Detection of Placenta Growth Factor (PIGF) Protein in Neonatal Rat Cardiomyocytes

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ABSTRACT

Angiogenesis, the formation of blood vessels, can provide blood to the heart when its normal arteries are compromised. Angiogenesis is highly dependent on the Vascular Endothelial Growth Factor (VEGF) family. Placental growth factor (PIGF) is a member of this family and is crucial for pathological angiogenesis in the adult. PIGF coupled with the more common VEGF could greatly increase angiogenesis in the heart tissue, thus providing oxygen to ischemic heart tissue. Previous research has shown that PIGF mRNA increases with 6 hours of hypoxia which models ischemia. However, little is known about PIGF protein expression on the protein level. We intend to establish that PIGF protein expression will increase in cardiomyocytes which have undergone 6, 12, and 24 hours of hypoxia compared to normoxic rat neonatal cardiomyocytes. This information will later be used in studies of potential protective treatments using PIGF.

 METHODS

Rat neonatal cardiomyocytes were isolated and cultured as described (Zhang et al., 2001). Ports of cultures were assessed by indirect immunocytochemistry using monoclonal antibody against sarcomeric myosin (SM-28: C-20 Santa Cruz Biotechnology) and propidium iodide counterstaining.

Culture Techniques: Hypoxic conditions were established by placing cell cultures in an anerobic puck (Bellco), and recirculating the cultures from the anerobic从前. Hypoxia times were 1, 5, 6, 12, 24 hours with each cell culture allowed to recover for ten minutes.

PIGF mRNA (PCR, RTPCR) and protein (Western blot) expression: a. RTPCR: Total RNA (500ng) was reverse transcribed (oligotex reverse transcriptase: Ambion, Inc.) and 2nd was subjected to cycles of PCR in the linear range of amplification. Relative differences in PIGF mRNA expression were assessed by semiquantitative multiplex RT-PCR (Torry et al., J Mol. Cell Cardiol, 2001) using the following primers (Biorad: 18S classic 600bp or classic H 332bp products: Ambion, Inc.): PIGF 224 bp product. The resultant products were separated by electrophoresis on a 2% agarose, stained with ethidium bromide and visualized with Kodak Gel Documentation System (DD-FDA). Denaturation was performed and the ratio of amplion in 18S was computed and compared statistically.

b. Western Blot: After resolution by SDS-PAGE, PIGF proteins were electrophoretically transferred to polyvinylidene difluoride (PVDF) membrane (Immobilon-P) at 100 V for 45 minutes. PVDF membranes were probed with [a-IGF-I antibody or 1:1,000 antibody from Santa Cruz Biotechnology, Inc., Donkey anti-goat IGF-I conjugated secondary antibody (1:200,000) Reticulocyte was used in conjunction with Chemiluminescent Substrate (Pierce) and film exposure to visualize the immunoreactivity.

 RESULTS

Cardiomyocytes were isolated from neonatal rats and cultured in DMEM supplemented with 10% FBS. Cells were cultured under hypoxic (≤5%) or normoxic conditions for 1, 3, 5, 6, 24 hours. Total RNA was isolated immediately and 500ng of total RNA was reverse transcribed. Resulting cDNA (12 cycles) was subjected to multiple PCR amplification using primers for rat VEGF (PIGF, VEGF-R1), and VEGF-R2, with 18S as an internal standard within the linear range of amplification for each primer. Amplification ratios were determined and fold changes in hypoxic cardiomyocytes compared to normoxic cultures within each time point were calculated. p < 0.06 from Normoxic.

 Western blot: PIGF protein expression in hypoxic cells

 SUMMARY AND CONCLUSION

PIGF mRNA expression in isolated cardiomyocytes:

1. PIGF mRNA expression was present in both normoxic and hypoxic cells at all time periods.

2. Hypoxia tended to increase PIGF mRNA expression but expression was significantly increased after 6 hours of hypoxia.

Immunohistochemistry: PIGF protein was present in the cytoplasm of neonatal rat cardiomyocytes.

Western Blot: We could not reliably detect cardiomyocyte PIGF protein expression by Western Blot in the study but our recent analysis appears encouraging; we are continuing to optimize the procedure.

Conclusion: Western blot detection of PIGF protein is still being sought. We are currently striving to find the correct antibody dilution to be able to detect the Western Blot. From it we will run Western Blots of hypoxic and normoxic cardiomyocytes in hopes to find differences in PIGF expression.

Supported by R15-HL76602-01 (KRF) and R01-HD44900 (INST).

BACKGROUND

Myocardial ischemia/infarction is a leading cause of morbidity and mortality in the United States. Although therapies to enhance coronary angiogenesis could substantially reduce the severity of ischemic heart disease, the endogenous mechanisms promoting blood vessel growth in the heart are not well known. One hypothesis involves the production of specific factors from cardiomyocytes that could lead to neovascularization and increase the diameter of the coronaries.

There is considerable evidence that members of the vascular endothelial growth factor (VEGF) family play an important role in the establishment, growth, and maintenance of blood vessels under a variety of physiological and pathological conditions (Herrera et al., 2001). One VEGF family member that has received relatively little attention in terms of its expression and function in heart tissues is placenta growth factor (PIGF). PIGF has been shown to act synergistically with vascular endothelial growth factor (VEGF) to promote angiogenesis and inhibit apoptosis. Although we and others have shown significant upregulation of VEGF expression in models of coronary angina (Torry et al., 2001), quantitative change in PIGF expression are unknown.

The present study sought to determine: 1) if cardiomyocytes express PIGF protein, 2) if hypoxia alters PIGF protein expression in cardiomyocytes.

Cultured Neonatal Rat Cardiomyocytes

Cardiomyocytes were isolated from 2-3 day old rats, selectively plated to enrich cardiomyocytes over non-cardiomyocytes. Representative aliquots were collected and cultured on chamber slides in DMEM/10% FBS for 48hrs. Panel A: The cultures were washed, fixed with 20% acetic, re-hydrated in PBS and reacted with goat polyclonal antibody to rat PIGF (Santa Cruz Biotechnology) and then FITC-conjugated donkey anti-goat IgG antibody (Dakopatts). Panel B: PBS was substituted for the primary antibody and the cultures were reacted with the FITC-conjugated donkey anti-goat IgG antibody.