Minireview

Nitric Oxide and its Effects on the Calcium Transport Systems in the Myocardium

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Abstract. Nitric oxide (NO) is an important modulator of many physiological processes, including myocardial contractile function The understanding of how the production of NO in the myocardium is regulated in response to physiological stimuli and pathological processes is evolving rapidly The main goal of this minireview is to summarize the current knowledge (i) about the enzymes that produce NO in the heart muscle, (ii) about the targets of myocardial NO that modulate calcium transport systems of the heart muscle cells, and (iii) about the role that NO-induced changes of calcium homeostasis play in the modulation of myocardial contractility

Key words: Nitric oxide — Cardiac cell — Calcium transport

The physiological importance of the cardiovascular system forced a strong interest in detailed studies of all processes in the heart Myocardial contraction is triggered by a transient increase in the cytoplasmic free Ca^{2+} concentration The process of coupling between excitation and contraction is mediated by the inward calcium current, which initiates calcium-induced calcium release (Fabiato 1989) through the calcium release channels of the sarcoplasmic reticulum (Beuckelmann and Wier 1988) The time course of the Ca^{2+} release and thus of contraction is modulated by all Ca^{2+} transport systems, which themselves are regulated directly or indirectly (via phosphorylation) by intracellular calcium The importance of calcium modulation in the heart is stressed by the fact that abnormal intracellular calcium handling is associated with several cardiovascular diseases and altered E-C coupling

Nitric oxide (NO) is widely recognized as a second messenger, and it has been shown to participate in physiological as well as pathophysiological functions of

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many organ systems Besides the vasodilatation effect in response to ischemia (Nonami 1997), evidence has indicated that NO contributes to a variety of functions (such as proliferation, apoptosis, mitochondrial electron transport, etc , Nathan and Qiao-Wen 1994), depending on the tissue Nitric oxide (NO) is one of the compounds which are known to be involved in the regulation of myocardial contractile function However, the physiological role and pathophysiological consequences of NO produced by the nitric oxide synthases (NOSs) in cardiac cells are not so clear Since cardiac NO is produced not only in cardiomyocytes, but also in the cardiac endothelium (Curtis and Pabla 1997) and intracardiac neurons (Clark et al 1994), it is difficult to specify which NOS is predominantly involved in the physiological and pathophysiological processes in the heart Therefore, the main goal of this minireview is to summarize the current knowledge about NOS produced by cardiac myocytes and to try to understand the mechanism of its action, more specifically the mutual interaction of NO, intracellular calcium, and consequently of Ca-transport systems

Production of NO

Over the past five years, a family of mammalian enzymes that produce NO, termed nitric oxide synthases (NOS), have been purified, cloned and characterized Up to now, three genetically distinct NOSs have been identified Constitutive NOSs have been originally described in neuronal tissue (nNOS or NOS1, Bredt et al 1991) and endothelial (eNOS or NOS3, Sessa et al 1992) cells, respectively Inducible NOS (iNOS or NOS2) was described for the first time in macrophages (Xie et al 1992) However, it was found that different NOS isoforms have much wider tissue distribution than originally appreciated Nitric oxide synthase enzyme forms NO and L-citrulline from the amino acid L-arginine via a complex oxidation-reduction reaction (Figure 1), requiring molecular oxygen and NADPH plus numerous cofactors (FAD, FMN, tetrahydrobiopterin), as well as the allosteric activator calmodulin (see below) All NOS isoforms have a high sequence similarity to cytochrome P-450 reductase and are unique because they are the only mammalian proteins

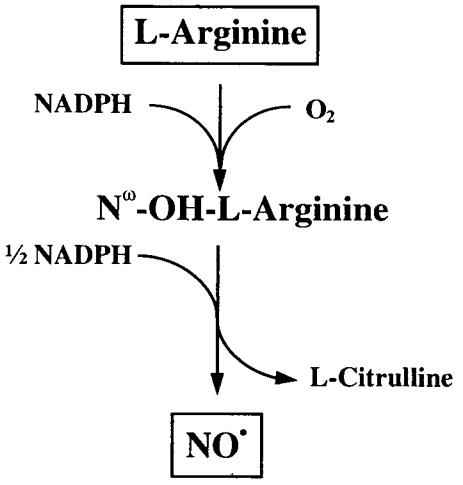

Figure 1. NO is produced from L-arginine and NADPH by the enzymes called nitric oxide synthases

that catalyze both hydroxylation reaction and NADPH reduction (Kelly et al 1996)

