# Polyamines in Cardiac Physiology and Disease

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**Abstract:** The natural diamine putrescine and polyamines spermidine and spermine belong to a family of low-molecular-weight organic polycations that are classically known to be important mediators of cell growth, proliferation and division. Several studies are nowadays available about the involvement of polyamines in various aspects - such as growth, differentiation and death - of cardiac cells, under physiological and pathological conditions. Polyamine metabolism and effects, and their relation with a number of extracellular signals and intracellular transductional cascades, have been investigated in cellular and animal models - comprising cultures of embryo, neonatal and adult primary cardiomyocytes, heart-derived cell lines, and stem cells, as well as wild-type and transgenic animals. Significant evidence for their critical role in (mal)adaptive cardiac (patho)physiology emerges from this extensive literature suggesting that, in principle, polyamine metabolism may constitute a target for treatment of cardiovascular diseases. In the present paper we have reviewed these studies.

**Keywords:** Apoptosis, cell growth, differentiation, heart hypertrophy, polyamines.

## INTRODUCTION

Putrescine, spermidine and spermine, are natural polyamines, aliphatic polycations occurring in all living cells. Putrescine is synthesized from the non protein amino acid ornithine by the enzyme ornithine decarboxylase (ODC, EC 4.1.1.17), rapidly induced upon several growth and differentiation stimuli. The product of ODC, is then converted to spermidine and spermine, by the sequential intervention of two constitutive, specific aminopropyl-transferases: spermidine synthase (EC 2.5.1.16) and spermine synthase (EC 2.5.1.22). S-adenosylmethionine is the aminopropyl donor for spermidine and spermine biosynthesis. It is provided by another inducible decarboxylase: S-adenosylmethionine decarboxylase (AdoMetDC, SAMDC, EC 4.1.1.50) [1]. Polyamine catabolism requires two enzymes. Spermidine/spermine N1-acetyltransferase (SSAT, EC 2.3.1.57) is rapidly induced by high polyamine levels as well as stress and inflammatory stimuli. N1-acetyl polyamine oxidase (PAO, EC 1.5.3.11) is constitutively expressed. More recently, the inducible enzyme spermine oxidase (SMO, EC 1.5.3.3) was identified as responsible for direct oxidation of spermine to spermidine. Polyamine catabolism not only contributes to the fine regulation of polyamine levels, but can also result in the production of potentially cytotoxic $H_2O_2$ and aldehydes as by-products of either PAO or SMO activity [2]. Although their synthesis and catabolism are a major route for balancing polyamine concentrations in mammalian cells, transport into and out of the cell also contributes to their homeostasis [3], which is relevant as everyday we take in a significant amount of polyamines from the diet [4]. Polyamines can specifically bind to nucleic acids, proteins and phospholipids *in vitro* thus affecting gene expression, signaling pathways and ionic transport in the cell [5, 6]. Although it has been well established that polyamines are essential factors for the proliferation of eukaryotic cells, increasing evidence indicates a role for polyamines or their metabolites in other cell responses, including differentiation and apoptosis [7, 8]. Indeed, increasing evidence indicates that polyamines, cell proliferation and apoptosis are tightly connected in a quite complex interplay. It appears that polyamines are Janus-faced regulators of cellular fate, promoting either cell proliferation or cell death, depending on the cell type, as well as on the environmental signals [9]. Polyamine metabolism is deregulated in cancer cells. Conversely elevated levels of polyamines can favour tumour promotion and progression [5]. The higher requirement of polyamines for tumour growth has made polyamine metabolism an attractive target in experimental cancer therapy and chemoprevention. However, α-difluoromethylornithine (DFMO), a specific ODC inhibitor, has so far been approved by the American FDA only for the treatment of African trypanosomiasis [1]. The role that ODC and polyamines play in cardiac biology and physiology is witnessed by the number of papers published in this field. In fact polyamine-mediated signaling is part of several transduction pathways, such as adrenergic- [10, 11] or androgen-activated [12-15] cardiac cellular responses. Moreover, a number of cardiac cellular functions are proven to be affected by polyamines, such as histone acetylation and ribonucleic acid synthesis [16] and $Ca^{2+}$ homeostasis [17-19]. In particular, studies from this and other laboratories have extensively explored the relationship between polyamines and heart hypertrophy, that is a major predictor of progressive cardiac disease, as pointed out in the classic Framingham Heart Study [20]. Pathological hypertrophy is often associated to stimulation by neurohormonal,

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stress and inflammatory signals, such as angiotensin II, tumor necrosis factor (TNF)α and catecholamines [21], involving the activation of complex intracellular pathways [22] – including polyamines – and promoting cardiac fibrosis and apoptosis, that may in turn contribute to the transition to overt heart failure.

