. Fatty

Key words: cardiomyocytes, energy metabolism, ET-1, HIF-1α.

Abbreviations: ET-1, endothelin-1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HIF-1α, hypoxia-inducible factor-1α; MHC, myosin heavy chain; PPAR-γ, peroxisome-proliferator-activated receptor-γ; PGC-1, PPAR-γ co-activator-1; RT-PCR, reverse transcription–PCR.

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acid β-oxidation is down-regulated in the failing heart in animal models and humans [1,2], clearly demonstrating that an impairment of cardiac energy metabolism is involved in heart failure. We showed previously that endothelin-1 (ET-1) gene expression is increased in the failing heart, and that ET-1 receptor antagonists can improve the survival rate in animal models of heart failure [3]. However, how ET-1 gene expression in the failing heart is up-regulated during the progression of heart failure was uncertain.

We have demonstrated that hypoxia-inducible factor-1α (HIF-1α), the master transcriptional factor of glycolytic metabolism, is responsible for the transcriptional regulation of the ET-1 gene [4]. However, that study did not investigate whether impaired energy metabolism in the heart is itself a principal regulator of the gene expression of ET-1. We hypothesize that the cellular energy state itself affects gene regulation in the heart. However, this has not yet been clearly demonstrated; therefore the purpose of the present study was to clarify which state of energy metabolism in cardiomyocytes, i.e. enhancement of glycolysis or of β-oxidation, is responsible for increased ET-1 gene expression.

METHODS

Primary culture of rat cardiomyocytes
Cardiomyocytes were isolated from the hearts of 2-day-old Sprague–Dawley rat neonates, and incubated on a fibronectin-coated dish in Dulbecco’s modified Eagle’s medium/Ham’s F12 medium, as described previously [4,5].

Reverse transcription–PCR (RT-PCR)
RNA isolation and RT-PCR were performed as described previously [4,5]. The synthesized cDNA was amplified with gene-specific primers for HIF-1α, ET-1 and peroxisome-proliferator-activated receptor-γ (PPAR-γ) co-activator-1 (PGC-1) [6], as well as a β-actin primer as an internal control. The upstream and downstream gene-specific primers were as follows: HIF-1α, 5’ AGTCAGCAACGTGGAAGG 3’ (sense) and 5’ GGAGGAAAAGGGAATCGTG 3’ (antisense); ET-1, 5’ TCTTCTCTCGTGTGTTGTG 3’ (sense) and 5’ TTAGTTTTCTTCCCTCCACC 3’ (antisense); PGC-1, 5’ GCTCTTCCITTAACCTCCGTG 3’ (sense) and 5’ CTTCTGCTTGCTGCTTCTCT 3’ (antisense); β-actin, 5’ GAAATCTGAGCCAGGCG- GTG 3’ (sense) and 5’ CTGACTCTCCTGCTGCTGAGTC- TCC 3’ (antisense); myosin heavy chain (MHC), 5’ CATGAGGAAGAGTGAGCGGC 3’ (antisense); glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 5’ CCTCAAATCATGCTCTACA 3’ (sense) and 5’ CCTTCCACAATGCCAAAAGTT 3’ (antisense).

PCR was performed, with the annealing temperature and number of cycles for each template as follows: 56 °C/28 cycles for HIF-1α, 52 °C/26 cycles for ET-1, 58 °C/30 cycles for PGC-1, 72 °C/24 cycles for β-actin, 68 °C/30 cycles for MHC, and 64 °C/24 cycles for GAPDH. MHC products amplified by PCR were treated with MspI to identify the α- and β-isoforms; only the β-isoform was digested by MspI, to produce 55 bp and 175 bp products.

As in our previous study [7], PCR was performed in the range that gave a linear correlation between the amount of cDNA and the yield of PCR products. The ratio of the RT-PCR product for each gene to that of β-actin was quantified and compared.

Electrical stimulation
Primary cultured cardiomyocytes were stimulated by electrodes set in a culture dish with frequency of 5 Hz and a duration of 10 ms, for 12 h [8].

Palmitate uptake
During the primary culture of cardiomyocytes in 20% or 50% oxygen, [3H]palmitate was added directly to the dish. After a fixed time interval, the cardiomyocytes were washed and lysed for measuring the uptake of [3H]palmitate.

Antisense oligonucleotide experiment
We prepared an antisense oligonucleotide (CCTCCATGCGCAATCGGTGC) against a transcriptional starting codon of HIF-1α. As a control, a scramble oligonucleotide was also prepared (ACTCGTACC-CGGCAGTTCG). It had already been confirmed that the sequence of the scramble oligonucleotide is not same as any sequence reported in a database. The antisense or scramble oligonucleotide (7.5 μM each) was added to primary cultured cardiomyocytes using Lipofectin (Life Technologies, Rockville, MD, U.S.A.) and incubated for 6 h, followed by a 48 h incubation in ordinary culture medium. Cardiomyocytes were finally harvested for RNA isolation. Total RNA was isolated from the cardiomyocytes for further evaluation of gene expression by RT-PCR.

RESULTS

MHC isoform gene expression during development and in the hypertrophied heart
We isolated RNA from the hearts of rat fetuses, neonates and adults to compare the gene expression of MHC. Increasing expression of the α-isoform of MHC was
shown during the development of the heart. Specifically, the gene expression of the MHC $\beta$-isoform decreased gradually from the fetus to the adult; therefore the MHC isoform in the normal adult heart is exclusively $\alpha$-MHC. However, under conditions of cardiac hypertrophy (e.g. monocrotaline-induced right ventricular hypertrophy), adult hearts show increased gene expression of the MHC $\beta$-isoform (Figure 1a). Because it has been reported that glycolysis is the main energy metabolic system in the fetal heart, and that the hypertrophied heart switches energy metabolism from $\beta$-oxidation to glycolysis, it is suggested that an alteration of MHC isoform expression reflects an alteration in the energy metabolic system.