NOSs share an overall amino acid sequence identity of approximately 50% A major difference between the constitutive and the inducible isoforms was found in the binding of calcium and calmodulin Binding of calmodulin appears to act as a "molecular switch" to enable electron flow from flavin prosthetic groups in the reductase domain to heme, thereby facilitating the conversion of O_2 and L-arginine to citrulline In nNOS and eNOS, physiological concentrations of calcium in cells regulate the binding of calmodulin, thereby initiating electron transfer from the flavins to the heme moieties In contrast, calmodulin remains tightly bound to the inducible isoform termed iNOS even at low physiological intracellular calcium levels, acting essentially as a subunit of this isoform (Abu-Soud et al 1995, Su et al 1995) Although the activity of iNOS was originally believed to be Ca^{2+}/calmodulin independent, owing to its tight binding of calmodulin, recent studies with purified recombinant iNOS protein have indicated that iNOS is twice as active in the presence of calcium as in its absence (Venema et al 1996)

The availability of L-arginine or tetrahydrobiopterin (BH4) may be rate limiting for NOS activity in some cell types, particularly after induction of iNOS (Simmons et al 1996a) Arginine concentration might be regulated by arginase, which is present in the heart of some species (Aminlari and Vaseghi 1992), including humans (Baranczyk-Kuzma et al 1980) Availability of arginine or BH4 is increased by coinduction of the respective systems together with iNOS, which was found to occur in the heart (arginine arginine transporter, Simmons et al 1996a, arginosuccinate synthase, Nussler et al 1994, BH4 GTP cyclohydrolase Hattori et al 1997) Conversely, the expression of the enzyme systems that increase the availability of arginine and BH4 is decreased by inhibitors of iNOS induction (Simmons et al 1996b)

NOS isoforms

Neuronal nitric oxide synthase (nNOS; NOS1): NOS1 gene was assigned to the human chromosome 12q24 21 (Marsden et al 1993) and with the molecular weight of 160 kDa is the largest NOS The expression of nNOS has been proven in neuronal (Bredt et al 1991) and skeletal muscle tissue (Kobzik et al 1994) In the human brain two molecular variants have been described which differ in their respective first exon and which are transcribed from different, alternative promotors (Bachmann et al 1997) Cardiac myocytes do not appear to express significant amounts of nNOS but low levels of nNOS were recently found to be constitutively expressed also in this tissue (Silvagno et al 1996) In addition, generation of NO by nNOS present in intracardial neurons (Clark et al 1994) might be of a special importance (Horackova et al 1995)

Inducible nitric oxide synthase (iNOS, NOS2): iNOS gene was assigned to the human chromosome 17q11 2 (Kone and Baylis 1997) and maintains the molecular weight of 135 kDa *In vitro* studies have demonstrated that a wide variety of mammalian cell types are capable of expressing iNOS following profound immune or cytokine stimulation iNOS was induced *de novo* in macrophages, hepatocytes, mesangial cells and endothelial cells by various cytokines (Ishiyama et al 1997) *Induction of NO in myocardial tissue* For a long time a controversy persisted in the literature, whether cardiac myocytes are able to produce iNOS after the appropriate stimulus, or whether the effects of iNOS in the myocardium result from its expression in other heart cells, i e endothelium or macrophages *In vitro* studies have demonstrated that endotoxin and cytokines can stimulate isolated myocytes to produce iNOS (Schulz et al 1992, Luss et al 1995, McKenna et al 1995, Pinsky et al 1995 Kinugawa et al 1997) Induction of iNOS might depend on the developmental state of the myocyte, rat neonatal (Kinugawa et al 1997) and adult (McKenna et al 1995) myocytes in culture were shown to express iNOS mRNA and protein, while adult dedifferentiated human myocytes only the respective mRNA (Luss et al 1997) Ikeda and colleagues (1995,1996) have shown that both angiotensin II and the α-adrenergic agonist phenylephrine augment cytokine-induced iNOS expression in cultured cardiac myocytes *In vivo*, iNOS was expressed as mRNA and protein in human failing heart tissue and the iNOS protein was colocalized with desmin, thus suggesting myocyte localization (Haywood et al 1996) However, the iNOS message in rat heart, which can be induced by endotoxemia (Robinson et al 1997, Sun et al 1997), was shown to be preferentially expressed in non-muscle cells (Robinson et al 1997)

