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Review

Regulation of basal myocardial function by NO

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1. Introduction

Nitrogen monoxide (nitric oxide, ˙NO) represents an important mediator within the cardiovascular system. Chemically, ˙NO is a lipophilic, highly reactive and unstable gaseous molecule. It is liberated from organic nitrates and other nitrovasodilators as the pharmacologically active principle of these drugs and has a therapeutic potential that has been in use for decades [1]. Ten years ago, it became evident that ˙NO is produced by different ˙NO-synthase (NOS) isozymes in the body [2]. Two of these enzymes, the endothelial (eNOS) and the neuronal (nNOS) isoform are constitutively expressed, while the inducible isoform (iNOS) is biosynthesized only after stimulation of cells with *Escherichia coli* lipopolysaccharide or cytokines such as interferon γ or tumour necrosis factor α [3].

Endogenous production of ˙NO is involved in a variety of physiological functions, such as local and systemic regulation of vascular tone, regulation of immune defense, synaptic plasticity and other neurologic functions [4–6]. Under certain pathological conditions, endogenous ˙NO synthesis can considerably increase and cause serious organ damage. Deleterious ˙NO production may contribute to cardiovascular failure in sepsis and to neurotoxicity following reperfusion after stroke [7–9]. By contrast, other pathologic conditions have been shown to be associated with a decreased capacity of normal vascular ˙NO synthesis or an impairment of its physiologic effects as measured by blunted endothelium-dependent vasorelaxation. These conditions include coronary artery disease, hypertension, diabetes and heart failure [4].

In contrast to numerous investigations on the effects of ˙NO on vascular smooth muscle, its actions on the cardiac muscle and the conduction system of the heart has only recently attracted scientific interest. This review summarizes the present knowledge about effects of exogenous and endogenous ˙NO on myocardial functions such as contraction, relaxation and beating rate. It is focused on investigations of isolated cells, tissues and in vivo set-ups in animals and man under basal conditions. The interactions between sympathoadrenal stimulation of the heart and myocardial effects of ˙NO, which have already been extensively reviewed [10,11], are discussed only briefly.

2. Exogenous ˙NO

Most of the present knowledge about the effects of exogenous ˙NO on cardiac functions stems from investigations with ˙NO donors. There are however important differences between the members of this class of drugs regarding the mechanism and kinetics of ˙NO release and the generation of toxic side products during degradation. The group of nitrovasodilators comprises compounds with very heterogeneous chemical structures, such as organic nitrates and nitrites, 3-morpholinosydnonimine (SIN-1), nitrosothiols such as *S*-nitroso-*N*-acetyl-D,L-penicillamine (SNAP), sodium nitroprusside (SNP) or N-nitroso derivatives, such as (*Z*)-1-(*N*-[3-aminopropyl]-*N*-[4-aminopropylammonio)butyl]-amino)diazen-1-ium-1,2-diolat (SPER/NO˙) or sodium (*Z*)-1-(*N*,*N*-diethylamino)diazen-1-ium-1,2-diolat (DEA/˙NO).

2.1. ˙NO, ˙NO-donors, organic nitrates

Exogenous ˙NO, delivered by organic nitrates, has been used for more than 100 years as a remedy to relieve pectanginal pain [12]. By contrast, the ˙NO-donating properties of these drugs were discovered much later [1]. The formation of ˙NO from organic nitrates such as glyceryl trinitrate (GTN), isosorbide-5-nitrate (ISMN),

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isosorbide-2,5-dinitrate (ISDN) or pentaerythritol tetranitrate (PETN) in tissues requires denitration by membrane-bound enzymes [13]. A similar situation holds true for SNP, which has been shown to release bioactive ˙NO in tissues only after enzymatic metabolization, although through a different type of enzyme [14]. The SNP molecule contains five moles of cyanide anion per mol of ˙NO. Thus, formation of cyanide anions during degradation of SNP is not surprising and is known to limit the clinical use of this drug [15]. This toxic side product complicates the interpretation of experimental and clinical data, since cyanide anions can impair the generation of adenosine trisphosphate (ATP) by inhibition of ferrocytochrome oxidase of the respiration chain. The release of cyanide from SNP also inhibits the biosynthesis of catecholamines [16]. Another clinically used nitrovasodilator, molsidomine, undergoes hepatic metabolization to form the active metabolite SIN-1. In vitro, spontaneous degradation of SIN-1 leads to the formation of equimolar amounts of both ˙NO and superoxide radicals [17]. In vivo, the superoxide radicals generated by SIN-1 are most likely scavenged by the abundantly present enzyme superoxide dismutase, leaving ˙NO as the mediator of the well known vasodilator properties of SIN-1.

