Effects of Small Molecules on Cardiomyocyte DNA Synthesis and Proliferation

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Introduction

The long established view has been that the adult mammalian myocardium is incapable of self repair and that cardiomyocytes lose their ability to proliferate shortly after birth. However, the success of future cell based cardiac therapies, including tissue engineering, will rely on a readily available source of proliferating cardiac cells. In light of this, the intrinsic ability of terminally differentiated adult cardiomyocytes to synthesize DNA has become a topic of intense interest. The limited amount of DNA synthesis that does occur in terminally differentiated cardiomyocytes results in karyokinesis and the formation of multinucleated cells. However, this is not the scenario observed in several organisms that have the capacity to regenerate, such as the adult zebra fish [1] and the axolotl salamander [2]. The ability to mimic a similar dedifferentiation process in the mammalian cardiomyocyte would be of enormous clinical value in the treatment of heart disease.

Several biologically active molecules have been found to influence the differentiation of a variety of cell types. Past studies have shown the ability of phorbol esters and growth factors to stimulate DNA synthesis, hypertrophy, and binucleation in terminally differentiated cardiomyocytes [3]. The use of dimethyl sulfoxide (DMSO) has also been found to inhibit differentiation of myoblasts and adipocytes [4, 5]. Additionally, several recent studies on substituted purines have shown novel effects on terminally differentiated skeletal myotubes. One of these molecules, myoseverin, has been found to cleave multinucleated skeletal myotubes and induce DNA synthesis in the resultant cells by binding to microtubules and remodeling the cytoskeleton [6, 7]. A similar molecule, termed reversine, has been found to dedifferentiate multinucleated skeletal myotubes into multipotent progenitor cells [8].

While the exact mechanisms that prevent binucleated cardiomyocytes from undergoing cytokinesis remain unclear, one possibility is that the intact myofibrils and cytoskeleton physically prevent cell cleavage [9]. To explore this possibility, cardiomyocytes were treated with PMA to encourage binucleation and subsequently treated with myoseverin to depolymerize microtubules that may physically restrict cytokinesis. Studies are currently underway to investigate the ability of reversine to dedifferentiate cardiomyocytes.

Experimental

Primary cardiomyocytes were isolated from the ventricles of 1-2 day old neonatal rat pups and cultured at 37 ˚C, 5% CO_2 as previously described [10]. After isolation, cells were plated on gelatin or dilute Matrigel® coated 8-well Permanox® chamber slides. After 24 hours, cells were treated with 100 ng/ml phorbol 12-myristate 13-acetate (PMA) for 24 hours. Cells were cultured in standard media for an additional 48 hours, and then treated with 40, 80, 100, 120, and 150 μM myoseverin delivered in a 0.1% DMSO carrier. Cardiomyocytes were also treated with 0.1, 0.5, and 1% DMSO for 24 hours.

Cardiomyocytes were identified by immunolabeling for sarcomeric actin (5C5, Sigma). DNA synthesis was monitored by incorporation of bromodeoxyuridine (BrdU) after a 24 hour pulse. Negative controls were stained simultaneously with equivalent IgM concentrations. Percentages were based on counts of approximately 600-700 cells from two chamber slides.

Results

Cultures treated with PMA showed a marked increase in DNA synthesis and binucleation (**Figure 1**). This effect was found to be greater in cardiomyocytes cultured on a

dilute Matrigel® substrate than on a gelatin substrate (data not shown). Myoseverin treated cardiomyocytes lost their typical striated morphology, however cytokinesis of binucleated cardiomyocytes in these treated cultures was not observed.