Luss et al (1997) have found that human adult dedifferentiated cardiac myocytes are capable of expressing iNOS mRNA This observation is consistent with those on the rat myocardial tissue and cardiac myocytes (Schulz et al 1992, de Belder et al 1993, Luss et al 1995, Pinsky et al 1995) However, iNOS enzyme assay and Western blot analysis for iNOS protein have shown that functional iNOS protein is not produced in such dedifferentiated preparation (Luss et al 1997) These data raised the possibility that in some human cell types, iNOS expression may be regulated at both the transcriptional and the translational level Selective inhibition of translation may represent an important mechanism of iNOS downregulation

Taken together, induction of iNOS in cardiac myocytes is probably involved in pathological processes such as myocardial infarction (Balligand et al 1994), and end-stage heart failure (Haywood et al 1996) It has been suggested that inducible iNOS is a part of primitive, but evolutionarily conserved inflammatory response (Geller et al 1993) The question arose, whether specific inhibition of iNOS will be of therapeutic benefit in the condition of vast overproduction of NO (Szabo et al 1994, Southan et al 1996a,b, Ishiyama et al 1997)

Endothelial nitric oxide synthase (eNOS, NOS3): eNOS was mapped to the human chromosome 7q35 36 (Marsden et al 1993) and the protein has a molecular weight of 133 kDa The primary structure of the full length eNOS lacks the NH_2-terminal amino acids present in nNOS (Kone and Baylis 1997) Recently eNOS has been discovered in diverse non-endothelial cells, including blood platelets hippocampal neurons and cardiac myocytes (Feron et al 1996)

Localization of eNOS in cardiac myocytes Cardiac myocytes express this isoform constitutively eNOS mRNA has been detected by RT-PCR in highly purified cardiac myocyte primary isolates (Balligand et al 1995) and in rat ventricular myocardium it was localized by *in situ* hybridization (Seki et al 1996) to myocytes and endothelial cells eNOS has been suggested to be involved in the physiological modification of myocardial contractility, mainly through parasympathetic signaling (Balligand et al 1995)

In cardiac myocytes as well as endothelial cells, eNOS has been shown to be targeted to specialized parts of the plasmalemmal membrane (Feron et al 1996) Recently it was shown that the amino terminus of eNOS contains a motif for myristoylation and reversible palmitoylation, modifications unique to eNOS in this gene family that contribute to its membrane association (Robinson et al 1995 Robinson and Michel 1995, Shaul et al 1996) Processing of the cytoplasmic 150-kDa isoform of eNOS to a palmitoylated 133-kDa isoform is essential for the targeting of eNOS to the caveolae, specific parts of the sarcolemma (Garcia-Cardena et al 1996) Mutagenesis of the motif, which prevents both myristoylation and palmitoylation, blocks eNOS targeting to caveolae (Shaul et al 1996) Elevation of intracellular cAMP inhibits transport of eNOS to the sarcolemma with concomitant protein kinase A dependent phosphorylation (Belhassen et al 1997) an effect that may have important implications for cardiac myocyte responsiveness to autonomic agonists *in vivo*

Chemical mechanisms of NO action

Nitric oxide can exert its effects by a variety of mechanisms The basic pathways of NO action which are important for calcium homeostasis in the myocardial cell are depicted schematically in Fig 2

Interaction with heme NO readily forms a complex with the heme, which makes it highly toxic if given in high concentrations (Ignarro 1996) One of the most important examples is the interaction of NO with guanylate cyclase, leading to the activation of this enzyme Two isoforms of guanylate cyclase are present in cardiac myocytes monomeric, membrane bound isoform and heterodimeric (soluble) isoform The monomeric isoform of guanylate cyclase is activated by atrial natriuretic factor (Rugg et al 1989), and is not regulated by NO The heterodimeric ($\alpha\beta$) isoform is activated by NO, which binds to the heme moiety in guanylate cyclase