We here aim to briefly outline the significant role for polyamines in triggering and/or modulating several aspects in cardiac biology, physiology and pathology.

## POLYAMINES AND CELL GROWTH

Polyamines are essential for cell proliferation as they affect the normal cell-cycle progression [23]. ODC activity and polyamine levels are increased in the heart of intact animals, or in isolated cardiac tissue and cells, by a large variety of stimuli [reviewed in 24], particularly those leading to cardiac hypertrophy, as previously mentioned. Although the adult mammalian myocardium has a limited ability of spontaneous and effective regeneration, recent studies have described cardiac stem or progenitor cells and reported that cardiomyocytes can still divide in response to specific extracellular stimuli [25]. DNA synthesis occurs in cardiomyocyte progenitors during embryonic and neonatal development [26] and possibly in the adult heart, at least in specific conditions [27]. Identification of extracellular stimuli and intracellular pathways leading to DNA replication in these cells may result in obvious therapeutic potential. In this light, the relation between polyamines and important mediators of inflammation such as TNFα, bacterial lipopolysaccharides (LPS) and NO, all of them able to affect cardiovascular functions and relevant for cardiovascular pathophysiology [12], was evaluated. In particular, we explored the effects of TNFα and LPS on polyamine metabolism and examined the interplay between NO and polyamines in heart cell cultures. Treatment of confluent chick embryo cardiomyocytes with TNFα plus LPS induces ODC and the inducible isoform of nitric oxide synthase (iNOS, NOS2, EC 1.14.13.39) as well as DNA synthesis. This mitogenic effect is strongly reduced inhibiting either NOS or ODC [28]. Two key signaling proteins mediate the activation of polyamine and NO biosynthesis by TNFα and LPS. The transcription factor NF-κB and the MAP kinase family member extracellular-signal-regulated-kinase (ERK, EC 2.7.1.37) are separately activated by TNFα plus LPS, but converge to favour proliferation of chick embryo cardiomyocytes. More in detail, nuclear translocation of NF-κB appears to be involved in both ODC and NOS induction, while ERK activation is required only for the induction of ODC [29]. NK-κB and ERK also cooperate to reduce caspase activity, thus favoring cell survival. TNFα plus LPS treatment enhance cGMP levels in chick embryo cardiomyocytes and polyamine biosynthesis appeared to be required for this effect. Putrescine and NO donors additively activated soluble guanylate cyclase (sGC) in cell-free extracts, and, accordingly, addition of exogenous polyamines to untreated cells raise the cGMP level in a NO-dependent fashion. Moreover, treatment of quiescent cells with NO donors, polyamines, cGMP analogues or sGC activators promote DNA synthesis. Pharmacological inhibition of sGC and cGMP-dependent protein kinase (PKG), show that cGMP-dependent pathways are required for the mitogenic action of TNFα plus LPS or polyamine treatment [30]. All together, these results outline a picture where the mitogenic effect of TNFα and LPS in chick embryo effect of TNFα and LPS in chick embryo cardiomyocytes involves the activation of ERK- and NF-κB-dependent pathways, leading to the induction of both ODC and NOS enzymes. In turn, downstream polyamines and NO cooperate to enhance cGMP levels, resulting in the stimulation of DNA synthesis [25, 30]. This evidence sustains the importance of polyamine biosynthesis, and of their interrelations with other key intracellular mediators and messengers, as a signaling pathway operating in the mitogenic response of the cardiac cell.