**Increased gene expression of the MHC $\beta$-isoform in cultured cardiomyocytes**

In primary cultured cardiomyocytes, the pattern of MHC gene expression in vitro contrasts with that of the heart in vivo. Even in cardiomyocytes isolated from the neonatal heart, $\beta$-MHC gene expression was greatly increased, following the same pattern as in the hypertrophied heart (Figure 1b). Therefore it is suggested that the in vitro condition itself alters the pattern of gene expression of MHC, and that cardiomyocytes in vitro alter their energy metabolic system in response to the culture condition from $\beta$-oxidation to a glycolysis-dominant state.
responsible for the gene regulation of ET-1. Gene expression in primary cultured cardiomyocytes, GAPDH (Figure 3). This suggests that HIF-1α produced into cardiomyocytes. The antisense oligonucleotide against HIF-1α transactivates ET-1 gene expression in cardiomyocytes. To evaluate how much HIF-1α contributes to ET-1 gene expression in primary cultured cardiomyocytes, an antisense oligonucleotide against HIF-1α was introduced into cardiomyocytes. The antisense oligonucleotide decreased the basal gene expression of both ET-1 and GAPDH (Figure 3). This suggests that HIF-1α is responsible for the gene regulation of ET-1.

**DISCUSSION**

This study presents novel findings. First, the energy metabolic system of primary cultured cardiomyocytes is remarkably different from that in vivo. Specifically, compared with the in vivo situation, cardiomyocytes in vitro alter their energy metabolic system from β-oxidation to glycolysis, i.e. a glycolysis-dominant cellular condition. Second, cardiomyocytes show alterations in gene expression, depending on the energy metabolism. Consequently, it is suggested that energy metabolism directly regulates gene expression as a cellular response in cardiomyocytes. Furthermore, ET-1 gene expression in cardiomyocytes is especially linked to a specific situation of energy metabolism in which glycolysis is dominant. Although many studies have investigated the regulatory mechanisms of ET-1 gene expression, the present study demonstrates another novel pathway of ET-1 gene expression, in terms of an effect of energy metabolism via HIF-1α.

In the progression of heart failure, we have reported that the failing heart shows increased gene expression of ET-1, and that ET-1 expression is especially enhanced in the advanced phase of heart failure [3,4]. The precise mechanism, however, remains to be investigated. Thus we have focused on the energy metabolic system in the heart. It has been reported that the failing heart shows down-regulation β-oxidation and activation of glycolysis [1,2]. The fact that ET-1 gene expression is generally increased in the failing heart suggests a correlation between glycolysis and the gene expression of ET-1. HIF-1α is a master transcriptional factor of the induction of glycolytic enzymes. In a recent study, we revealed that HIF-1α directly transactivates ET-1 gene expression [4]. Therefore in the present study we further investigated this relationship between HIF-1α and ET-1 gene expression in terms of glycolysis-dominant energy metabolism.

Primary cultured cardiomyocytes under standard culture conditions produce energy mainly by glycolysis, as demonstrated by their predominant expression of the β-isoform of MHC, as is also the case in cardiac hypertrophy and in the fetal heart. However, electrical stimulation of cardiomyocytes drastically decreased GAPDH and HIF-1α gene expression; on the other hand, PGC-1, which is a cofactor of PPAR-γ involved in adipocyte differentiation, was not affected. This suggests that electrical stimulation attenuates glycolysis. Electrical stimulation is reported to activate mitochondrial function in cardiomyocytes [7], suggesting that glycolysis is down-regulated and β-oxidation is enhanced. Another alteration in culture conditions, i.e. culture in 50% oxygen, similarly decreased the gene expression of GAPDH, HIF-1α and, surprisingly, ET-1. It is suggested that cardiomyocytes cultured in 50% oxygen switch their energy metabolism from glycolysis to β-oxidation, which was further supported by an increase in [3H]palmitate uptake by the cardiomyocytes. These findings suggest that ET-1 gene expression is closely related to the energy metabolic state in cardiomyocytes, i.e. increased ET-1 gene expression occurs in cardiomyocytes in which glycolysis predominates. To further investigate this relationship, an antisense oligonucleotide experiment was performed, showing that direct inhibition of HIF-1α causes greatly decreased expression of ET-1. This suggests that a predominant energy metabolic state of glycolysis increases the gene expression of ET-1 through the master transcriptional factor of glycolytic enzymes, HIF-1α.

Many actions if ET-1 have been reported in cardiomyocytes, including hypertrophy and a positive inotropic action. In addition, it is reported that ET-1
stimulates the glycolytic system via glucose transporters, resulting in an increase in glucose uptake [8]. Therefore, in terms of the cardiac energy metabolic system, activation of the ET system is closely related to the shift in intrinsic energy metabolism from \( \beta \)-oxidation to glycolysis. HIF-1\( \alpha \) is a principle factor involved in activation of the glycolytic system. The failing heart adapts by increasing glycolysis via HIF-1\( \alpha \) to compensate for the depressed \( \beta \)-oxidation. However, such an adaptation also results in increased ET-1 gene expression via the regulatory promoter region. Activation of the ET system in turn will further shift the intrinsic energy system to glycolysis, forming a 'vicious cycle' in the failing heart. Therefore the present study presents another factor that is involved in the transcriptional regulation of ET-1, i.e. the energy metabolic system of glycolysis.

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