Polyamines, NO and cGMP mediate stimulation of DNA synthesis by tumor necrosis factor and lipopolysaccharide in chick embryo cardiomyocytes

Benedetta Tantini\textsuperscript{a,*}, Flavio Flamigni\textsuperscript{a}, Carla Pignatti\textsuperscript{a}, Claudio Stefanelli\textsuperscript{a}, Monia Fattori\textsuperscript{a}, Annalisa Facchini\textsuperscript{a}, Emanuele Giordano\textsuperscript{a}, Carlo Ciò\textsuperscript{b}, Claudio Marcello Caldarera\textsuperscript{a}

\textsuperscript{a}Department of Biochemistry “G. Moruzzi”, School of Medicine, University of Bologna, via Irnerio, 48 40126 Bologna, Italy
\textsuperscript{b}Institute of Biological Chemistry, School of Medicine, University of Parma, Parma, Italy

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Abstract

Objective: We have recently shown that tumor necrosis factor-α (TNFα) and lipopolysaccharide (LPS) stimulate DNA synthesis in chick embryo cardiomyocytes (CMs). The aim of the present research was to investigate the pathways involved in this mitogenic response.

Methods: CMs were isolated from 10-day-old chick embryos and grown to confluence. After 20 h of serum starvation the cells were treated with TNFα and LPS, and/or specific agonists and antagonists to manipulate the levels of polyamines, NO, cGMP and their biosynthetic enzymes ornithine decarboxylase (ODC), nitric oxide synthase (NOS) and soluble guanylate cyclase (sGC). ODC, NOS, sGC activities and cGMP contents were determined by radiochemical procedures. DNA synthesis was determined by incorporation of [\textsuperscript{3}H]-thymidine. Results: Treatment of CMs with TNFα and LPS increased cell number and [\textsuperscript{3}H]-thymidine incorporation. Addition of TNFα and LPS provoked an induction of ODC, with consequent polyamine accumulation, and a more delayed enhancement of NOS activity, which appeared to be independent of the activation of the ODC–polyamine system. TNFα and LPS treatment also enhanced cGMP level in CMs and both polyamine and NO biosyntheses appeared to be required. Experiments with specific inhibitors of ODC and NOS, as well as with inhibitors of sGC and cGMP-dependent protein kinase (PKG), showed that polyamine-, NO- and cGMP-dependent pathways are required for the mitogenic action of TNFα and LPS. Moreover, addition of exogenous polyamines to untreated cells raised the cGMP level in a NO-dependent fashion, and enhanced [\textsuperscript{3}H]-thymidine incorporation. The latter effect was inhibited by sGC or PKG inhibitors. Treatment of quiescent cells with NO donors, 8-bromo-cGMP or YC-1, an sGC activator, also promoted DNA synthesis. Furthermore, putrescine and NO donor can additively activate sGC in cell-free extracts. Conclusion: TNFα and LPS stimulate DNA synthesis in chick embryo CMs and this effect is mediated by polyamines, NO and intracellular cGMP. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Cytokines; Myocytes; Nitric oxide; Second messengers

1. Introduction

Cardiomyocytes (CMs) differentiated from the very early phases of the embryo, continue to increase in cell number up to the first 2–3 days after birth [1], although the number of DNA synthesizing myocytes begins to decrease during late embryonic development [2]. Recent observations suggest that CM regeneration occurs in the adult heart, albeit at a low rate, and that DNA synthesis of CMs increases under certain conditions, such as cardiac overload and failure or viral myocarditis [3–5], contributing to the remodeling of the pathological heart. In the light of the relevance of DNA synthesis in CMs during embryonic and neonatal development and possibly even in the adult heart,
it may be important to identify extracellular mediators and intracellular pathways leading to DNA replication in CMs.

