Mini-review

Insulin-like growth factor-1 and myocyte growth: the danger of a dogma.
Part I. Postnatal myocardial development: normal growth

Piero Anversa *, Jan Kajstura, Wei Cheng, Krzysztof Reiss, Elena Cigola, Giorgio Olivetti

Department of Medicine, Vosburgh Pavilion Room 302, New York Medical College, Valhalla, NY 10595, USA

Received 19 October 1995; accepted 15 December 1995

Keywords: Development; Myocyte proliferation; IGF-1; IGF-1R

1. Introduction

The possibility that the human heart may respond to an increase in pressure and volume loads not only by hypertrophy of pre-existing myocytes but also by myocyte cellular hyperplasia has been a matter of controversy for several decades. The critical heart weight theory introduced by Linzbach in the late 1940s and early 1950s [1] has suggested that in the presence of a cardiac weight of nearly 500 g or greater, hyperplasia of myofibers begins and this cellular process constitutes the prevailing form of myocyte growth in the heart. These initial findings were restricted to the left ventricle, but similar adaptations subsequently have been shown at the level of the right ventricle [2], indicating that the entire heart may react to a sustained increase in workload by the generation of new myocytes through mitotic division of the stressed muscle cells. In contrast, experimental studies have demonstrated that short-term cardiac hypertrophy in the adult heart results from an enlargement of myocytes with little DNA synthesis in myocyte nuclei, possibly representing the formation of polyploid cells [3-5]. Thus, it has become a general belief that no proliferation of myocytes occurs once cell division has ceased immediately after birth in the mammalian myocardium. These studies in the pressure-overloaded rat heart have examined thymidine incorporation early after the imposition of the overload and the lack of autoradiographic labeling in myocytes has been interpreted as a definitive indication of the terminally differentiated state of these cells [3-5]. On this basis, the work of Linzbach [1] as well as several new observations [2,6-12] were dismissed and the dogma was created asserting that adult cardiac myocytes are unable to divide and the growth reserve mechanism of these cells is dependent exclusively on their capacity to develop cellular hypertrophy. In this regard, ventricular myocytes repeatedly have been compared to neurons and the contention that the life span of myocytes in man can last for 100 years or more has been introduced and accepted without question. On the assumption that adult myocytes can increase only in size, a major effort has been performed in the last several years to identify the mechanisms of this cellular hypertrophic response. This work has focused mostly on the α1-adrenergic receptor and effector pathway linked to this receptor [13] and on the local renin-angiotensin system [14-18]. More recently, our laboratory has analyzed the role of insulin-like growth factor-1 (IGF-1) and its receptor (IGF-1R) in myocyte hypertrophy and proliferation during normal postnatal development [19] and following ischemic myocardial injury [20-22]. These forms of cardiac growth were examined, in combination with in vitro experiments [23], to establish whether physiologic maturational growth differs from pathologic myocardial growth, and whether the IGF-1/IGF-1R system is involved in the regulation of myocyte hypertrophy, proliferation, or both. The discussion below reviews current information contrary to the contention that myocytes are terminally differentiated as well as present knowledge on the potential effects of IGF 1 on cardiac muscle cell growth.

* Corresponding author. Tel.: (+1-914) 993-4168; fax: (+1-914) 993-4406.

0008-6363/96/$15.00 Copyright © 1996 Published by Elsevier Science B.V. All rights reserved
PII S0008-6363(96)00035-1
2. Postnatal myocardial development: normal growth

Maturational growth represents the response of the heart to the hemodynamic overloads that accompany cardiac development and the progression of life before the deleterious effects of aging become apparent. The fetal heart differs from the adult heart in structure and function. The work required by the two ventricles is approximately equal during intrauterine life, whereas after birth the workload on the left ventricle is significantly greater than that on the right ventricle [24]. This condition leads to faster growth of the left ventricular myocardium resulting in its relatively larger muscle mass typical of the adult heart [25]. The dimension and weight of the right and left ventricles change disproportionally after birth [25]. The left ventricle gets larger and the wall thickens rapidly. The right ventricle also becomes larger, but with a relatively thinner wall. These anatomical modifications are the consequence of an increase in pressure and volume loads on the left side of the heart and an increase in volume load only on the right. Thus, the growth of the myocardium during the early postnatal period must accommodate not only the increasing demands of the growing body mass, but also the abrupt changes in the patterns of blood flow and circulatory resistance occurring shortly after birth [24]. When these major adaptations have taken place, the process of maturation continues and the heart is influenced by the expansion in circulating blood volume which increases the preload on both chambers [26].