Other ˙NO donors, aqueous ˙NO solutions and ˙NO gas are mainly used in experimental medicine. There are substantial differences between these application forms of ˙NO. Very high concentrations of ˙NO (100 μM) can be achieved by application of aqueous ˙NO solutions. However, these solutions are rarely used because of their chemical instability in the presence of light and oxygen [18]. Free ˙NO rapidly reacts with other radicals to form toxic side products, such as peroxynitrite [19]. The rapid generation of peroxynitrite from ˙NO and superoxide implies that a delayed release of ˙NO from ˙NO donors prevents a rapid increase of the peroxynitrite concentration. Furthermore, a considerable part of the ˙NO-donor molecules diffuses to the target cells before ˙NO is released. This reduces the probability of oxidation of ˙NO before induction of pharmacological actions. Nitrosothiols such as SNAP can generate ˙NO in the presence of trace amounts of the heavy metals that are present in normal buffer solutions [20] or by transnitrosation reactions with free thiols, yielding rapidly degrading nitrosothiols [21]. Interestingly, there is one report about the therapeutic use of nitrosothiols to treat the HELLP syndrome [22]. By contrast, N-nitroso derivatives, the so-called ˙NOates, are exclusively used in experimental medicine. DEA/NO and SPER/NO are stable in alkaline solutions but spontaneously degrade with different half-lives at physiological pH, giving a total of 2 mol of ˙NO per mol of compound [23]. So far, there is no evidence demonstrating the generation of toxic or any other interfering side products during the spontaneous degradation of these compounds. Another advantage of ˙NOates is their well-defined half-life in aqueous solutions under physiological conditions.

2.2. Mechanisms of action of ˙NO in cardiac muscle

The major target of ˙NO in the cardiovascular system is the soluble guanylate cyclase [1]. This holds true for almost any cell type in which exogenous ˙NO can elicit a response. Alternatively, ˙NO can circumvent soluble guanylate cyclase and affect other cellular structures such as transmembrane ion channels or mitochondrial enzymes [24,25]. The various ˙NO-signalling pathways differ substantially in their sensitivity to ˙NO and their influence on myocardial function (Fig. 1, Table 1).

2.2.1. cGMP-dependent ˙NO-signalling pathways

Activation of soluble guanylate cyclase results in conversion of guanosine trisphosphate (GTP) to the second messenger cyclic guanosine monophosphate (cGMP) [26]. The enzyme has been detected in many different cell types throughout the body and is most abundant in smooth muscle, neurones and platelets. It is also expressed in cardiomyocytes of animals and man [27–29]. Once biosynthesized, cGMP binds to different effector proteins. In mammalian cardiomyocytes, the most important effector proteins are cGMP-dependent protein kinase (PKG) and cGMP-inhibited cAMP–phosphodiesterase (PDE III) [27].

Investigations in tissues of rat, guinea pig and frog have shown that stimulation of PKG results in inhibition of voltage-dependent calcium channels. This effect was evident at high concentrations ($>$10 μM) of cGMP and/or cGMP analogs [30–34]. The underlying mechanism of action remains to be elucidated. Results of an earlier study provided evidence for the involvement of phosphorylation of voltage-dependent calcium channels [35]. A further investigation performed in frog cardiomyocytes using the whole-cell patch–clamp technique showed that inhibition of voltage-dependent calcium channels induced by ˙NO and mediated by cGMP is independent of membrane potential and is not associated with a modification of the inactivated state of the channel [36]. These observations suggest a mechanism of action that is different from that of dihydropyridine calcium channel blockers, such as nifedipine. An additional and different PKG-dependent mechanism was evaluated by Shah et al. [37], who proposed that stimulation of PKG mediates a desensitization of cardiac myofilaments to calcium. Finally, PKG might mediate cardiac effects of ˙NO by impairment of receptor-operated transmembrane and sarcoplasmatic calcium channels [38], but so far, there are no data supporting this hypothesis.