Tumor necrosis factor-α (TNFα) is a proinflammatory cytokine that has been detected in myocardium during sepsis, transplant rejection, heart failure and viral myocarditis [6–8]. In these settings ischemia and bacterial lipopolysaccharide (LPS) are some of the clinically relevant stimulants that induce TNFα production in the heart [9,10]. TNFα has also been detected in chick embryo heart and its expression was not restricted to cardiac regions where cell death occurred [11]. Interestingly, mice deficient in FADD or casper, which are signal transducers of the TNFα receptor family, exhibit impaired heart development [12,13]. Although the precise biological role for TNFα expression within the heart is not clear, TNFα has been proposed as an autocrine/paracrine mediator in myocardial remodeling [14]. Experimental evidence indicates that TNFα can favour cell growth or death, depending on the cell type, the developmental stage and the association with other stimuli such as LPS [15–17]. In adult rat CMs, treatment with TNFα can lead to apoptosis [6,18] but this effect was not observed in neonatal proliferating CMs [18]. On the other hand, another report [14] shows that TNFα can provoke a hypertrophic growth response in adult CMs. Actually, transgenic mice with cardiac-specific overexpression of TNFα develop dilated cardiomyopathy characterized by inflammation and hypertrophy, without abundant CM necrosis or apoptosis [19]. Moreover protective effects of TNFα in cardiac injury and hypoxic stress have been proposed [20].

TNFα can induce NO synthase (NOS) activity in different cell types, including CMs [21] and NO is an important mediator of TNFα in the heart. In most cases, the expression of inducible NOS is enhanced by LPS [22,23]. Arginine, the substrate for NOS, may also be converted to ornithine, which in turn can be decarboxylated by ornithine decarboxylase (ODC). Interestingly, increased activity of ODC, the first and key enzyme in polyamine biosynthesis [24], is an early event related to DNA replication and cell proliferation [25]. Besides, we have shown that like NO, polyamines can stimulate cGMP production [26,27].

This background prompted us to investigate whether TNFα may stimulate DNA synthesis in CMs and whether polyamines, NO and cGMP are involved. In this regard confluent chick embryo heart cells may represent a useful model, since they can be rapidly recruited into the cell cycle by mitogens. In a preliminary report [28] we have shown that TNFα and LPS induce ODC and NOS activities and stimulate DNA synthesis in chick embryo CMs cultures. In the present study the effects of TNFα and LPS on the induction of ODC and NOS were further investigated focusing on the possible relevance of cGMP synthesis. The results suggest that polyamine and NO biosyntheses may cooperate to enhance intracellular cGMP content, resulting in stimulation of DNA synthesis.

2. Methods

The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication NO.85-23, revised 1996).

2.1. Materials

Recombinant human TNFα was purchased from CABRU: (±)-S-nitroso-N-acetylpenicillamine (SNAP) was from Calbiochem and spermine NONOate (SPM-NO) from Cayman. α-Difluoromethylornithine (DFMO) was kindly provided by the Merrell Centre (Strasbourg). L-N-Monomethylarginine (L-NMMA), LPS, sodium nitroprusside (SNP), methylene blue and hemoglobin were from Sigma. LY8353, KT5823 and YC-1 were purchased from Alexis and 8-bromo-cyclic GMP was from Boehringer Mannheim.

2.2. Preparation of cardiomyocyte cultures

Preparation of monolayer cultures of spontaneously beating embryo CMs from the hearts of 10-day-old chick embryos was carried out by a trypsin disaggregation procedure [29]. Myocytes were purified by differential adhesion by a 2 h preplating of the initial cell suspension at 37°C. Then nonadherent cells (CMs) were counted with a hemocytometer and their viability was verified at 85% by Trypan Blue exclusion. Cells were seeded at a density of 2.5×10^6 per 35 mm plate (Falcon) in 3 ml of Dulbecco modified Eagle’s medium (Gibco) containing a high concentration of glucose (25 mM) supplemented with 10% foetal calf serum, 1% streptomycin and 1% penicillin. The cultures were incubated at 37°C in a humidified atmosphere containing 5% CO₂ and grown to confluence, with a change in serum-containing medium every 48 h, in order to replenish cell culture medium constituents as well as to remove any dead cells and cell debris. The viability was always maintained greater than 95% under all experimental conditions. Using this method, we routinely obtained within 5 days a confluent monolayer of spontaneously beating cells with <5% noncardiomyocytes. Confluent cultures were then serum-starved for 24 h before treatment with TNFα and/or LPS or the different agonists dissolved in PBS. Enzymatic inhibitors were dissolved in PBS or dimethylsulfoxide and control cells received an equal amount of the vehicle.

2.3. Ornithine decarboxylase assay

At the end of the incubations a crude enzyme extract was prepared from cells which were previously washed with phosphate buffered saline (PBS) and scraped in a buffer consisting of 0.1 mM EDTA, 0.02 mM pyridoxal phosphate, 2.5 mM dithiothreitol in 10 mM sodium...
phosphate buffer, pH 7.2. The cells were disrupted by freeze–thawing three times and then centrifuged at 15 000 g for 15 min. The ODC activity was measured by estimation of the release of $^{14}$CO$_2$ from L-[1-$^{14}$C]-ornithine, as previously described [28]. Data are expressed as pmol/mg protein/min. Proteins were determined according to Bradford [30].