3. DNA synthesis in the developing heart

The cellular processes of myocyte hypertrophy and hyperplasia are the major factors of ventricular remodeling in the developing heart. Cardiac myocytes in the rat retain their capacity for proliferation up to the age of weaning, although significant hyperplasia may cease earlier. Average size of myocytes also increases in parallel with postnatal body growth [27], and myocytes possess the capacity for additional hypertrophy in response to an added workload [25]. However, little information is available concerning these structural adaptations in the late stages of fetal life. Two essential processes occur during the prenatal morphogenesis of the heart: myocyte mitotic division and maturational changes intracellularly, consisting of the synthesis and organized alignment of myofibrillar and other sarcoplasmic structures [28]. Myocyte cellular hyperplasia may account for most of the increase in chamber volume and wall thickness in the fetal heart, but quantitative data are lacking and this possibility has to be confirmed by morphometric studies. In an attempt to evaluate the magnitude of myocyte proliferation prenatally and postnatally, the extent of DNA synthesis in myocytes recently has been measured [19].

Bromodeoxyuridine (BrdU) labeling of left ventricular myocytes involves nearly 17% of cells in the fetal rat heart and this parameter decreases to a value of 13% at 1 day after birth (Fig. 1). Subsequently, the percentage of BrdU-positive myocyte nuclei diminishes progressively with postnatal development, and only 0.2% of cells are labeled at 2 months of age in the young adult rat heart. The major reductions in the degree of BrdU labeling of cardiac myocytes occur from 1 to 5 days and from 11 to 21 days. The low level of labeling detected at 3 weeks after birth appears to persist in the young adult myocardium. It is important to note that no statistically significant difference has been found in the magnitude of BrdU labeling between the fetal and the 1-day-old heart. During the interval from 1 day to 2 months postnatally, the fraction of BrdU-positive myocyte nuclei in the left ventricle decreases by 98.5% [19]. These and previous observations [29] document that the level of DNA synthesis is markedly diminished in adult rat ventricular myocytes. However, the number of cells and nuclei increases rapidly with maturation [27,30,31], so that a decrease in the fraction of BrdU-labeled myocytes does not necessarily reflect a comparable reduction in the absolute number of cells synthesizing DNA in the entire ventricle.

The left ventricle of the rat heart contains approximately $7.5 \times 10^6$, $13.1 \times 10^6$, and $21.8 \times 10^6$, $30.2 \times 10^6$, and $40.3 \times 10^6$ myocyte nuclei at 1, 5, 11, 21, and 60 days after birth [25], so that the percentage of BrdU-positive cells measured would imply that nearly 1 million myocytes in the entire left ventricle are undergoing DNA synthesis at 1, 5, and 11 days postnatally. At 21 and 60 days, $157 \times 10^3$ and $85 \times 10^3$ myocytes are engaged in DNA replication, respectively. A value of $140 \times 10^3$ also has been reported for the adult myocardium [29]. In addition, recent results have demonstrated that increases in workload on the rat heart may activate DNA synthesis in myocytes, leading to nuclear mitotic division and cellular hyperplasia [20,22,32]. The magnitude of DNA synthesis in myocyte nuclei can-
not be equated with the extent of myocyte proliferation. Binucleation is an important aspect of myocyte growth postnatally in the rat [27,30]. Ploidy changes and DNA repair both may influence the degree of DNA synthesis observed by BrdU labeling. A few examples of mitosis in myocytes have been detected in the normal rat heart [33], supporting the notion that the attenuation in the DNA synthetic machinery of these cells with postnatal development may be reversed, and cell proliferation may be re-instated. It is noteworthy that the reduction in myocyte cellular hyperplasia with maturation appears to occur when volume and pressure loads on the ventricle reach the adult values, at 3 to 4 weeks of age in the rat [25]. Such a phenomenon raises the possibility that a relationship may exist between the magnitude of ventricular loading and the activation of the replicatory machinery of myocytes in the adult heart.