The second major target protein of cGMP is the PDE III [27]. This enzyme is an important type of phosphodiesterase in mammalian cardiomyocytes [39]. Activation of PDE III has been observed at low concentrations of cGMP ($<$1 μM). Binding of cGMP to PDE III reduces the activity of the enzyme and subsequently results in a measurable increase of the cAMP content of cardiomyocytes [40]. Earlier investigations in frog and guinea

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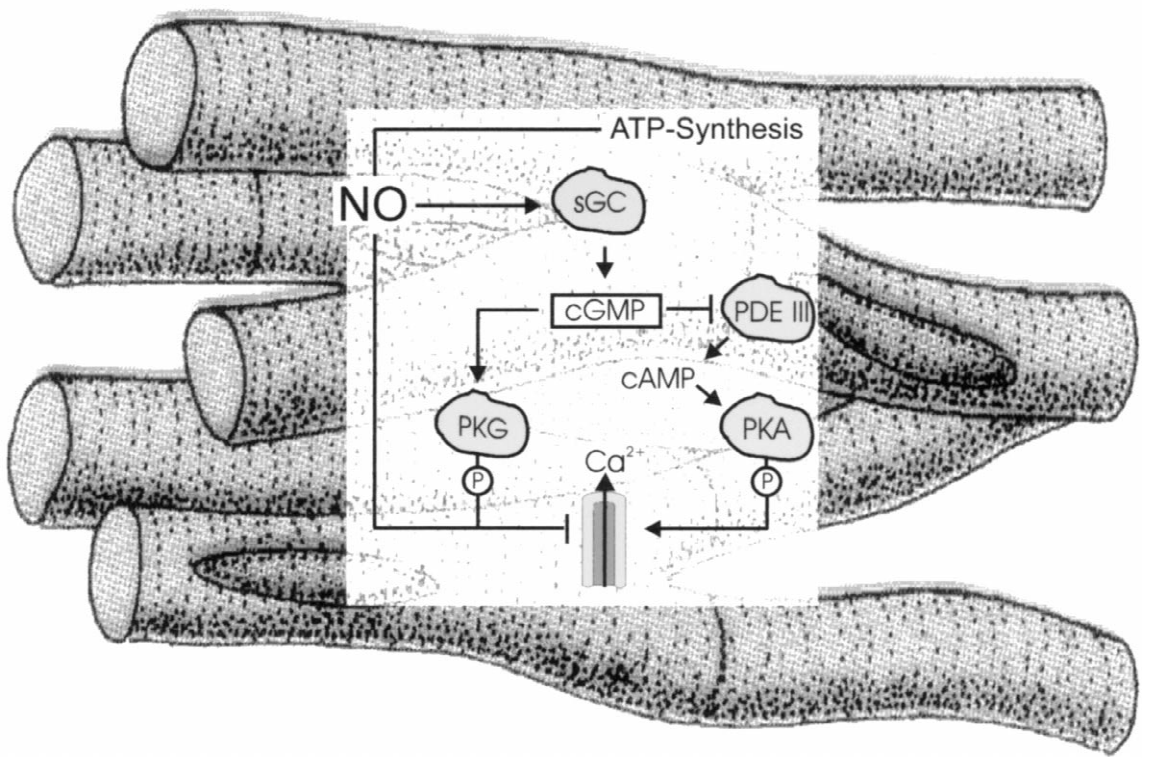

Fig. 1. Mechanism of action of ˙NO in the myocardial muscle. Activation of soluble guanylate cyclase (**sGC**) and subsequent formation of cGMP can result in both (1) inhibition of cGMP-inhibited cAMP–phosphodiesterase (**PDE III**), accumulation of cAMP and stimulation of cAMP-dependent protein kinase (**PKA**), promoting calcium entry via L-type calcium channels and (2) activation of cGMP-dependent protein kinase (**PKG**), inhibiting calcium entry via L-type calcium channels. ˙NO is also able to directly inhibit L-type calcium channels or to diminish mitochondrial ATP synthesis.

pig cardiomyocytes have shown that inhibition of PDE III initiated by cGMP stimulates transmembrane calcium current [33,36]. A similar effect was shown to occur in myocytes derived from human atrial tissue [28]. Interestingly, inhibition of PDE III by 8-bromo-cGMP in intact tissues is unlikely, because this widely used cGMP analogue is a ten-fold stronger stimulator of PKG but a 60-fold weaker inhibitor of PDE III compared with cGMP [41].