2.4. Nitric oxide synthase assay

NOS activity was tested monitoring L-[3H]-citrulline formation from L-[2,3-3H]-arginine. At the end of the incubation periods, the cells were washed once with HEPES buffer and then incubated for 30 min at 37°C with 1 ml of the same buffer containing 10 mM l-arginine and 1 μCi L-[2,3-3H]-arginine (NEN, 40.5 Ci/mmol specific activity)/plate. The reaction was stopped by washing the cells with cold PBS containing 5 mM l-arginine and 4 mM EDTA. After supernatant removal, 0.5 ml ethanol was added to each monolayer and allowed to evaporate. A 2-ml volume of 20 mM HEPES, pH 5.5, was then added. After 5 min, 1 ml of supernatant was mixed with 0.4 ml of slurry Dowex AG50W-X8 Na+ form equilibrated in stop buffer and vortexed for 30 min. A 0.5-ml volume was collected from the supernatant and counted in a liquid scintillation spectrometer. Proteins were determined after alkaline hydrolysis of the cells. Data are expressed as pmol/mg protein/min.

2.5. Guanylate cyclase assay

Serum starved cultures were washed twice with cold 0.85% NaCl, harvested by scraping in 50 mM NaCl, 10 mM Hepes (pH 7.6), 0.1 mM dithiothreitol, 0.1 mM EGTA and homogenized with a PTFE glass homogenizer at 4°C. The homogenates were centrifuged at 105 000 g for 60 min to separate the soluble fraction. The soluble guanylate cyclase activity was estimated by cGMP generated. The reaction mixture contained 30 mM Hepes (pH 7.6), 25 mM NaCl, 0.5 mM dithiothreitol, 0.05 mM EGTA, 2.5 mM phosphocreatine, 0.05 mg/ml creatine phosphokinase, 0.5 mM 3-isobutyl-1-methyl-xanthine, 1 mM MnCl$_2$, and 20 μl of appropriately diluted enzyme (20–40 μg of protein) in a final volume of 200 μl. After 10 min of preincubation at 30°C in the presence of 100 μM putrescine, 100 μM SNP or both, the reaction was started by the addition of 1 mM GTP, carried out for 10 min, stopped by adding 0.6 ml of 50 mM Tris–HCl (pH 7.4) containing 4 mM EDTA, boiling and centrifuging at 2000 g for 10 min. Aliquots of the supernatant were assayed for cGMP by using a specific kit from Amersham. Proteins were determined as described by Bradford [30].

2.6. cGMP and polyamine determination

At the end of the incubation at 37°C, the medium was aspirated and the cells were rinsed twice with cold 0.85% NaCl, deproteinized with 0.6 M HClO$_4$, pooled by scraping, frozen and thawed twice and then centrifuged at 15 000 g for 10 min. The pellets were used for protein determination, while the supernatants were neutralized with 5 M K$_2$CO$_3$ and then assayed for cGMP content by a radioimmunoassay kit (Amersham). Polyamines were detected in the supernatant by HPLC, after dancyl derivatization [31].

2.7. [3H]-Thymidine incorporation and cell count

DNA synthesis was quantified by [3H]-thymidine incorporation of subconfluent CM cultures. The cells, maintained for 20 h in a serum-free DMEM, were then treated with the different drugs, and pulsed during the last 2 h with 3 μCi of [3H]-thymidine per dish (Amersham, 5.0 Ci/mmol specific activity). The cells were then washed twice with ice-cold PBS, collected by scraping in cold 0.6 M HClO$_4$, frozen and thawed twice and centrifuged at 15 000 g for 10 min. The precipitate, dissolved in 1 M NaOH, was used for radioactivity analysis. Data are expressed as a percentage of the radioactivity measured under basal conditions. Cell counting was performed after trypsinization of monolayers and resuspension of pelleted cells in PBS and transfer to a hemocytometer.

2.8. Statistical analysis

Values are given as means±S.D. All experiments were performed with at least three independent cardiomyocyte cultures. Comparison among two groups was performed using t-test. Differences were considered significant for $P<0.05$.

