SHORT COMMUNICATION

INSULIN-LIKE GROWTH FACTOR-II INDUCES HYPERTROPHY OF ADULT CARDIOMYOCYTES VIA TWO ALTERNATIVE PATHWAYS

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Insulin-like growth factor (IGF)-II is known to induce hypertrophy of isolated adult rat ventricular cardiomyocytes cultured in the absence of serum. However, it is not known how the growth factor exerts this hypertrophic effect. We show here that IGF-II induces hypertrophy of the cultured cardiomyocytes via two alternative pathways: (1) an IGF-I receptor-dependent pathway, or (2) a lysosome-dependent pathway when the IGF-I receptor-dependent pathway is blocked.

KEYWORDS: cardiomyocyte hypertrophy; insulin-like growth factor (IGF)-II; IGF-I receptor; lysosomes.

INTRODUCTION

In response to mechanical and physiological stimuli, the myocardium adapts by hypertrophy of its cardiomyocytes (Morgan and Baker, 1991). Cardiomyocytes in cell culture have proved especially useful for identifying multiple growth factors that mediate their hypertrophy (Hefti et al., 1997). Recently, we established a serum-free system for culturing adult rat ventricular cardiomyocytes and showed that insulin-like growth factor (IGF)-II rapidly and directly induces hypertrophic growth in these cultured cells as evidenced by their increased size, total protein synthesis, and transcription of muscle-specific genes (Huang et al., 2002). IGF-II also induces hypertrophic growth of cultured neonatal rat cardiomyocytes (Adachi et al., 1994). However, the manner by which IGF-II exerts these hypertrophic effects is not known.

Cardiomyocytes contain receptors on their surfaces for both IGF-I (IGF-IR) and IGF-II (IGF-IIR) (Guse et al., 1992). The existence of both receptors on cardiomyocytes complicates attempts to understand how IGF-II affects the heart be-
The experimental protocol was approved by the Laboratory Animal Care Advisory Committee of this institution.

**Cell isolation, culture and sizing**

For each experiment, ventricular cardiomyocytes were isolated from 2 or 3 adult male rats (250–295 g) and cultured in serum-free medium at 37°C on mouse laminin-treated cell plates as described (Huang et al., 2002). Cytosine arabinoside · HCl (Sigma Chemical Co., St Louis, MO, U.S.A.) at a final concentration of 10 μM in the serum-free medium was added to prevent the growth of any residual fibroblasts still present (Haddad et al., 1988). The following were added daily to the 4 ml culture medium: 10^{-8} M IGF-II (Roche Molecular Biochemicals, Indianapolis, IN, U.S.A.), 5 μl chicken anti-human IGF-IR α-subunit antibodies (polyclonal, in PBS-0.05% sodium azide, Upstate Biotechnology, Lake Placid, NY, U.S.A.), 100 μM chloroquine (Sigma), and 20 μM genistein (Sigma). When 5 μl PBS-sodium azide alone were added, results were the same as obtained with control plates lacking the addition. Chloroquine was dissolved in ddH2O and 10 μl were added to the culture medium. Genistein was dissolved in 50 μl ethanol, diluted with 4.95 ml ddH2O, and 40 μl were added to the culture medium. When the same amount of ethanol-H2O alone was added, results were the same as obtained with control plates lacking the addition.

Cardiomyocytes were sized on the culture plates as described (Huang et al., 2002).

**Statistical analysis**

Statistical differences were assessed by one-way ANOVA with Fisher’s LSD test. *P<0.05* was considered statistically significant. Data are expressed as the mean ± standard error (SE).
RESULTS AND DISCUSSION

Recently, we showed that IGF-II induces hypertrophy of adult rat cardiomyocytes cultured on serum-free medium (Huang et al., 2002). In the present study, by 2 days of culture with \(10^{-8}\) M IGF-II, these cardiomyocytes were hypertrophied by 54\% (Fig. 1) similarly to the 57\% we reported previously (Huang et al., 2002). We first tested whether this IGF-II-induced hypertrophy resulted from the growth factor interacting with the IGF-IR which is a tyrosine kinase and is known to mediate growth-promoting effects of IGF-II on many different cells (Moxham and Jacobs, 1992; Butler and LeRoith, 2001). For this, we added either neutralizing antibodies against the IGF-IR or genistein, a tyrosine kinase inhibitor (Akiyama, et al., 1987), along with the growth factor to the cultured cardiomyocytes. In the presence of the antibodies or genistein, IGF-II still induced the cardiomyocytes to hypertrophy (Fig. 1). This result indicates that when the IGF-IR-mediated pathway is blocked, IGF-II induces cardiomyocyte hypertrophy via an IGF-IR-independent pathway. In this regard, we noted that IGF-II also binds to the IGF-IR which lacks tyrosine kinase activity but has a major physiological role in the trafficking of proteins to lysosomes (Kornfeld, 1992; Moxham and Jacobs, 1992). Therefore to test for the involvement of lysosomes in the postulated IGF-IR-independent pathway, we added chloroquine, a lysosome inhibitor (Herschko and Ciechanover, 1982), along with the growth factor to the cardiomyocytes. In the presence of chloroquine, IGF-II still induced hypertrophy of the cardiomyocytes (Fig. 1). Notably, however, when we exposed the cardiomyocytes to chloroquine together with either antibodies against the IGF-IR or genistein, the hypertrophy induced by IGF-II was eliminated (Fig. 1). We interpret these latter results as showing that when chloroquine alone is present, IGF-II still interacts with the IGF-IR, thus initiating hypertrophy; however, when an IGF-IR inhibitor is present together with chloroquine, IGF-II-induced hypertrophy does not occur because the IGF-IR signal transduction pathway and a necessary lysosome-mediated step associated with an alternative pathway are both inhibited. Thus, lysosomes do appear to be involved in the progression and/or maintenance of IGF-II-induced hypertrophy of adult rat ventricular cardiomyocytes when the IGF-IR signaling pathway is blocked. In line with this finding is the report that IGF-II-treatment leads to an accumulation of protein in cultured adult rabbit ventricular cardiomyocytes and that the growth factor does this by inhibiting protein degradation by lysosomes (Decker et al., 1995).

In sum, the present study shows for the first time that IGF-II induces hypertrophy of adult rat cardiomyocytes via two alternative pathways: (1) an IGF-IR-dependent pathway, or (2) a lysosome-dependent pathway when the IGF-IR-dependent pathway is blocked. To prevent the induction of hypertrophy by IGF-II, both pathways must be blocked.

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REFERENCES