## POLYAMINES AND DIFFERENTIATION

Polyamines are involved in the regulation of cellular differentiation. In recent studies, ODC activity and putrescine levels were correlated with mieloid cell differentiation induced by retinoic acid treatment [31] and polyamine depletion caused by α-difluoromethylornithine (DFMO), an irreversible inhibitor of ODC, was shown to prevent adipocyte differentiation [32]. Inasmuch as muscle cell differentiation is concerned, a recent paper demonstrated that the impaired skeletal muscle function in male mice with genomic androgen receptor knockout, was consistent with a reduced expression of genes encoding polyamine biosynthetic enzymes. This suggests that androgen-driven muscle development also involves the regulation of polyamine biosynthesis [33]. This work corroborates early results showing that polyamine depletion inhibits the differentiation of L6 myoblast cells [34]. We recently studied the effect of insulin on the activity of ODC while inducing H9c2 embryonal rat cardiomyoblasts to differentiate into myotubes. ODC activity increased in H9c2 cells upon insulin treatment, consistently with their differentiation in myotubes. DFMO, while reducing ODC activity induced by insulin in H9c2 cells, also delayed the increase in myogenin expression driven by insulin treatment. H9c2 cells overexpressing ODC ($ODC^+$) showed a significant increase in polyamine synthesis and differentiated into myotubes without the need for any external stimulus and faster than the wild type cells treated with insulin. Treatment with DFMO slowed down $ODC^+$ cell differentiation, showing the specific role played by polyamines [35]. These results confirm and extend the evidence of a pivotal role of polyamines in myogenic process and address a prospect suggestion of protocols for muscle regeneration.

In this respect, high expectations for regenerative medicine of cardiac muscle rely on the potential offered by mesenchymal stem cells (MSCs), a self-renewing cell population able to differentiate into several cell lineages [36]. Their use to regenerate the damaged tissue is however hindered by the fact that most of them, once transplanted in the infarcted heart, die because of apoptosis upon 24 hours [37]. In the search for new approaches to limit MSCs death, we investigated how polyamine may affect apoptosis in MSCs. Apoptosis was induced in rat bone marrow-derived MSCs with TNFα administered together with MG132, able to blunt the prosurvival pathway mediated by TNFα, which results in a prompt activation of the executioner caspases. Pre-treatment of MSCs with DFMO determined a four-fold reduction in the activation of caspase-3 and the occurrence of other markers of apoptosis (annexin V, TUNEL). On the other hand, addition of putrescine or spermidine to MSCs restored caspase activity induced by TNFα stimulation. Therefore, polyamines seem to be closely involved in the regulation of

basal and cytokine-stimulated caspase activity in rat MSCs. Thus the depletion of these natural polycations may be useful to counteract the apoptotic process [38]. MSC pre-treatment with DFMO significantly affected also their differentiation towards a cardiac lineage. MSCs grown in culture in presence of adult cardiomyocytes show a faint immunostaining for cardiac troponin I and cardiac myosin light chain. Pre-treatment of MSCs with DFMO enhances the expression of these cardiac markers. Sarcomeric protein expression significantly increases when MSCs are cultured with cardiomyocytes subjected to an ischemic-like insult. Under this condition, intended to stimulate *in vitro* an infarct damage, pre-treatment of MSCs with DFMO further enhanced production of cardiac specific sarcomeric proteins. These effects on cardiac differentiation were partially suppressed by treating MSCs with anacardic acid, an inhibitor of histone acetylation, indicating a potential mechanism involved in the DFMO-mediated cardiac commitment [39]. Differentiation of MSCs to an early cardiac phenotype seems therefore to be improved by pre-treating MSCs with DFMO, especially when they establish contacts with post-ischemic cardiomyocytes. These findings indicate that the *ex-vivo* pre-treatment of MSCs with DFMO could represent a useful strategy to improve regeneration of the damaged cardiac tissue. The potential of this treatment is also emphasized by the absence of DFMO toxicity *in vivo* and by the observation that polyamine depletion is maintained in MSCs for some days after DFMO removal, a time which coincides with the early phase of MSCs transplantation in the heart. The two above-mentioned described examples of the involvement of polyamines in cell differentiation may sound puzzling: increasing their cellular concentrations [35] may lead to differentiation as well their depletion [39] does. To reconcile these opposite evidences one can consider that different cell types may integrate polyamine-mediated signaling within their peculiar intracellular transductional network, with the final emergence of defined properties resulting from the role exerted by other (possibly hidden) players [40]. This is not unusual for cellular functions where a clear implication of polyamine is described, such as e.g. apoptosis [41].