3. Results

3.1. TNFα and LPS induce ODC and NOS activities in cardiomyocytes

Treatment of confluent and serum-starved chick embryo CMs with TNFα and LPS induced ODC activity, with a significant peak at 4 h (Fig. 1A). This increase in ODC led to the accumulation of polyamines, particularly putrescine and spermidine (Table 1). Fig. 1B shows that NOS activity, present in untreated, serum-starved cells, progressively increased after exposure to TNFα and LPS with a maximum at 24 h. This was accompanied by enhancement of NO level, detected by the Griess colorimetric method (not shown). Fig. 2A shows that both ODC and NOS inductions required the concomitant presence of TNFα and LPS. TNFα alone was without effect, but strongly enhanced both the stimulation of ODC and the increase in NOS elicited by LPS. In order to establish if the early activation of the ODC–polyamine system could
be involved in the more delayed induction of NOS, cells were preincubated with DFMO, a specific ODC inhibitor. The presence of DFMO did not affect the induction of NOS by TNFα and LPS (Fig. 2B). Likewise, the exposure of cells to exogenously added polyamines could not increase basal NOS activity (not shown). Conversely, L-NMMA, a competitive NOS inhibitor, did not influence ODC induction by TNFα and LPS (Fig. 2B).

3.2. Interplay between polyamines and nitric oxide modulates cGMP level in cardiomyocytes

The treatment of cells with TNFα and LPS was followed by increased cGMP levels (Fig. 1C), in accordance with the notion that NO can stimulate soluble guanylate cyclase (sGC) [26]. Since we previously showed that polyamines can stimulate sGC in heart cell cultures [27], some experiments were performed to verify whether polyamines cooperate with NO in modulating cGMP level in CMs. Fig. 3A shows that the increase in intracellular cGMP induced by TNFα and LPS was dependent on NO generation, since it was abolished either by pretreatment with L-NMMA or by the presence of hemoglobin, a known NO scavenger [32]. In addition, pretreatment with DFMO prevented the effect of TNFα and LPS indicating that endogenous polyamines are also implicated in the NO-dependent increase in intracellular cGMP. Interestingly, the treatment of CMs with single exogenous polyamines, at the concentration most effective in stimulating sGC [27], increased the cGMP content, particularly evident in the case of putrescine, the product of ODC (Fig. 3B). However, pretreatment of the cultures with L-NMMA blunted the increase in the level of cGMP elicited by exogenous polyamines, suggesting that NO is required in their action. Besides, exposure of the cells to NO donors such as SNAP, SPM-NO or SNP not only increased NO content (not shown), but also cGMP level (Fig. 3C). This latter effect was prevented by the sGC inhibitor LY83583. Altogether these results indicate that both polyamine and NO biosyntheses are required for cGMP production in CMs.

3.3. In vitro effects of polyamines and NO on soluble guanylate cyclase activity

We have previously shown that polyamines stimulate the activity of particulate and more remarkably sGC activity in cell-free extracts [33]. Polyamines increased the affinity of sGC for the substrate, as well as its Vₘₐₓ. Moreover polyamines seemed to substitute for Mn as cofactor of the enzyme, probably by interacting with its cation site [27]. Therefore we performed some experiments to establish if polyamines may cooperate with NO donors to activate sGC directly in vitro. Table 2 shows that putrescine as well as SNP directly stimulate the activity of sGC in cell-free extracts of CMs. The stimulating effect of SNP has been attributed to the release of NO which binds to the heme group of the enzyme [34]. Interestingly, putrescine and SNP exerted an additive effect in increasing sGC activity when added directly to the incubation mixture. This suggests that putrescine and SNP stimulate the enzyme activity through different mechanisms.

3.4. Polyamines, NO and cGMP mediate the mitogenic response of cardiomyocytes to TNFα and LPS

TNFα and LPS stimulated the re-entry of CMs into the

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**Table 1**

Effect of TNFs and LPS on polyamine content in confluent chick embryo cardiomyocytes

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time (h)</th>
<th>Polyamine (nmol/mg protein)</th>
<th>Putrescine</th>
<th>Spermidine</th>
<th>Spermine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0.20±0.01</td>
<td>5.37±0.55</td>
<td>12.05±1.15</td>
<td></td>
</tr>
<tr>
<td>TNFα+LPS</td>
<td>8</td>
<td>1.37±0.10*</td>
<td>9.15±0.85*</td>
<td>15.37±1.16*</td>
<td></td>
</tr>
<tr>
<td>TNFα+LPS</td>
<td>16</td>
<td>1.78±0.13*</td>
<td>10.95±1.12*</td>
<td>17.40±1.65*</td>
<td></td>
</tr>
</tbody>
</table>

* Serum starved cardiomyocytes were treated for 8 or 16 h with 500 U/ml TNFα and 10 μg/ml LPS. Data are means±S.D. of three independent experiments.