2.2.2. cGMP-independent ˙NO-signalling pathways

NO might influence the function of cardiac muscle by signalling pathways that circumvent the generation of cGMP by soluble guanylate cyclase [10]. Indeed, two recent studies provided evidence for such pathways in cardiomyocytes. Investigations of the oxygen consumption of isolated dog ventricular tissue have shown that the ˙NO donor SNAP can inhibit cellular respiration [25]. This effect, which occurs at a SNAP concentration below 1 μM,

Table 1
Concentration-dependent action of ˙NO on myocardial contractility

Half maximal effective ˙NO concentration	Mediator	Cellular target	Inotropic action	Reference
0.05 μM	cGMP	cGMP-inhibited cAMP–phosphodiesterase	Positive inotropic action (via increased cAMP-level in cardiomyocytes)	[28,34,36,40]
10 μM	˙NO (cGMP)	Unknown	Negative inotropic action (via inhibition of mitochondrial electron transport)	[25]
>10 μM	cGMP	cGMP-dependent protein kinase	Negative inotropic action (via inhibition of voltage-dependent calcium channels)	[30–35,38]
>100 μM	˙NO	L-type calcium-channel	Negative inotropic action (via inhibition of voltage-dependent calcium channels)	[42]

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was suggested to be the consequence of an inhibition of mitochondrial electron transport. Another signalling pathway that was independent of cGMP was reported by Hu et al. [42]. In transfected cells expressing cardiac voltage-gated calcium channels, treatment with the ·NO donor SNAP resulted in a cGMP-independent inhibition of calcium currents. This inhibition was concentration-dependent and the half maximal effective concentration was >100 μM.

2.3. Effects of exogenous ·NO on myocardial contraction

Some evidence for the suggestion that ·NO might have effects on myocardial contraction is based on observations that have been made more than 25 years ago. Raff et al. [43] demonstrated a direct positive effect of GTN in the dog in vivo and Strauer [44] was able to reproduce this effect in isolated human ventricular myocardium. The initial results were confirmed by others who showed the positive inotropic effects of SNP in cat papillary muscle [45]. On the other hand, it was shown that cGMP can reduce the transmembrane calcium flux in cardiomyocytes, which is expected to induce a negative inotropic effect [34].

2.3.1. In vitro studies

Investigations in isolated rat cardiomyocytes using SNP showed that exogenous ·NO is capable of reducing the contractile response of ventricular muscle to electrical field stimulation [46]. A similar result was obtained after subjection of the same cell type to high concentrations of various ·NO donors, giving ·NO concentrations of approximately 30 μM [40]. The ·NO donors induced a concentration-dependent increase in cGMP levels in cardiomyocytes. Inhibition of PKG not only blunted the negative inotropic effect of high concentrations of ·NO donors but reversed it to a positive inotropic effect. In addition, 100-fold lower concentrations of the ·NO donors also amplified the contractile response. The positive inotropic responses to ·NO donors were completely abolished after inhibition of cAMP-dependent protein kinase, suggesting a cAMP-dependent mechanism of action. These results indicate that a small increase in cGMP induced by nitrovasodilators improves the contractility of myocardial muscle, while a large increase in cGMP has the opposite effect. However, the inotropic effects of ·NO in isolated cardiomyocytes were small compared to the effects of catecholamines.

Small effects of ·NO on myocardial contractility were also found in various studies with isolated cardiac muscle and Langendorff preparations. Investigations in rat papillary muscle with ·NO concentrations below 1 μM showed a small increase in twitch tension between 12 and 15% [40]. In accordance, maximal inotropic effects of ·NO donors, not exceeding a 15% increase in contractility, were observed in rat constant-flow Langendorff preparations and cat papillary muscle [47,48]. Subjection of rabbit papillary muscle to a very high concentration (100 μM) of ·NO in aqueous solution induced a maximal reduction of twitch tension, of approximately 20% [49]. A similar situation was found in preparations of human atrial and ventricular myocardium, where high concentrations of SNP induced a maximal negative inotropic effect of 10–15% [50]. In this study, lower concentrations of ·NO donors, which increase calcium entry in human atrial myocytes [28], have not been investigated. However, earlier studies in human ventricular myocardium demonstrated small positive inotropic effects of submicromolar concentrations of GTN [44].