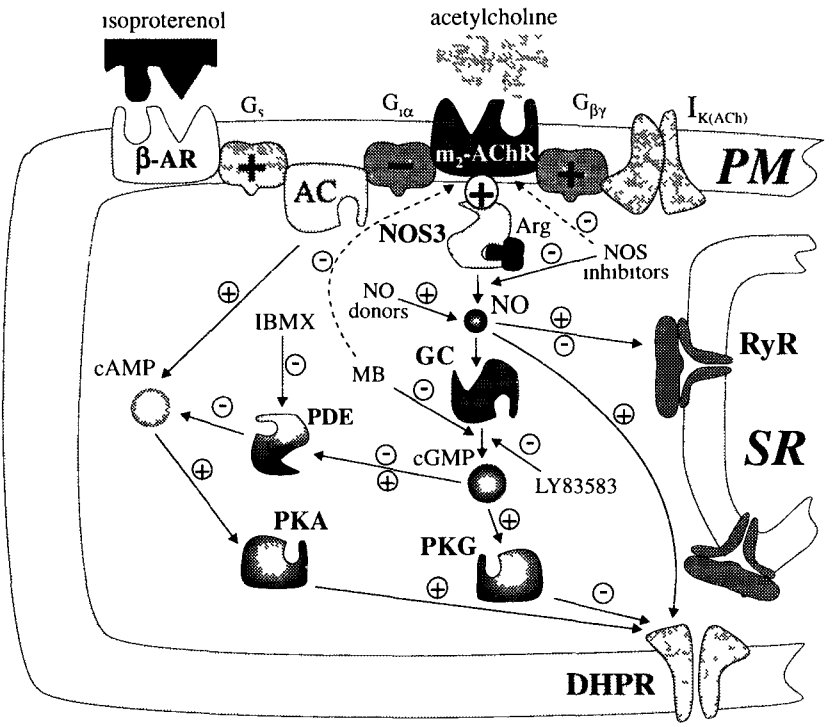

Figure 2. The principal pathways involved in regulation of calcium homeostasis in the cardiac myocyte by NO. PM – plasma membrane, SR – sarcoplasmic reticulum, NOS3 – endothelial type nitric oxide synthase, DHPR – dihydropyridine receptor, RyR – ryanodine receptor, β-AR – β-adrenoceptor, AC – adenylate cyclase, GC – guanylate cyclase, PDE – phosphodiesterase, PKG – cGMP-activated protein kinase, PKA – cAMP-activated protein kinase, MB – methylene blue. Side effects of drugs are shown as dashed arrows.

(Ignarro 1990, Stamler et al. 1992). The presence of this NO-sensitive isoform in the guanylate cyclase-rich membrane fraction was also demonstrated (Méry et al. 1993). Activation of guanylate cyclase in the cardiac cell has three sequelae: (i) activation of cGMP-dependent protein kinase, which inhibits I_{Ca} in mammalian cells (Levi et al. 1989, Méry et al. 1991), (ii) activation of the cGMP-stimulated cAMP-phosphodiesterase (cGA-PDE, type II, Beavo and Reifsnyder 1990), which inhibits cAMP-stimulated I_{Ca} (Hartzell and Fischmeister 1986, Fischmeister and Hartzell 1987), and (iii) inhibition of the cGMP-inhibited cAMP-phosphodiesterase (cGI-PDE, type III, Beavo and Reifsnyder 1990) which potentiates the effect of cAMP (Fischmeister and Hartzell 1990, 1991, Ono and Trautwein 1991). As the activation of the three above-mentioned enzymes has different effects on the cardiac calcium current, the overall effect produced by changes in the concentration

of NO depend on the activities of all the involved enzymes The inhibition of the cGI-PDE occurs at much lower concentrations of NO donors than the activation of the cGA-PDE (Méry et al 1993)

Other interactions with transition metals NO readily forms complexes with transition metal ions, including those found in metalloproteins Such iron-sulfur centers are found in mitochondrial enzymes (Stamler et al 1992) and therefore NO also modulates mitochondrial energetics (see Henry et al 1993, and the review in Kelly et al 1996)