* P<0.05 vs. control cells.
Fig. 2. ODC and NOS activities in confluent chick embryo cardiomyocytes treated with TNFα and/or LPS. Effect of DFMO and L-NMMA. (A) Serum starved cardiomyocytes were treated with 500 U/ml TNFα and/or 10 μg/ml LPS for 4 h (ODC) or for 8 h (NOS). (B) Serum starved cardiomyocytes, pretreated for 20 h with 4 mM DFMO or for 1 h with 100 μM L-NMMA, were then incubated with TNFα and LPS, as described above. The results are expressed as relative activity with respect to the activities of control cells, taken as 1.0. Control ODC activity was 0.30±0.1 pmol/mg protein/min; control NOS activity was 2.4±0.3 pmol/mg protein/min. *, P<0.05 vs. control cells.

cell cycle, as indicated by an increase in cell number by about 200% (from $98 \times 10^4$/ml to $197 \times 10^4$/ml) and enhanced $[^3H]$-thymidine incorporation after 1 day of treatment (Fig. 4A). This mitogenic response was prevented by the presence of the sGC inhibitors LY83583 and methylene blue, the specific cGMP-dependent protein kinase (PKG) inhibitor KT5823, the NOS inhibitor L-NMMA or the ODC inhibitor DFMO, suggesting that cGMP-, NO- and polyamine-dependent pathways are all required for the proliferative process in CMs. This is supported by the fact that the sGC activator YC-1 [35], the cell permeant cGMP analogue 8-br-cGMP and the two NO donors SNAP and SPM-NO were each effective in increasing basal $[^3H]$-thymidine incorporation (Fig. 4B). Furthermore, addition of exogenous polyamines (putrescine, spermidine or spermine) also increased DNA synthesis (Fig. 4C), and their effect was mediated by cGMP, since it was prevented by LY83583 and KT5823. SPM-NO, which can release both spermine and NO, was the most effective among the agents tested. Finally, either TNFα or LPS alone were hardly effective in stimulating DNA synthesis (not shown). Therefore, not only polyamines and NO are necessary for the mitogenic response of TNFα and LPS, but these intracellular mediators may mimic the action of the cytokines. Moreover, polyamine and NO effects may be mediated by cGMP.

4. Discussion

It has been reported that TNFα and LPS can be mitogenic or cytotoxic according to the cell type and the developmental stage [15,17]. This study demonstrates that TNFα, in the presence of co-stimuli such as LPS, can stimulate CM proliferation, suggesting its potential mitogenic action, which may be relevant to issues such as the development of embryonic heart or the remodeling of pathological heart. It should be taken into consideration that in vivo the presence of other cytokines might condition the response of CMs to TNFα. However it should also be stressed that these results were obtained with chick embryo CMs and the effects on mammalian embryonic or adult CMs remain to be assessed. TNFα and LPS act in concert in several experimental systems and may activate multiple signal transduction pathways, but their mode of action is only partially defined [15,17,36]. To our knowledge, the present study is the first indicating that increased polyamine and NO biosyntheses induced by treatment with
TNFα and LPS cooperate to enhance cGMP level leading to stimulation of DNA synthesis. An involvement of NO and cGMP generation in mitogenesis has been evidenced in endothelial cells [37,38], but in other cell types, generally, NO and cGMP exert an antiproliferative effect [39,40].

On the other hand, it is known that the induction of ODC and polyamine biosynthesis are early events closely related to DNA synthesis [25]. Polyamines are known to influence a number of cellular pathways relevant for proliferation [41], but their mechanism of action is not yet fully clarified. In cultured CMs polyamines have been reported to activate sGC [27] and some effects of these polycations may be related to the increase in cellular cGMP content [34]. Indeed, the activation of sGC also represents an important step in the mechanism of action of NO and cytokines [26].