2.3.2. In vivo studies

In vivo, direct inotropic effects of ·NO on myocardial contractility are overshadowed by the vascular actions of nitrovasodilators leading to hemodynamic changes that alter contractility by various mechanisms, such as Frank-Starling and baroreceptor reflex. Thus, investigations on the direct inotropic effects of ·NO require the measurement of contractility shortly after the regional application of ·NO donors, to avoid artefacts induced by systemic effects. By recording changes in regional contractility after the intracoronary application of ·NO donors, Preckel et al. [51] were able to demonstrate a direct positive inotropic effect of ·NO in the dog in vivo. Another methodological approach is to compensate for the nitrate-induced hemodynamic effect by volume loading before measurement of changes of myocardial contractility is performed. Using this method, direct positive inotropic effects of GTN were found in dogs and patients with coronary artery disease long before it was known that this drug is a ·NO donor [43,52]. Another clinical study described positive inotropic effects after intracoronary application of GTN in the absence of volume loading [53]. Overall, the positive inotropic effect of ·NO in vivo is as small as that observed in vitro. By contrast, there is no evidence allowing us to conclude that exogenous ·NO can diminish myocardial contractility (dP/dt_{max}) in vivo.

2.4. Effects of exogenous ·NO on myocardial relaxation

Exogenous ·NO has been shown to accelerate myocardial relaxation. The first evidence for a lusitropic action of ·NO derived from studies with ferret papillary muscle [54]. The relaxation-hastening effect also induced a reduction in peak tension developed during ventricular contraction but had no effect on dP/dt_{max}. A lusitropic action was also seen after direct activation of PKG by 8-bromo-cGMP. Further investigations in isolated cardiomyocytes, guinea pig working hearts and constant flow perfused rat hearts confirmed these interesting results [37,55,56]. A similar relaxation-hastening effect was observed in patients with coronary artery disease after intracoronary infusion of SNP

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[57]. The underlying mechanism might involve an activation of PKG, which desensitizes cardiac myofilaments to the effects of calcium [37]. The lusitropic effect of ˙NO is small, as already noted for the direct inotropic effects.

3. Endogenous ˙NO

3.1. Myocardial ˙NO-synthases

During the last few years, it has become evident that cardiomyocytes can express two isoforms of NOS, eNOS and iNOS. In general, NOSs convert L-arginine to citrulline and ˙NO. The first evidence for a myocardial activity of NOSs was published by Schulz et al. [58] who showed that cardiomyocytes isolated from normal rats convert L-arginine to citrulline, indicating the presence of a constitutive NOS. Furthermore, pretreatment of rats with various cytokines for 6 h resulted in a great increase in calcium-independent NOS activity, which is typical for induction of iNOS. Another study confirmed and extended these results by demonstrating that treatment of isolated guinea pig cardiomyocytes with endotoxin decreases their contractile response to electrical field stimulation and that this effect could be reversed by pharmacological inhibition of NOS [59]. These investigations strongly indicated the existence of at least two different isoforms of NOS in ventricular myocardium. Further detailed studies by Balligand et al. [60,61] in rat isolated cardiomyocytes provided evidence for the constitutive expression of eNOS and for induction of iNOS expression following treatment with cytokines.

Normal cardiomyocytes selectively express eNOS, while expression of iNOS is detectable only after incubation with various cytokines [10]. These two isoforms of NOS show several major differences [3]. The activity of eNOS is strictly dependent on the cytosolic calcium concentration and the enzyme produces ˙NO in nanomolar concentrations. By contrast, iNOS produces ˙NO in the micromolar concentration range and its activity is not regulated by the cytosolic calcium concentration. The intracellular concentration of ˙NO in cardiomyocytes as an indicator of the activity of eNOS is unknown. A recent study in normal isolated rat cardiomyocytes showed a concentration of ˙NO at its cell surface that was below 150 nM [62]. Interestingly, exposure of the cells to β-adrenergic agonists increased endogenous ˙NO production and resulted in a cell surface concentration of 700 nM ˙NO, indicating a β-adrenergic stimulation of eNOS activity. Other investigators measuring cGMP as an indicator for ˙NO production in rat cardiomyocytes showed a ten-fold increase in cGMP levels after stimulation with either bradykinin or acetylcholine [63]. These results suggest that several mediators can induce a stimulation of basal eNOS activity in cardiomyocytes.

3.2. Effects of endogenous ˙NO on myocardial contraction

The results of several investigations in isolated cardiomyocytes, multicellular myocardial preparations and in vivo models suggest that the activity of myocardial NOSs plays a role in the regulation of myocardial contractile function. Two recent reviews have comprehensively summarized the interactions with the autonomic control of myocardial function [10,11]. This review focuses on the effects of myocardial ˙NO production under basal conditions.