4. Myocyte growth in vivo and the IGF-1/IGF-1 receptor system

Insulin-like growth factor-1 belongs to the insulin family of peptides and acts as a growth factor in many tissues and tumors [34]. Of the two major forms of IGFs, IGF-1 is important for postnatal growth, whereas IGF-2 is involved in fetal growth and development. Although the role of IGF-1 in promoting growth and differentiation of skeletal muscle has been investigated extensively [35], more limited information is available concerning the effects of IGF-1 on the growth of cardiac myocytes. In neonatal ventricular myocytes in culture, IGF-1 activates DNA synthesis [23,51] and the expression of myosin light chain-2, troponin and skeletal α-actin [36], which are consistent with a hyperplastic and hypertrophic response of these cells. However, long-term cultures of adult myocytes react to the addition of IGF-1 by increasing only the formation of myofibrils in the cytoplasm [37]. Moreover, an upregulation of IGF-1 mRNA in the myocardium occurs in pressure overload hypertrophy in vivo [38], and this adaptation has been linked to myocyte hypertrophy exclusively. The density of IGF-1 receptors is increased in the decompensated human heart [39] and this phenomenon has been associated with muscle cell hypertrophy. Unfortunately, these in vivo studies did not attempt to distinguish the differential growth patterns of the myocyte and non-myocyte compartments of the myocardium nor did they recognize that the activation of the IGF-1/IGF-1R system may be coupled with the stimulation of the DNA synthetic machinery of myocytes and interstitial cells in the pathologic heart. Similarly, the IGF-1/IGF-1R system has been implicated in the postnatal development of the myocardium [40], although the growth-promoting effect of this pathway on myocyte size and number, and fibroblasts and endothelial cell proliferation remained to be determined. These issues can only be addressed by the use of enzymatically dissociated myocytes with high levels of purity and yields to constitute an acceptable sampling of the ventricular myocardium [19–22]. In view of the known mitogenic effects of IGF-1 in various cell systems [34], it is surprising that the potential ability of this growth factor to trigger DNA replication and cell proliferation was rarely considered for the myocyte population. However, before the description of recent results concerning the relationship between IGF-1 and myocyte growth, some information on the importance of IGF-1 and its receptor, IGF-1R, on cell growth will be discussed.

It is well established that ligand activation of IGF-1R is necessary for proliferation of fibroblasts, smooth muscle cells, keratinocytes, chondrocytes, osteoblasts and hematopoietic cells [34]. IGF-1 and high concentrations of insulin are frequently used in chemically defined culture media to induce cell proliferation [41]. Balb/3T3 fibroblasts require at least two growth factors to activate DNA synthesis. In the presence of PDGF, these cells increase the number of IGF-1R and, under these conditions, IGF-1 and insulin induce early-growth-related genes, late-growth-related genes, DNA replication and cell division [34]. Thus, stimulation of IGF-1R by its ligand is the only requirement for cell cycle progression when Balb/3T3 fibroblasts possess a critical number of IGF-1R [34]. In this regard, Balb/3T3 cells transfected with expression vectors, where IGF-1 and IGF-1R cDNAs are controlled by the SV40 promoter (i.e., P12 cells), proliferate in serum-free medium without exogenous growth factors [41]. Conversely, cells transfected with IGF-1R expression vector only (i.e., P6 cells) require IGF-1 or insulin for proliferation to occur [42]. The number of IGF-1R in Balb/3T3 cells has to increase approximately 2-fold before ligand activation can promote cell division [42]. In contrast, inhibition of the translation of IGF-1R mRNA into receptor protein by antisense technique markedly attenuates cell proliferation [34,42]. Similarly, inactivation of IGF-1R by peptide analogs produces an inhibitory effect on cell proliferation. The importance of the IGF-1R in cell growth has been confirmed in vivo by the finding that mouse embryos with a targeted disruption of the IGF-1R gene and the IGF-2 gene at birth are only 30% of the size of wild-type littermates [43]. This finding provides the demonstration that IGF-1R and its ligands are required in vivo, where they control 70% of murine embryonal growth [45]. In contrast, when the metallothionein promoter is employed to drive the expression of an IGF-1 cDNA in transgenic mice, an overexpression in IGF-1 occurs in several organs and this phenomenon is coupled with an increased IGF-1 concentration in the serum [44]. These animals have an increased body weight and larger visceral organs [44].