We provide here the evidence that exposure of CMs to TNFα and LPS progressively increases the cellular cGMP level up to 20 h. This effect is dependent on both NO...
Table 2
Effect of putrescine or SNP on sGC activity in vitro

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Guanylate cyclase activity (pmol cGMP/mg protein)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>4.18±0.5</td>
<td>100</td>
</tr>
<tr>
<td>Putrescine</td>
<td>8.67±0.9*</td>
<td>207</td>
</tr>
<tr>
<td>SNP</td>
<td>11.04±1.0*</td>
<td>264</td>
</tr>
<tr>
<td>Putrescine + SNP</td>
<td>19.67±1.6*</td>
<td>470</td>
</tr>
</tbody>
</table>

* sGC was preincubated for 10 min at 30°C in the presence of 100 μM putrescine, 100 μM SNP or both. Therefore the assay was initiated by the addition of GTP and carried out for further 10 min. Data are means±S.D. of three independent experiments.

In summary, we propose that NOS and ODC inductions elicited by TNFα and LPS in CMs are two events related to the re-entry into the cell cycle and to increased cell proliferation, but not linked by a cause–effect relationship. We also suggest that both NO and polyamines must be accumulated at a ‘critical’ level in order to obtain a mitogenic response of CMs to TNFα and LPS. The activation of sGC is strictly required for the mitogenic generation and polyamine biosynthesis, since it was abrogated by L-NMMA, hemoglobin or DFMO. Increased cGMP content has been also observed following treatment of CMs with exogenous polyamines or different NO donors. Moreover, the addition of TNFα and LPS to CMs induces ODC and NOS activities independently. In fact, pretreatment with L-NMMA or DFMO did not affect ODC or NOS inductions, respectively. Therefore, in CMs, polyamines may cooperate with NO in increasing cGMP levels and appear to be involved in the NO–cGMP pathway at the level of sGC activation, rather than NO generation. In fact we showed that putrescine and a NO donor exerted an additive effect in stimulating the activity of sGC directly in cell-free extracts of CMs.

Fig. 4. TNFα and LPS, cGMP, NO donors and polyamines stimulate DNA synthesis in confluent chick embryo cardiomyocytes. (A) Serum starved cardiomyocytes were incubated for 20 h with 500 U/ml TNFα and 10 μg/ml LPS in the absence or presence of 10 μM LY83583, 10 μM methylene blue, 1 μM KT5823, 100 μM L-NMMA or 4 mM DFMO; (B) serum starved cardiomyocytes were incubated for 20 h with 10 μM YC-1, 2 mM 8-Br-cGMP, 100 μM SNAP or 100 μM SPM-NO; (C) serum starved cardiomyocytes were incubated for 20 h with 100 μM putrescine, spermidine or spermine in the absence or presence of 1 μM KT5823 or 10 μM LY83583. *, P<0.05 vs. untreated cells.
response of CMs, and NO and polyamines closely cooperate in increasing cellular cGMP, which could represent the common mediator of two separate pathways leading to DNA synthesis. The mechanism of cGMP-mediated stimulation of DNA synthesis is not clear, but requires PKG activity, as judged by the efficacy of KT5823. Our preliminary data suggest that p44/p2 mitogen-activated protein kinase is also involved in the cGMP effect, as has been reported for endothelial cells stimulated by vascular endothelial growth factor [38]. Besides, it may be interesting to verify whether an ‘excess’ of, or a long-term exposure to cellular NO and polyamines can, on the contrary, favour cell death instead of proliferation. Recently it has been observed that apoptosis can be induced in neonatal CMs through a delayed and sustained NO generation following a 72–120 h treatment with a combination of cytokines [42].

Both polyamines and NO affect cardiovascular functions. Polymines are thought to protect the myocardium against the harmful effect of ischemia. A decrease of cardiac polyamine content has been observed after myocardial infarction [43], whereas increased ODC activity and polyamine levels take place as cellular responses to cardiac hypertrophy [44,45]. In this regard, it may be interesting to verify whether polyamines could be involved in the hypertrophic effect of TNFα observed in adult CMs [14]. The expression of NOS as well as the levels of circulating cytokines are high in systemic inflammatory response syndrome and in many cardiac-related pathogenic conditions [46–48]. The evidence provided here of a mitogenic effect of TNFα and LPS in embryonal CMs could be of some relevance for the clinical approach to a variety of cardiac disorders related to increased levels of circulating cytokines and/or altered polyamine biosynthesis, and it may be helpful in the development of new therapeutic strategies in the cardiovascular field.

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