3.2.1. Endothelial ˙NO-synthase

Investigations in rat and guinea pig isolated cardiomyocytes have shown a basal NOS activity [58] but failed to detect an influence on the contractile response to electrical field stimulation [59,64]. By contrast, studies in spontaneously beating or paced multicellular preparations of ventricular myocardium showed that pharmacological inhibition of NOS decreases myocardial contractility. This effect was elicited by different NOS inhibitors in different experimental settings, such as the rat heart in vitro [48,65] and in vivo [66] and in the dog heart in vivo [67–69]. The effect of the NOS inhibitors were concentration-dependent and the maximal reduction of myocardial contractility varied between 5 and 25%. If endogenous NO production by a constitutive NOS affects myocardial contractility, one would suggest that at least a part of these effects are mediated by cGMP generated upon stimulation of soluble guanylate cyclase (Fig. 1). Recently, a new and specific inhibitor of soluble guanylate cyclase, the compound ODQ, has become available [70]. The effects of this drug on myocardial contractility have been investigated in a constant flow perfused Langendorff preparation of the rat heart. In these experiments, ODQ induced a marked reduction of myocardial contractility at a low concentration (0.1 μM), suggesting that cGMP production stimulated by basal NOS activity supports the development of myocardial contraction [48]. Investigations in patients with coronary artery disease have shown that pharmacological inhibition of NOS induces a reduction in cardiac output that cannot be explained by the concurrent increase in blood pressure, as evidenced by phenylephrine infusions [71]. In summary, these studies in animals and man provided evidence for the existence of a basal activity of endothelial NOS in the ventricular myocardium that results in a mild to moderate positive inotropic effect.

However, the results of investigations on the influence of basal endogenous ˙NO production are not homogeneous. Other studies in isolated cardiomyocytes, isolated papillary muscles and in vivo preparations failed to detect any influence on myocardial contraction or obtained opposite results. The contraction amplitude of hamster papillary muscles decreases with increasing beating rate. Inhibition

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of NOS enhanced the contractile response of these muscles at any frequency, although the effect declined with increasing beating rates [72]. A similar amplification of the contraction amplitude after inhibition of NOS was observed in isolated rat cardiomyocytes [73]. Another study in the dog heart in vivo reported that the NOS inhibitor N^G-monomethyl-L-arginine (L-NMMA) had no effect on baseline myocardial contractility [74]. These results indicate that basal endogenous ˙NO production inhibits rather than supports myocardial contraction. The reason for this apparent discrepancy is unknown. It might be related to species differences or experimental conditions, such as culturing techniques that might influence the basal expression of NOSs, the time point of measurement of contractile effects, the technique of electrical field stimulation, including stimulation frequency, possible counter-regulating hemodynamic changes, the remaining degree of autonomic control or effects on coronary perfusion. Another explanation would be that the expression or activity of PDE III, which is essential to mediate positive inotropic effects of ˙NO (Fig. 1), varies depending on the species and experimental conditions.

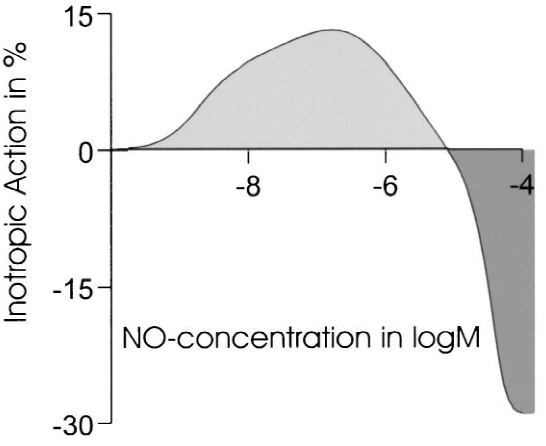

Fig. 2. Scheme of the effects of exogenous ˙NO on myocardial contractility under basal conditions. The effects of ˙NO are summarized from the results of a variety of investigations (see chapter 2.3) and are given as the percentage variation in contractile parameters such as dP/dt_{max} or maximal left ventricular pressure. Overall, the maximal positive inotropic effects of ˙NO under basal conditions are observed at physiological concentrations. These effects are small compared to the inotropic effects of catecholamine. By contrast, the negative inotropic effects of ˙NO are somewhat more pronounced but occur only at very high concentrations.