The observations summarized above formed the basis for the analysis of the myocyte IGF-1/IGF-1R autocrine system during postnatal myocardial growth [19]. In addition, the claim has been made that ventricular tissue produces IGF that may act in an autocrine or paracrine...
fashion to influence the growth of myocytes during maturation [40]. However, the contribution of the different cell populations of the myocardium was not examined. Similarly, in the early phases of postnatal myocardial growth in the spontaneously hypertensive rat, the number of IGF-1 receptors increases [40] and this reaction is considered to modulate cardiac hypertrophy. Changes in the proportion between type 1 and type 2 IGF receptors may also occur in the myocyte and non-myocyte cell populations of the myocardium, and this phenomenon may affect the growth characteristics of cardiac muscle cells prenatally and postnatally [45]. Moreover, the properties of myocyte growth with maturation offer a unique opportunity for the evaluation of the role of the IGF-1/IGF-1R system in myocyte hypertrophy and proliferation. This is because in the rat, from birth to 2 months of age, myocyte cell volume increases nearly 25-fold and myocyte cell number by only 3-4-fold [25,27,30]. The phenomenon of cell proliferation takes place mostly in the immediate postnatal period and becomes rapidly attenuated shortly after birth. Therefore, a progressive decrease in the expression of IGF-1 and IGF-1R in myocytes postnatally, in spite of ongoing cellular hypertrophy, would indicate that the IGF-1/IGF-1R system is involved predominantly in myocyte proliferation. Conversely, a continuous increase in the expression of IGF-1 and IGF-1R in myocytes, in spite of a marked decrease in DNA synthesis and cell proliferation, would suggest that the IGF-1/IGF-1R system is implicated primarily in myocyte cellular hypertrophy. Finally, the persistence of high levels of expression of IGF-1 and IGF-1R in myocytes postnatally would support the involvement of this effector pathway in both forms of myocyte growth.

As illustrated in Fig. 2, the expression of IGF-1 and IGF-1R in myocytes is comparable in the fetal heart and in the left ventricular myocardium during the first few days of postnatal life. However, the message for IGF-2 in these cells decreases sharply at birth. With maturation, the induction of IGF-1 and IGF-1R also becomes attenuated so that adult myocytes exhibit low levels of IGF-1, IGF-2, and IGF-1R mRNA. Similarly, IGF-1 receptor protein in these cells is down-regulated postnatally. Thus, the decrease in the expression of IGF-1 and IGF-1R in myocytes parallels the decrease in DNA replication [19,29] and cell proliferation [30], strongly suggesting that the IGF-1/IGF-1R autocrine system regulates the hyperplastic growth capacity of myocytes with maturation more than myocyte cellular hypertrophy [19]. In this regard, marked increases in myocyte volume occur after birth [27] despite the progressive reduction in IGF-1 mRNA and IGF-1R mRNA and protein in the cells. However, the impact of IGF-2 on myocyte growth should not be minimized. IGF-1 and IGF-2 in the plasma are bound to specific proteins, and this phenomenon limits their free concentration to approximately 1 to 5% of the total amounts [46]. The fetal concentration of IGF-2 is greater than that of IGF-1 [46], but the concentration of IGF-1 increases after birth, whereas the plasma level of IGF-2 decreases sharply during the first 3 weeks of postnatal life in the rat [46]. The observation that IGF-2 mRNA in myocytes is attenuated at birth is consistent with these results as is the notion that IGF-2 is implicated mostly in prenatal cell and organ growth [43]. Conversely, it cannot be excluded that the downregulation of IGF-2 in myocytes postnatally may influence the ability of these cells to proliferate, because IGF-1 and IGF-2 can operate through the same IGF-1 surface receptor [45].

