Decrease of PKB/Akt Phosphorylation is Partially Mediated by SAPK/JNK Activation in Serum-free L6 Myoblasts Starved with Low Glucose

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Abstract. [Purpose] Studies have been using cell cultures of muscle cells to mimic atrophy in vivo and in vitro tests. However, changes in the activation of atrophy-related PKB/Akt is not fully understood in serum-free starved skeletal muscle cells. The purpose of the present study was to determine the change of PKB/Akt phosphorylation in L6 myoblasts under serum-free starvation conditions. [Methods] We used western blotting to examine PKB/Akt expression and phosphorylation in atrophied L6 myoblasts. [Results] The phosphorylation of PKB/Akt was significantly lower in L6 myoblasts under serum-free starvation than that of the control group. Serum-free starvation for 6, 12, 24, 36, 48, 72, 96, and 120 hours significantly decreased the phosphorylation of PKB/Akt. Furthermore, the decrease of PKB/Akt phosphorylation under serum-free starvation was partially restored by SP600125, an inhibitor of SAPK/JNK. [Conclusion] These results suggest that decrease of PKB/Akt phosphorylation due to serum-free starvation with low glucose is partially related to the activity of SAPK/JNK in L6 myoblasts.

Key words: PKB/Akt, Serum-free starvation, L6 myoblasts

INTRODUCTION

Akt, also called protein kinase B (PKB/Akt), is a pleckstrin homology domain-containing serine/threonine kinase which is activated by various stimuli, such as growth factors and agonists1–4). Akt has also been widely reported to have key roles in muscle hypertrophy and the prevention of atrophy5, 3, 5). Our previous study demonstrated that the phosphorylation of PKB/Akt in atrophied muscle tissues significantly diminishes in a time-dependent manner in cast-immobilized rats5). Starvation in culture induces loss of muscle mass6–8). To study the signal transduction of atrophy, various cell culture models have been developed9–12). The elevated degradation of proteins in skeletal muscle atrophy and serum-free starvation is coupled with the activation of atrophy markers such as muscle-specific RING finger-1 (MuRF-1) and the muscle atrophy F-box protein (MAFbx, also called atrogin-1), and these are greatly up-regulated in the initiation and development of skeletal muscle atrophy9–12). However, changes in the phosphorylation of PKB/Akt in starvation-induced atrophy and its temporal characteristics are not fully understood. Therefore, we investigated the changes in the phosphorylation of PKB/Akt in L6 myoblasts grown under serum-free starvation conditions with low glucose.

MATERIALS AND METHODS

The L6 myoblasts were purchased from the American
Type Culture Collection (Rockville, MD, USA) and cultured in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum (FBS), 100 U/ml penicillin, 100 μg/ml streptomycin, 200 mM glutamine and 4,500 mg/L D-glucose (high concentration). To induce serum-free starvation, groups of cells were grown to 60–70% confluence in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum (FBS), 100 U/ml penicillin, 100 μg/ml streptomycin, 200 mM glutamine and 4,500 mg/L D-glucose (low concentration) without FBS for 6, 12, 24, 36, 48, 72, 96, and 120 h, respectively. 

Table 1. Changes in the expression and phosphorylation of PKB/Akt in L6 myoblasts grown under serum-free starvation with low glucose

| Experimental period | PKB/Akt (%) | p-PKB/Akt (%) | HG+10% FBS | p-PKB/Akt (%) |
|---------------------|-------------|---------------|------------|---------------|
| 0 hour              | 100±0.0     | 100±0.0       | HG+10% FBS | 100±0.0       |
| 6 hours             | 100.7±6.1   | 18.7±6.6*     | LG+2% FBS  | 99.3±1.8      |
| 12 hours            | 99.3±1.2    | 11.3±4.5*     | LG+0% FBS  | 98.7±0.9      |
| 24 hours            | 98.7±2.6    | 10.3±3.5*     | HG+10% FBS | 100.0±0.0     |
| 36 hours            | 101.0±4.9   | 10.7±3.8*     | LG+0% FBS  | 103.3±5.8     |
| 48 hours            | 99.3±3.5    | 13.3±4.3*     | SB203580 10 μM | 102.7±2.3 |
| 72 hours            | 100.7±5.8   | 11.3±4.1*     | SP600125 10 μM | 102.3±2.4 |
| 96 hours            | 101.0±3.5   | 10.3±6.0*     | LY294002 10 μM | 103.3±2.3 |
| 120 hours           | 102.3±4.1   | 10.7±5.9*     |            |               |

Data are presented as the mean ± SEM. HG, glucose of high concentration; LG, glucose of low concentration; p, phosphorylated protein; PKB/Akt, protein kinase B/Akt; SB203580, an inhibitor of p38MAPK; SP600125, an inhibitor of SAPK/JNK; LY294002, an inhibitor of phosphatidylinositol 3-kinase. The basal levels of abundance and phosphorylation in the control (0 hour) were considered to be 100%. *: vs. 0 hour control, p<0.05

The phosphorylation of PKB/Akt was significantly lower in L6 myoblasts grown under starvation conditions with low glucose and 0% FBS than that in the control group cultured with high glucose and 10% FBS (n=4; Table 1, Fig. 1A). Serum-free starvation with low glucose for 6, 12, 24, 36, 48, 72, 96, and 120 hours significantly decreased the phosphorylation of PKB/Akt (n=5; Table 1, Fig. 1B). Furthermore, the decrease of PKB/Akt phosphorylation in serum-free starvation with low glucose was partially restored by 10 μM SP600125, an inhibitor of SAPK/JNK (n=4; Table 1, Fig. 1C). However, the starvation did not influence the abundance of PKB/Akt expression in any group (n=4–5; Table 1, Fig. 1A, 1B, and 1C).

DISCUSSION

Maintenance of muscle mass and of muscle function are important for healthy life, and is important in the rehabilitation of musculoskeletal disease in the field of physical therapy. The maintenance mechanisms of muscle mass include anabolic and catabolic signal transduction via PKB/Akt, a protein with a critical role in the ability to optimally use ingested nutrients in the maintenance of muscle mass. In contrast, as muscle mass decreases, there is an accompanying loss in muscle strength and use of the nutrients that contributes to reduced muscle function and quality of activities of daily living (ADL). Malnutrition and chronic diseases such as diabetes mellitus, heart failure, and chronic obstructive pulmonary disease are also directly associated with a dramatic reduction in the phosphorylation of PKB/Akt with decrease of muscle mass. Our previous study reported that increases in atrophy markers and a decrease in PKB/Akt phosphorylation occur in gastrocnemius muscle strips atrophied by cast-immobilization, and PKB/Akt phosphorylation is involved in the development of serum-free starvation-induced MuRF-1 expression in L6 myoblasts. Exocellular sig-
muscles and L6 myoblasts. However, further systematic and scientific studies in the fields of electrotherapy, neureotherapy, hydrotherapy and others are needed to confirm the mechanisms of PKB/Akt in atrophied muscle strips and cells. In summary, the phosphorylation of PKB/Akt decreased in starved L6 skeletal muscle cells. The present results suggest that serum-free starvation-induced atrophy is partially mediated by PKB/Akt via the SAPK/JNK pathway in L6 myoblasts.

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