〈Original Article〉

Epinephrine upregulates renalase expression in cultured C2C12 muscle cells

Yasuko Yoshida[1,2], Takehito Sugasawa[3], Katsuyuki Tokinoya[1], Shunji Namba[2] and Kazuhiro Takekoshi[3]

Summary Renalase is a flavin adenine dinucleotide (FAD)-dependent soluble monoamine oxidase that was discovered in recent years. The main function of renalase is to metabolize circulating catecholamines. Previously, we reported, for the first time, that renalase expression can fluctuate based on the exercise load in humans, and that renalase expression increased significantly on running for 10 or 20 km. We found that renalase expression increased on exercise, but the mechanism underlying this phenomenon is unknown. Therefore, we speculated that an increase in catecholamines owing to exercise could stimulate renalase expression in skeletal muscle. The purpose of this study was to investigate whether renalase expression is induced by epinephrine in cultured C2C12 muscle cells.

Myotubes formed from C2C12 cells were incubated with epinephrine. The concentrations of epinephrine were 10^{-6} g/L, 10^{-5} g/L, and 10^{-4} g/L, and the incubation times were 15 min, 30 min, and 45 min. As a result, renalase expression was significantly increased at an epinephrine concentration of 10^{-5} g/L and incubation time of 30 min. This finding in muscle cells has not been previously reported and could help elucidate the mechanism of renalase secretion in skeletal muscle in more detail. Renalase is said to degrade catecholamines and is involved in the regulation of blood pressure. These findings may help clarify the mechanism underlying the control of blood pressure through exercise therapy in the future.

Key words: Renalase, Epinephrine, C2C12

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1. Introduction

Renalase is a flavin adenine dinucleotide (FAD)-dependent soluble monoamine oxidase that was discovered in recent years[1,2]. The renalase-encoding gene, *RNLS*, is on chromosome 10 q 23.33 and comprises 10 exons and at least 4 isoforms; the most frequently expressed isoform (renalase 1) consists of 342 amino acids. The human renalase protein is detected in blood, kidney, heart, liver, and skeletal muscle. It has a signal peptide at the amino terminus, and it contains a FAD-binding region and an amine oxidase domain. The main function of renalase is to metabolize circulating catecholamines. If renalase is deficient, blood pressure is believed to increase owing to excessive levels of non-metabolized catecholamines. Therefore, renalase is reported to be involved in the regulation of cardiac function and systemic blood pressure[2,3,4].

Previously, we reported, for the first time, that renalase expression fluctuates based on the exercise load in humans, and that renalase expression increased significantly on running for 10 or 20 km[5]. While we observed that renalase expression increased on exercise, the underlying mechanism is unknown.

A previous paper suggested that catecholamines stimulate renalase expression and secretion in an isolated perfusion kidney model[6]. However, in our previous studies, levels of renalase in blood were inversely proportional to renal function. Therefore, we speculated that an increase in catecholamine levels owing to exercise could stimulate renalase expression in skeletal muscle.

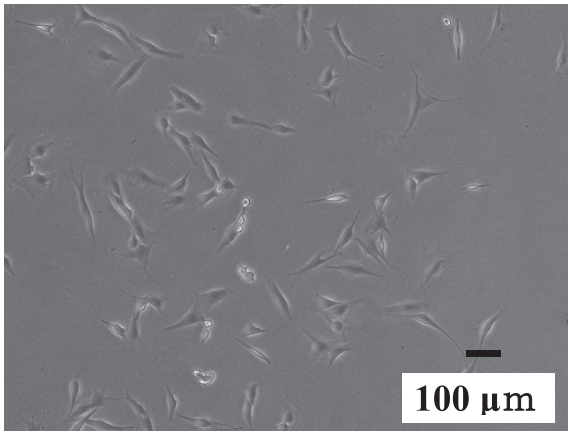
The purpose of this study was to investigate whether renalase expression is induced by epinephrine in cultured C2C12 muscle cells.