Nitrosylation of thiols NO is not very reactive towards thiols, and this was postulated to give an intrinsic level of regulation to this pathway (Stamler 1994) The nitrosylation pathway utilizing reaction with O_2 or O_2^- is very slow under physiological conditions when NO $<$ 1 μmol/l and O_2 $<$ 200 μmol/l (Goldstein and Czapski 1996) Therefore, the most likely mechanism of nitrosylation by NO is one-electron oxidation to NO^+, which then acts as the nitrosating species (Stamler 1994)

Free radicals Being a radical, NO takes part in radical reactions in which superoxide and peroxide radicals are formed These mechanisms might be important for the role of NO in ischemia and NOS preconditioning They are extensively reviewed elsewhere (Stamler 1994, Gross and Wolin 1995, Kelly et al 1996)

Inhibition of NOS function

Numerous animal studies have demonstrated systemic and other effects of NOS blockade using L-arginine analogues These compounds act as competitive inhibitors of the enzyme, as they bind to the arginine binding site, but some of them may act as substrates which produce NO very slowly (Klatt et al 1994) They inhibit the conversion of L-arginine to citrulline, and thus NO production, in a dose-dependent manner, and mostly are not isoform-selective NO synthesis inhibitors include N-monomethyl-L-arginine (L-NMMA), N^G-nitro-L-arginine methyl ester (L-NAME), N-iminoethyl-L-ornithine (L-NIO), and N^G-nitro-L-arginine (L-NA) (Moncada 1992, Moore and Handy 1997) Some of these compounds have been shown to have a side effect of inhibiting also arginine transport, at least in neurons (Westergaard et al 1993) and endothelial cells (Bogle et al 1992), and they were shown to act as muscarinic receptor antagonists as well (Buxton et al 1993)

Isothiourea and thiazine derivatives are also potent competitive inhibitors of NOS, acting at the arginine binding site (Nakane et al 1995) Some of them, such as aminoethylisothiourea or S-methylisothiourea, are relatively selective towards the iNOS isoform (Szabo at al 1994, Jang et al 1996, Southan et al 1996a) Mercaptoalkylguanidines (Southan et al 1996b) and aminoguanidines (Ishiyama et al 1997) also serve as relatively selective inhibitors of iNOS The above compounds were shown to decrease the severity of the pathophysiological sequelae of excess NO

production, e g in experimental autoimmune myocarditis in rats (Ishiyama et al 1997) or after endotoxin treatment (Szabo et al 1994, Southan et al 1996b) Another iNOS-selective inhibitor is L-N6-(1-iminoethyl)-lysine (Stenger et al 1995)

Two of the inhibitors – 2-aminoethylthiazoline (Southan et al 1996b), and N^G-aminoarginine (Gross et al 1990) – display selectivity towards the eNOS isoform

The compound 7-nitroindazole belongs to relatively NOS-isoform-selective inhibitors for nNOS (Kone and Baylis 1997) This compound as well as other indazoles also inhibits the other two isoforms (Bland-Ward and Moore 1995), and apparently the selectivity is achieved by selective uptake of this compound by neuronal cells (Southan and Szabo 1996) In contrast to other NOS inhibitors, indazole derivatives bind to the heme moiety in the NOS enzyme and block the conversion of O_2 (Wolff and Gribin 1994)

Effects of NO on the L-type Ca channel

Effects through the guanylate cyclase In frog, where activation of the cGMP-stimulated phosphodiesterase is prominent, NO generated from SIN-1 in higher doses seems to exert its effect by this pathway on the forskolin- or cAMP-stimulated I_{Ca} (Mery et al 1993) Low doses of NO stimulated I_{Ca} elevated by non-maximal doses of forskolin via the cGMP-inhibited cAMP phosphodiesterase However, the same authors were not able to demonstrate the effect of *in situ*-activated NOS (Méry et al 1996) To our knowledge, expression of NOS in frog heart myocytes has not been studied, although the presence of NOS was shown in intracardial ganglion neurons (Clark et al 1994), suggesting a possible role for NO regulation of frog heart function consistent with the findings of Mery et al (1993, 1996)