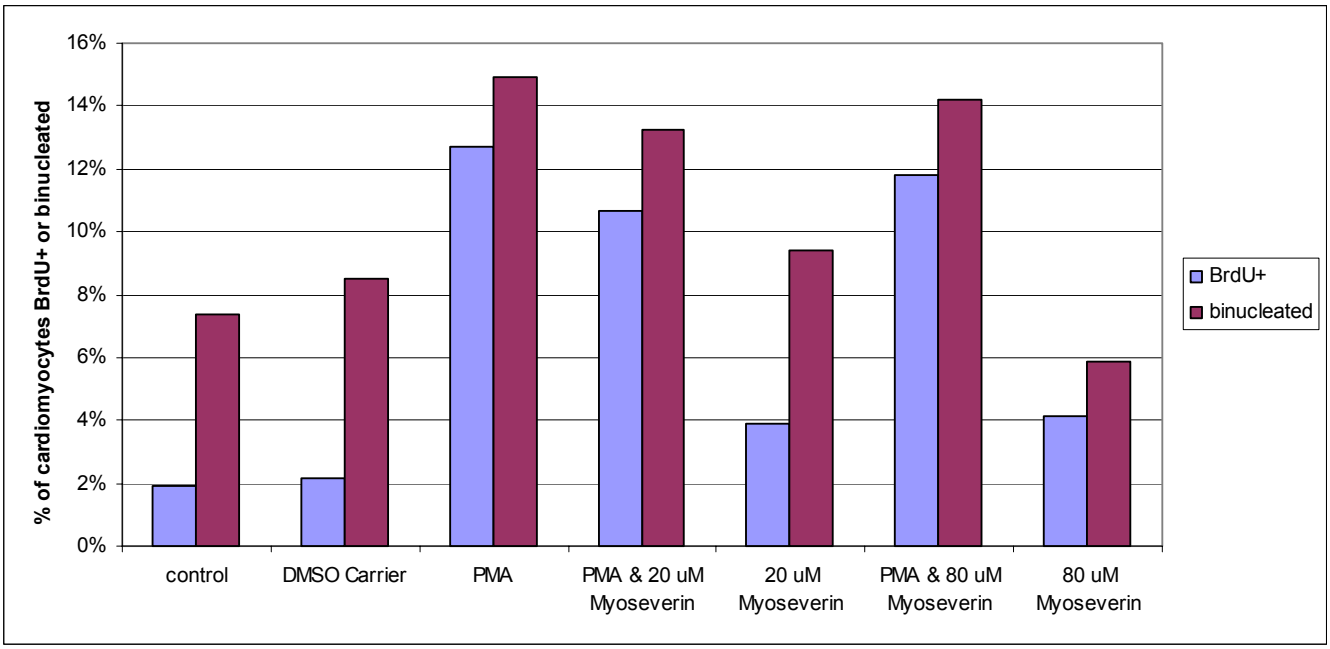

Figure 1: Effect of PMA and myoseverin on cardiomyocyte DNA synthesis and bi-nucleation in cells cultured on dilute Matrigel®.

Discussion

Cell based therapeutics for cardiac infarcts demand an available cell source, and the terminally differentiated phenotype of adult cardiomyocytes precludes their direct use in such therapies. However, recent discoveries concerning the effect of small molecules on the differentiation of skeletal muscle cells have encouraged exploration on the effect that these molecules may have on cardiomyocytes. These studies have shown that terminally differentiated rat cardiomyocytes have the ability to reproduce their genetic material; however these treatments have thus far failed to stimulate cytokinesis. Phorbol esters have long been known to activate protein kinase C and cause hypertrophy of cardiomyocytes, however the exact mechanism in which DNA synthesis is stimulated is unclear. Plating cells on Matrigel® has also shown a positive influence on the number of cells undergoing DNA synthesis. This may be a result of growth factors present in Matrigel®, the influence of substrate specific integrins, or a combination of the two factors. Studies on fibroblasts have shown a distinct influence of the substrate on DNA synthesis and proliferation [11], and it is reasonable to think that similar factors may be at work in cardiomyocytes. These studies represent important steps in exploring DNA synthesis in cardiomyocytes, and raise the possibility that DNA synthesis may be controlled in this terminally differentiated phenotype.

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