## POLYAMINES AND APOPTOSIS

Apoptosis is a regulated form of cell death under genic control. Deregulated apoptosis is involved in several fields of medicine, including cardiovascular medicine [42]. It is now evident that apoptosis is deeply involved in the pathophysiology of almost all kinds of heart disease. Apoptosis of cardiac myocytes has been recognized as a cellular mechanism of injury in cardiac ischemia/reperfusion, hypertrophy and failure [43, 44]. Several studies showed that polyamines are involved in pathways leading to either cell death or survival in a number of cell types [for reviews on polyamines and apoptosis see refs. 1, 7, 8]. Based on growing experimental work, it may be concluded that polyamines can doubtless affect the apoptotic process. However the relationship appears to be complex and dependent on the cell type and death stimulus, as well as on the actual levels and activated polyamine pathways. Both up-regulation and down-regulation of polyamine levels have been reported during apoptosis [41]. Whereas excessive intracellular polyamines in overproducing cells or following exogenous addition generally induce apoptosis [45-47], polyamine depletion caused by a specific polyamine biosynthesis inhibitor like DFMO can reduce or enhance the susceptibility to apoptosis even in the same cell type, depending on the specific death stimulus [48, 49]. Two main phases of apoptosis have been described: an initiation phase during which specific signal transduction pathways are activated and a mitochondrial phase, modulated by Bcl-2 family members and involving the release of cytochrome c and the activation of caspase cascade. Today we know that polyamines can interfere with both these phases [for review see ref. 8] and recent studies focused on cardiac cells suffering ischemic stress [50-53]. In particular, activity of ODC is rapidly and transiently induced, causing increased polyamine levels [50] in H9c2 cardiomyoblasts exposed to a condition of simulated ischemia that leads to apoptosis [54]. In this same model, intracellular DFMO-driven polyamine depletion protects against apoptosis, inhibiting several molecular events at the level of the mitochondrial phase of apoptosis following simulated ischemia, such as release of cytochrome c from mitochondria, caspase activation, down-regulation of Bcl-xL and consequent DNA fragmentation. However, DFMO was also shown to prevent apoptosis at the level of the pre-mitochondrial phase, by affecting key signaling proteins such as ERK, JNK and AKT, in different cell types [reviewed in refs. 8 and 55]. In addition, cardiomyocytes isolated from transgenic mice overexpressing cardiac ODC, resulting in a 4-fold increased putrescine intracellular level, show a higher caspase activation with respect to cells from control mice, in accordance with the view of these amines as apoptosis-facilitating factors [50]. The complexity of the actions of polyamines in apoptosis is indicated by a recent report on simulated ischemia/reperfusion injury where the addition of putrescine to cultured neonatal rat cardiomyocytes was shown to enhance the rate of apoptosis, whereas the treatment with spermine or spermidine resulted to decrease it [51]. Again, involvement of the anti-apoptotic protein Bcl-2 and a modulation of cytochrome c release from mitochondria was described in this setting. Perturbation of polyamine metabolism was also observed in isolated, perfused rat heart subjected to ischemia and reperfusion [52]. Increased ODC and SSAT activities and putrescine accumulation were detected after acute heart ischemia; however, a loss of spermine was observed after reperfusion, attributed to an increase of NO content and associated with reduced myocardial cell viability. In fact a negative correlation was found between NO and spermine levels; moreover, treatment with the exogenous NO donor sodium nitroprusside (SNP) decreased cardiac ODC activity, and increased SSAT activity, resulting in spermine reduction. Indeed, it was reported that SNP inhibits ODC induction in cardiomyocytes, being also able to provoke cytotoxicity, which was prevented by antioxidants, suggesting the involvement of toxic radical derivatives [33]. Actually NO may act in pathways leading to either cell survival or cell death [56, 57] and a complex cross-talk between NO and polyamine metabolism occurs as discussed in various parts of this article. Very recently, upregulated expression of SSAT, a key enzyme in polyamine catabolism, was found in the ischemic myocardium upon coronary ligation in rats [58]. SSAT appeared induced under conditions of ATP depletion *via* AMPK signaling, but resulted to exert a cardioprotective action in this context, which was not related to changes in polyamine content. As a final thought, despite a