In addition, in mammalian cells, NO also affects the cGMP-dependent protein kinase and thus inhibits I_{Ca} (Wahler and Dollinger 1995)

Direct effects L-type Ca channel has been shown to be activated by NO donors in a guanylate cyclase-independent way (Campbell et al 1996) The molecular substance producing activation was probably peroxynitrite, as the effect was inhibited by addition of superoxide dismutase Nitrosothiols produced a similar activation, while thiol reducing agents inhibited I_{Ca} Therefore, S-nitrosylation and/or disulfide reactions were proposed as the mechanism of I_{Ca} activation

Effects of NO on other calcium transport systems

The cardiac ryanodine receptor (RyR) is subject of phosphorylation (Hain et al 1995), and has several reactive cysteins whose redox state is important for the function of RyR (Pessah et al 1990) Thus, RyR is a likely candidate for both, direct modulation by NO (through modification of thiol groups) or indirect modulation (through the extent of RyR phosphorylation) Yet, reports on the effect of NO on

RyR are scarce and controversial Using relatively high concentrations of NO and NO donors, Stoyanovsky et al (1997) demonstrated activation of calcium release from cardiac sarcoplasmic reticulum (SR) vesicles, as well as an increase in RyR open probability at basal levels of intracellular calcium Conversely, a reduction in open probability of the cardiac RyR was found after application of low concentrations of NO donors, and furthermore, also by *in situ* activation by L-arginine of the eNOS present in the SR membrane preparation, at elevated levels of calcium at the cytoplasmic face of the membrane (Zahradníková et al 1997) In both reports, the effects of NO or NO donors were ascribed to a direct action on reactive cysteins of the RyR, as they were reversed by addition of thiol-containing reagents

Interestingly, there are also contradictory reports on the effects of NO on the large-conductance Ca^{2+}-activated potassium (BK-Ca) channels These channels don't seem to be present in the cardiac myocytes, but they are prominent in both vascular and endothelial cells In smooth muscle they play an important role in the regulation of vascular tone by calcium-dependent mechanisms (Nelson et al 1995) Whereas in vascular myocytes BK-Ca channels were shown to be directly (Bolotina et al 1994) or indirectly (George and Shibata 1995) regulated by NO, no such a regulation was observed in endothelial cells (Haburčak et al 1997) The degree of homology between the endothelial and the vascular BK-Ca channels is not known so that the difference in direct NO action between the two preparations cannot be correlated to eventual structural differences between channel isoforms

NO stimulates the Na^+-Ca^{2+} exchanger in neuronal preparations and astrocytes via a cyclic GMP-dependent mechanism (Asano et al 1995, Takuma et al 1996) However, the effect of NO on the cardiac Na^+-Ca^{2+} exchanger remains to be studied

Effects of NO on myocardial contractility

Induction of iNOS can increase production of NO in cardiac myocytes, resulting in attenuated contractile function of cardiomyocytes (Balligand et al 1994) NO generated by the iNOS isoform induced by endotoxin caused attenuation of the inotropic response of the whole heart to isoprenaline (Sun et al 1997) this effect was prevented with dexamethasone and inhibited by L-NA (but not by D-NA) or mercaptoethylguanidine (a selective iNOS inhibitor) No effects on the baseline contractile parameters were observed in this work although in another report, endotoxin (lipopolysaccharide, LPS) induced iNOS was described to decrease spontaneous beating rate, contraction amplitude, and peak systolic Ca in cultures of rat myocytes (Kinugawa et al 1997)

Even if NO was shown to regulate the calcium current as well as other calcium transport systems in the myocardial cells, studies of the role of NO in altering myocardial contractility without iNOS expression have produced conflicting results