3.2.2. Inducible NOS

Myocardial expression of iNOS can be stimulated by treatment with cytokines or *E. coli* lipopolysaccharide [58–60,64,75]. Subsequent to iNOS expression, the reactivity of the myocardial muscle changes and a decrease in basal myocardial contractility [59,75,76], a diminished response to β-adrenergic stimulation [60,64] and higher rate of cell deaths have been observed [77]. These observations suggest that iNOS expression strongly increases the intracellular concentration of ˙NO in cardiomyocytes, leading to an inhibition of myocardial contraction. This interpretation is consistent with cardiodepressive effects that occur at high concentrations of exogenous ˙NO (Fig. 2). A more direct prove of this hypothesis would be the detection of large amounts of ˙NO production in cytokine-treated cardiomyocytes [62].

As already described for eNOS, the results of investigations on the influence of iNOS on myocardial contraction are not homogeneous. There is evidence for myocardial depression induced by cytokines that could be abolished by NOS inhibitors, although the effects were observed after a 30-min incubation period, which almost excludes expression of iNOS as an underlying cause [78]. The mechanism of this effect of cytokines is not known and there is no evidence that cytokines directly activate NOSs to produce ˙NO. A similar situation seems to hold true for lipopolysaccharide. Treatment of guinea pigs with lipopolysaccharide can induce a strong decrease in left ventricular contractility in the absence of any detectable expression of iNOS [79]. Similar results have been obtained in an experimental model of heart failure. Isolated constant flow perfused hearts of aged 'stroke-prone' spontaneously hypertensive rats with significant hemodynamic and humoral signs of severe heart failure and with massive myocardial hypertrophy showed a strongly inhibited response to catecholamines but a completely identical response to ˙NO donors and NOS inhibitors compared to control animals [56].

In spite of this apparent diversity, it is attractive to assume that expression and activity of iNOS may be involved in the development of contractile dysfunction of the ventricular myocardium in different diseases and evidence for this interesting hypothesis has been summarized recently [10,11,80]. Accordingly, expression of iNOS may play a significant role in heart failure or rejection after transplantation. In transplanted hearts, the reduction of the inotropic response to the β-mimetic dobutamine correlates with the degree of myocardial iNOS expression [81]. Expression of iNOS has also been detected in different forms of heart failure, but the results are somewhat contradictory. In the human myocardium, a significant expression of iNOS was found in dilatative cardiomyopathy and myocarditis, as evidenced by histology and mRNA-level quantitation [82–84]. However, this expression is also detectable in normal human myocardium [85] and absent in another group of patients with dilatative cardiomyopathy [86]. These data suggest that iNOS is not consistently expressed in the myocardium of heart failure patients. Furthermore, the expression and the activity of enzymes involved in the ˙NO–cGMP pathway of the cardiac muscle (Fig. 1) might be changed in heart failure [87]. Thus, the role of iNOS expression in heart failure remains controversial.

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3.3. Effects of endogenous ˙NO on myocardial relaxation

The lusitropic action that has been described for exogenous ˙NO was also observed after stimulation of endogenous ˙NO synthesis [88]. Infusion of bradykinin and substance P elicited a lusitropic effect in the guinea pig working heart, which was completely abolished by the ˙NO scavenger, oxyhemoglobin. A similar effect was observed in man. Intracoronary infusion of substance P in the presence of dobutamine was associated with accelerated myocardial relaxation [89]. The lusitropic effect of endogenous ˙NO observed in both animals and man are similar to the lusitropic effects of exogenous ˙NO.