2. Materials and methods

2.1. Cell culture

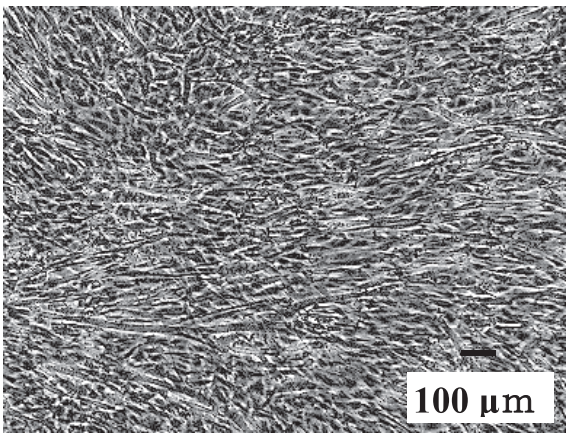
Mouse muscle myoblasts were used C2C12 cells (RIKEN BioResource Center, Tsukuba, Japan). The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; Wako Pure Chemical Industries, Ltd., Osaka, Japan) containing 10% fetal bovine serum and maintained at 37 °C under a continuous 5% CO_2 stream in 6-well plates. After the cultures reached confluence, the medium was replaced with DMEM containing 2% horse serum (differentiation medium) and incubated for a further 7 days to stimulate myotube formation (Fig. 1).

A Myoblasts



40× magnification

B Myotube cell



40× magnification

Fig. 1 C2C12 cells.

2.2. Addition of epinephrine

The formed myotubes in serum-free medium were incubated with epinephrine (Sigma-Aldrich Co., St. Louis, MO, USA). In the first study, the epinephrine concentrations were 10^{-6} g/L, 10^{-5} g/L, and 10^{-4} g/L and the incubation time was 30 min. In

the next study, the epinephrine concentration was 10^{-5} g/L and the incubation times were 15 min, 30 min, and 45 min.

2.3. Quantitative real-time PCR

Renalase genes was measured in C2C12 cell using realtime reverse transcription–polymerase chain reaction (RT–PCR). Total RNA was extracted from the samples using Sepasol-RNA I Super G (Nacalai, Kyoto, Japan) according to the manufacturer's instructions. Total RNA was reverse transcribed into cDNA using PrimeScript RT Master Mix (Perfect Real Time; TAKARA BIO INC., Siga, Japan). To quantify gene expression levels, PCR was carried out using a KAPA SYBR FAST qPCR kit (kapa biosystems, Wilmington, USA) and the Applied Biosystems 7500/7500 Fast Real-Time PCR System (Thermo Fisher Scientific, Waltham, USA) according to the manufacturer's instructions. The primers used for renalase were 5'-GGGTGGGGATATAGGGGGAAG-3' (forward) and 5'-GCTGTGCATCGGGGATTATG-3' (reverse). The cycling programme involved preliminary denaturation at 95 °C for 20 seconds, followed by 40 cycles of denaturation at 95 °C for 3 seconds, annealing and elongation at 60 °C for 3 seconds. Then, by melting curve analysis, it was confirmed that non-specific by-products were not contained in the PCR product. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the internal normalizer control for mRNA.

2.4. Statistics

Statistical analysis was conducted using SPSS statistical software (version 24.0; SPSS Inc., Chicago, Illinois, USA). The data was subjected to one-way analysis of variance (ANOVA) and subsequent post-hoc test for comparison between the four groups, and t-test for the comparison between the two groups. P values below 0.05 were considered significant.

3. Results

3.1. Concentration-dependent epinephrine-induced renalase expression

C2C12 cells were cultured with three different concentrations of epinephrine for 30 min. Compared to controls, only cells incubated with epinephrine at a concentration of 10^{-5} g/L showed a significant increase in renalase mRNA levels ($P < 0.05$, Fig. 2).

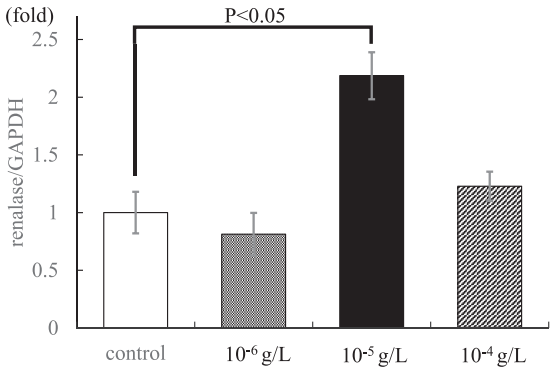


Fig. 2 Concentration-dependent epinephrine-induced renalase expression.

C2C12 cells were cultured with three different concentrations of epinephrine for 30 min. Compared to controls, only cells incubated with epinephrine at a concentration of 10^{-5} g/L showed a significant increase in renalase mRNA levels.

3.2. Time-dependent epinephrine-induced renalase expression

C2C12 cells were cultured with epinephrine at a concentration of 10^{-5} g/L for three different time periods. Compared to controls, only cells incubated for 30 min showed a significant increase in renalase mRNA levels ($P < 0.05$, Fig. 3).

As a result, these experiments demonstrate a significant increase in epinephrine-stimulated renalase expression in C2C12 cells when cells were cultured at an epinephrine concentration of 10^{-5} g/L for 30 min.