No effect of NOS inhibitors on spontaneous myocardial contractility (Klabunde et al 1992 Thelen et al 1992) was found, although there was a decrease in the basal cGMP concentration (Klabunde et al 1992) On the other hand activation of the constitutive NOS was shown to increase the production of NO in cardiac myocytes concomitantly with inducing a depression of myocardial contractility (Balligand et al 1993) Furthermore, both potentiation (Balligand et al 1993) and reduction (Klabunde et al 1992) of the effect of β-adrenergic agonists were observed as the result of NO generation NOS inhibitors were also observed to be able to reverse the effect of muscarinic agonists (Balligand et al 1993) This effect could be ascribed to their antimuscarinic properties (Buxton et al 1993) only partially, as addition of L-arginine partially restored the response to carbachol (Balligand et al 1993) It was demonstrated that in isolated myocytes, NO donors as well as muscarinic agonists increased the levels of both cGMP and cAMP At the same time muscarinic agonists decreased contractility, while NO donors had no effect (MacDonell et al 1995)

The above mentioned contradictions might stem from several reasons Some of the effects ascribed to inhibition of NOS by arginine derivatives or to inhibition of guanylate cyclase by methylene blue could actually be caused by the antimuscarinic actions of these compounds (Buxton et al 1993, Abi-Gerges et al 1997) Moreover, the preparations in the "basal" state might differ in their overall levels of individual enzymes taking part in the response In this respect, the action of NO might depend on the developmental status of the myocytes It was shown that in newborn rat heart, the ability of both, cGA-PDE and cGI-PDE, to be regulated by cGMP is markedly reduced as compared to adult hearts (Picq et al 1995) Therefore, the PDE-mediated effects of NO might be much less prominent in newborn animals Another factor might be the localization of proteins involved in NO actions *in vivo* The subcellular localization of eNOS (Feron et al 1996) is regulated by cAMP (Belhassen et al 1997), the concentration of which is altered by two NO-regulated processes The muscarinic cholinergic receptor, to which eNOS activation is coupled, is translocated to the same plasma membrane site upon binding of muscarinic agonists (Feron et al 1997), and this translocation is prevented by muscarinic antagonists In addition, the localization of the NO-sensitive guanylate cyclase in the plasma membrane (Méry et al 1993) as well as the membrane localization of the cGI-PDE (Weishaar et al 1987) might be important to produce the *in vivo* effect

Nitric oxide and pathological conditions in heart

From the current knowledge it is clear that the dysregulation of NO production in the heart can raise serious pathological consequences Dysregulation of constitutive NOS or induction of iNOS may play a role in the etiology and pathogenesis

of several cardiovascular disorders NO overproduction has been linked to primary vascular and inflammatory diseases, including end-stage heart failure (Haywood et al 1996), myocardial infarction (Balligand et al 1994), acute inflammation, hypertension, atherosclerosis, and the myocardial depression associated with septic shock (Kiechle and Malinski 1993, Gross and Wolin 1995) On the other hand, NO deficiency caused by reduced NO synthesis or increased NO degradation may contribute to chronic hypertensive disease (Gross and Wolin 1995), and thus indirectly to cardiac hypertrophy Nitric oxide contributes to both protective and impairing processes during ischemic/reperfusion injury (Gross and Wolin 1995)

The pathological effect of NO on the cardiac myocytes is brought about through the endothelial or inducible nitric oxide synthase overproduction in the myocyte The microvascular endothelium, which is in a close proximity to cardiac myocytes may influence myocardial contractility through NO release as well Basal release of NO and consequent modest elevation in cGMP may preserve myocardial function while large increases would depress the myocardial contractility Under pathological conditions, overproduction of nitric oxide by an inducible nitric oxide synthase may be detrimental for contractile function (Balligand et al 1994)

Low concentrations of endogenous NO are useful in reducing myocardial injury Higher concentrations of endogenous NO that are produced in response to cytokine stimulation have been reported to have negative inotropic effects on the heart (Finkel et al 1992, Barry 1994)

These observations show that the mechanism of NO action on the cardiac tissue is very intricate The research in this area therefore opens still new perspectives

In summary there is ample evidence that the NO producing enzymes are present and functional in cardiac myocytes as well as in non-muscle cells of the myocardial tissue The existence of several targets of NO action in heart muscle cells is also clearly established Among these the mechanisms by which NO modulates the activity of guanylate cyclase are most clearly understood In spite of this understanding the exact nature of the regulation of myocardial contractility by NO remains a controversial issue Part of the contradictions encountered might be caused by the local nature of NO action and by the dynamics of cellular localization of the proteins that compose the NO pathway

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