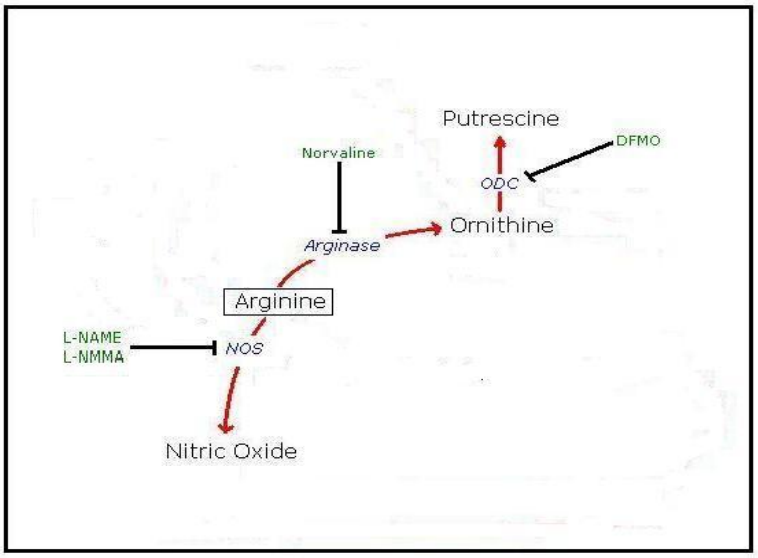


**Fig. (1). Arginine between nitric oxide and polyamine biosynthetic pathways.**
*DFMO*, difluoromethylornithine; *L-NAME*, L-arginine-methyl ester; *L-NMMA*, $N^G$-monomethy-L-arginine; *NOS* nitric oxide synthases; *ODC*, ornithine decarboxylase.

variety of results, partially related to different experimental models and protocols, all these studies underline the importance of polyamines and polyamine-related enzymes in affecting survival and death of cardiomyocytes.

## POLYAMINES AND HEART HYPERTROPHY

Heart muscle hypertrophy is an adaptive response to both physiological (chronic exercise) and pathological (chronic hypertension, cardiac valve stenosis) stimuli, aiming to increase ventricular pump function and to decrease cardiac wall tension. Individual myocyte growth sustains the hypertrophic phenotype at the cellular level, where activation of gene expression and protein synthesis are typical features. A role for ODC and polyamine metabolism in cardiac hypertrophy was described since long ago [59-61]. More specifically, an increase in polyamine concentrations was reported in cardiac tissue or cells after ascending aortic stenosis [62], stress [63], physical exercise [64, 65], administration of adrenoceptor agonists [66-68] - all inducers of cardiac hypertrophy. This increase in cellular polyamine content was consistent with histone hyperacetylation [69], and elevated RNA [64, 69] and protein [70] synthesis. On the other hand, administration of DFMO, reduced polyamine content and attenuated isoproterenol (ISO)- and clenbuterol-induced cardiac effects [71, 72]. Today a number of transgenic and knockout mouse models, with altered levels of polyamine metabolizing enzyme is available, as reviewed in [73]. Recently, the targeted overexpression ODC to the heart has been shown to produce a moderate baseline cardiac hypertrophy. β-adrenergic stimulation with ISO increases the left ventricular hypertrophy in these transgenic mice [74]. Interestingly, we found that ISO also dramatically induces arginase activity in these transgenic hearts [75]. Arginase catalyzes the conversion of arginine to ornithine, the ODC substrate. On the other hand, arginine is also the substrate for nitric oxide synthases (NOS), which lead to the synthesis of NO (Fig. **1**) [76]. Reduced levels of NO are consistent with development of cardiac hypertrophy [77, 78], and overexpression of the endothelial isoform of NOS has been shown to attenuate the hypertrophic effect of ISO [79]. Moreover, NO can inhibit ODC activity [80] and reduce polyamine content [81], and polyamines can inhibit NOS [82]. The activity of arginase is thus likely to play a regulatory role in the biosynthesis of both NO and polyamines and these metabolic pathways may therefore crosstalk in regulating a variety of cardiovascular functions [32, 33, 83, 84].

## CONCLUDING REMARKS

Natural polyamines have been credited of several specific roles within cardiac cells and tissue, in a large variety of experimental models ranging from cell cultures to whole animals. Accumulating evidence indicates that polyamines are involved in various cellular aspects of cardiac development and remodeling, including proliferation, differentiation and apoptosis of cardiac cells. Ongoing investigations will test out if they may represent a potential target for the treatment of cardiovascular diseases.

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