4. Discussion

This study showed that epinephrine induced renalase expression in differentiated myocytes. This finding in skeletal muscle has not been previously reported and may help elucidate the mechanism of renalase secretion owing to exercise in more detail.

This result was also consistent with reports on

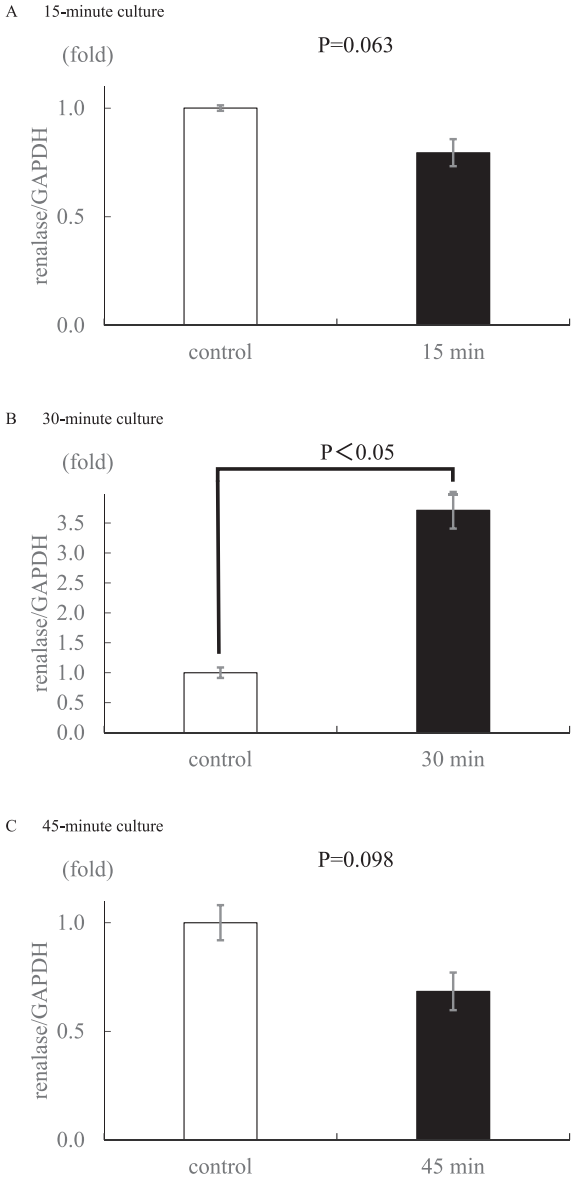


Fig. 3 Time-dependent epinephrine-induced renalase expression.

C2C12 cells were cultured with epinephrine at a concentration of 10^{-5} g/L for three different time periods. Compared to controls, only cells incubated for 30 min showed a significant increase in renalase mRNA levels.

renalase expression in renal cells in vivo and in vitro[6,7]. However, here we noted differences between kidney cells and myocytes in terms of the time periods during which epinephrine addition caused a significant increase in the expression of the renalase gene. In addition, there was a difference also in the concentration of significantly increased epinephrine. Renalase expression in renal cell culture was significantly increased 6–24 h after addition of epinephrine. However, experiments with long-term epinephrine addition were conducted, but no significant differences were observed in muscle cells. The time required for epinephrine to upregulate renalase expression may be shorter in muscle cells than in renal cells. Further, the concentration of epinephrine increased significantly in the renal cells at 10^{-6}, 10^{-7}, and 10^{-8} g/L, however in muscle cells the concentration was not significant. During vigorous exercise, the blood flow rate of skeletal muscle is 80% of the total cardiac output, but on the contrary the blood flow rate of the kidney and internal organs is greatly reduced[8]. That is, the muscle cells are thought to change in high concentration and in a short time to epinephrine so as to cope with such a drastic change.

The two cell types differ in the adrenergic receptors involved in renalase expression. A previous paper has shown that the adrenaline α1 receptor is involved in renal cells, while it is possible that adrenergic β2 receptors are involved in myocytes; however, this has not yet been investigated and requires further study. Furthermore, the pathways mediating renalase expression are still unknown in each cell type[7].

Renalase is a monoamine oxidase that decomposes catecholamines and is involved in the regulation of blood pressure[6,7,9-11]. In recent years, association between renalase and various diseases has also been studied[12-17], but there is almost no relation with skeletal muscle. These findings on renalase expression in skeletal muscle may help elucidate the mechanism underlying the control of blood pressure through exercise therapy in the future.

Conflicts of interest

The authors have declared that no conflict of interest exists.

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