An additional important aspect that links the myocyte IGF-1/IGF-1R autocrine system to the attenuation in cell proliferation with postnatal development concerns the changes in the expression of late-growth-related genes in the maturing myocytes [42]. The message for PCNA, histone-H1, and DNA polymerase-α in these cells decreases after birth, reaching very low levels by 2 months of age (Fig. 3). PCNA, histone-H1, and DNA polymerase-α were evaluated because the induction of these genes is required for DNA replication and cell cycle progression. Although the changes in the quantity of mRNA encoded...
by these genes continue with time, the major reduction occurs from 11 to 21 days. Thus, the decrease in PCNA, histone-H\(_3\), and DNA polymerase-\(\alpha\) mRNAs parallels the downregulation in the IGF-1/IGF-1R system in myocytes [19]. Although the mechanisms responsible for the temporal changes in the expression of late-growth-regulated genes in myocytes with maturation are not clear, these genes are considered essential in the modulation of the cell cycle [42,47]. The level of mRNA for histone-H\(_3\) is low in G\(_0\) and during G\(_1\), but it increases rapidly at the G\(_1\)-S boundary, decreasing at the G\(_2\) phase of the cell cycle. Similarly, PCNA mRNA markedly increases when cells are stimulated to proliferate, decreasing sharply at the end of the S-phase. DNA polymerase-\(\alpha\) is the principal polymerase in eukaryotic DNA synthesis and plays a key role in the replication of the eukaryotic genome [48]. Moreover, inhibition of this enzyme inhibits DNA synthesis and cell proliferation. The postnatal decrease in the expression of genes encoding PCNA, histone-H\(_3\), and DNA polymerase-\(\alpha\) in myocytes is consistent with the progressive attenuation in cell proliferation and the corresponding increase in the myocyte cellular hypertrophic response after birth.