4. Chronotropic effects of ˙NO

4.1. Exogenous ˙NO

There are only a few reports about the chronotropic effects of ˙NO in the absence of autonomic stimulation. An early study investigated the effects of the cGMP analogue dibutyryl-cGMP in cultured rat neonatal cardiomyocytes. Subjection of these cells to high concentrations of dibutyryl-cGMP resulted in a 15% reduction of their spontaneous beating rate of approximately 100 beats/min [90]. In contrast, a later study in this cell type failed to detect any influence of the ˙NO donors SNP or SIN-1 on the basal beating rate [91]. Similar results with ˙NO donors were obtained in isolated rat right atria [92], while another study reported that low concentrations of SNP induced an increase in the spontaneous beating rate of the rat isolated heart of approximately 20% [93]. Furthermore, clinical investigations in patients after heart transplantation indicated a direct positive chronotropic effect of ˙NO. Infusion of SNP increased the heart rate in these patients before reinnervation of the heart occurs, which strongly speaks against baroreceptor activation as an underlying mechanism [94,95]. These results indicate that chronotropic effects of ˙NO are dependent on the species and on the concentration of ˙NO. A recent study in the isolated guinea pig sinus node showed that ˙NO donors, such as SNAP, SNP and SIN-1, biphasically alter the spontaneous beating rate of this preparation [96]. Low concentrations (10 nM–10 μM) induced a 30% increase in beating rate, while this effect declined at higher concentrations. Both effects were dependent on cGMP, suggesting a role for soluble guanylate cyclase in the regulation of heart rate. Further experiments of this study were performed by the single cell patch–clamp technique and demonstrated that the positive chronotropic effect of ˙NO is caused by a stimulation of the hyperpolarization-activated inward current (I_f). Thus, studies in animals and man suggest that submicromolar concentrations of exogenous ˙NO can increase heart rate under basal conditions. However, it is not clear if the positive chronotropic effect of exogenous ˙NO is important in a heart that is beating under autonomic control.

4.2. Endogenous ˙NO

The first report describing the chronotropic effects of endogenous ˙NO was performed with cultured neonatal rat cardiomyocytes [91]. In this study, the NOS inhibitor L-NMMA had no significant effect on the spontaneous beating rate of these cells. In contrast, the NOS inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) induced a considerable reduction of beating rate in constant pressure perfused isolated rat hearts and this effect was reversed by L-arginine [93]. The negative chronotropic effect of L-NAME was confirmed in a later study investigating rat constant flow perfused Langendorff preparations [48]. Conversely, application of the NOS substrate L-arginine alone induced a small positive chronotropic effect. These results are consistent with the observation that mice lacking the eNOS gene have a significant bradycardia [97]. Furthermore, several weeks of oral application of L-NAME to normal mice also results in a significant reduction of heart rate [98,99]. These effects have been measured in awake trained mice using an automated tail cuff method. Using the same method, it was shown that three weeks of oral application of L-NAME to mice lacking the eNOS gene induces an augmentation of their bradycardia [99]. It is of note that the NOS inhibitor L-NAME is also capable of blocking muscarinergic receptors [100], an effect that might have attenuated the observed reduction of heart rate in mice. Taken together, the present evidence suggests that heart rate can be increased by the activity of both constitutively expressed NOS isoforms. Of these, eNOS is much more important than nNOS. These results are consistent with the positive chronotropic effects that have been described and explained for exogenous ˙NO.

5. Summary

The effects of exogenous and endogenous ˙NO on myocardial functions such as contraction, relaxation and heart rate have recently gained considerable scientific interest. ˙NO stimulates myocardial soluble guanylate cyclase to produce cGMP, which activates two major target proteins. A small increase in cGMP levels predominantly inhibits phosphodiesterase III, while high cGMP levels activate cGMP-dependent protein kinase. Accordingly, submicromolar ˙NO concentrations improve myocardial contraction, while submillimolar ˙NO concentrations decrease contractility. The latter action includes direct inhibitory ˙NO effects on ATP synthesis and voltage-gated calcium channels. Overall, the inotropic effects of exogenous ˙NO are small and probably of minor importance for myocardial contractility. Cardiomyocytes are capable of

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expressing eNOS and iNOS. Endogenous $^{\cdot}$NO has effects on myocardial contraction, similar to that of exogenous $^{\cdot}$NO. Various NOS inhibitors can substantially reduce myocardial contractility in vitro and in vivo, suggesting that basal endogenous $^{\cdot}$NO production supports myocardial contractility. There is also evidence for a $^{\cdot}$NO-dependent cardiodepressive effect of cytokines that is mediated by expression of iNOS. This is consistent with the negative inotropic effects of $^{\cdot}$NO at high concentrations. Cardiodepressive actions of endogenous $^{\cdot}$NO production may play a role in certain forms of heart failure. Finally, $^{\cdot}$NO also has an effect on heart rate. Physiologic $^{\cdot}$NO concentrations can stimulate heart rate by activating the hyperpolarization-activated inward current (I_f) and this effect decreases at submillimolar $^{\cdot}$NO concentrations. In summary, physiological concentrations of $^{\cdot}$NO increase contractility and heart rate under basal conditions, while high $^{\cdot}$NO concentrations induce the opposite effects.

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