5. Myocyte growth in vitro and the IGF-1/IGF-1 receptor system

Although the decrease in the expression of IGF-1, IGF-1R and late-growth-related genes in myocytes with maturation [19] correlates with attenuation in the proliferative capacity of these cells [30,31], a cause-and-effect relationship between these two events remains to be demonstrated. Myocyte cellular hypertrophy occurs currently during physiologic and pathologic myocardial growth [25,27,31], making the separation of these two cellular processes at the molecular level very complex in an in vivo system. Moreover, with the exception of the work performed in our laboratory [19–22], the other in vivo studies utilized ventricular tissue so that the adaptation of fibroblasts and endothelial cells was not distinguished from that of the muscle compartment of the myocardium [36–40,49]. In an attempt to characterize the growth-promoting effect of IGF-1 on myocytes, primary cultures of neonatal rat ventricular myocytes were employed [23]. This cell system was selected because, in the presence of serum (FCS), 1-day-old cardiac myocytes proliferate and undergo cellular hypertrophy [13]. Surprisingly, when myocytes maintained in serum-free medium (SF) are stimulated with 100 ng/ml IGF-1, DNA replication occurs and its magnitude is similar to that obtained with 10% FCS. In contrast, IGF-1 has no impact on myocyte cellular hypertrophy [23], raising the possibility that myocyte replication may be regulated by a single growth factor. This would suggest that myocytes behave in a unique manner and cannot be compared to interstitial fibroblasts [41]. Moreover, the degree of DNA synthesis in vitro is almost identical to that measured in vivo in 1-day-old rats [19,23], further supporting the notion that the IGF-1/IGF-1R system modulates most of the proliferative capacity of myocytes. Importantly, DNA replication and mitotic division of myocytes are completely inhibited when FCS-stimulated cells are treated with antisense oligodeoxynucleotides to IGF-1R mRNA (Fig. 4). Conversely, the antisense intervention fails to attenuate myocyte hypertrophy chronically and the expression of ANF which is commonly used as a molecular marker of cellular hypertrophy [50]. Thus, activation of IGF-1R on myocytes is required for DNA synthesis and myocyte cellular hyperplasia. In this regard, thymidine incorporation in myocytes is
enhanced following IGF-1 stimulation [23,51]. Basic fibroblast growth factor (bFGF) also induces DNA replication in neonatal cardiac myocytes in culture [51]. However, bFGF may stimulate the IGF-1/IGF-1R system increasing the expression of IGF-1R on myocytes, a phenomenon shown to be operative in skeletal muscle [52] and smooth muscle cells [53]. Moreover, acidic fibroblast growth factor (aFGF) has been claimed to exert growth-promoting properties on fetal and neonatal cardiac myocytes [54]. Whether aFGF may affect myocyte proliferation independently from IGF-1R receptors is an important unanswered question. It should be recognized that other growth factors such as aFGF [54], TGF-β and IGF-2 [51] may influence the response of cardiac myocytes and interstitial cells during postnatal myocardial development. In addition, these multiple growth factors may interact in the modulation of cell growth in normal and pathologic states of the heart. However, it is beyond the purpose of this review to describe the growth-promoting properties of these growth factors and their influence on myocyte size and number in the maturing and decompensated heart.

6. Conclusions

If the IGF-1/IGF-1R system is not involved in myocyte cellular hypertrophy, other effector pathways have to be implicated in this form of myocyte growth. Stimulation of α1-adrenoreceptors in vitro induces hypertrophy of cardiac myocytes [13]. Additionally, ventricular myocytes possess the various components of the renin–angiotensin system and its activation leads to myocyte cellular hypertrophy [14,17]. These observations suggest that distinct regulatory mechanisms may modulate myocyte proliferation and myocyte cellular hypertrophy in vitro. Although the possibility that the IGF-1 effector pathway may be implicated in myocyte cellular hypertrophy in vivo has been raised [37,38,49,55], conclusive evidence remains to be obtained. In this regard, myocyte cellular hypertrophy in the failing heart has been linked to the activation of surface angiotensin II receptors on the cells [15,16]. Adult ventricular myocytes have all the molecular constituents required to synthesize angiotensin II [18]. In addition, these cells exhibit surface receptors for angiotensin II [15,16,18,56], establishing the necessary requirements for an autocrine signaling pathway. Importantly, in vivo conditions of overload are coupled with an upregulation of this autocrine mechanism [18], which may modulate cellular responses supporting myocyte cellular hypertrophy and contractile function [56]. Moreover, the hypothesis has been advanced that the increase in myocyte length in the pathologic heart may be related to α1 adrenergic receptors and effector pathways coupled with these receptors [57], whereas the lateral expansion of myocytes may be associated with regulatory modifications of angiotensin II receptors [15,18]. Since IGF-1 stimulation may be implicated in myocyte proliferation exclusively, it is tempting to speculate that different molecular control systems may exist for the induction of myocyte hypertrophy and hyperplasia not only in vitro, but also in the overloaded adult heart in vivo.

Acknowledgements

This work was supported by grants HL-38132, HL-39902, and HL-40261 from the National Heart, Lung, and Blood Institute. The expert technical assistance of Maria Feliciano is greatly appreciated.

References (Part I)


Overy HR, Priest RE. Mitotic cell division in postnatal cardiac growth. Lab Invest 1980;43:1100–1103.


Wong SW, Wall A, Yuan PM, et al. Human DNA polymerase α gene expression is cell proliferation dependent and its primary structure is similar to both prokaryotic and eukaryotic replicative DNA polymerase. EMBO J 1988;7:37–47.


