Role of nitric oxide in the neural control of cardiovascular function

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Abstract

The discovery in 1990 that nitric oxide (NO) acts as a neuromodulator within the central and peripheral nervous system triggered intensive research which considerably extended our understanding how this factor regulates cardiovascular functions. In addition to its direct effects on blood vessels NO has additional targets at all levels of the neural control of circulation. When not scavenged by hemoglobin, NO is relatively stable and diffuses over large distances (>500 μm) so that one NO-producing cell can influence several thousands of adjacent cells in vivo. In different brain regions, NO and its metabolites have excitatory as well as inhibitory effects. The modulation of autonomic functions by these factors is therefore highly complex and often variable between the different levels from the brain to postganglionic nerve endings. This review is focused on the available evidence derived from animal studies and will summarize the current discussion about (i) the modulation of the generation of sympathetic and parasympathetic activities within the brain stem by NO; (ii) the actions of NO on cardiovascular reflexes and (iii) the role of NO as a modulator of autonomic functions within the target organs. Finally, the available evidence from human studies and some pathophysiological implications of altered NO-mediated modulation of the neural control of circulation will be discussed. © 1999 Elsevier Science B.V. All rights reserved.

1. Introduction

The neural control of circulation represents a complex network of regulatory influences on heart and blood vessels provided by the activity of postganglionic sympathetic, parasympathetic and so-called non-adrenergic non-cholinergic (NANC) nerves, and by circulating catecholamines from the adrenal gland. Since the discovery that nitric oxide (NO) is not only a regulator of smooth muscle tone but also a neuromodulator within the central and peripheral nervous system [1,2] it is clear that the cardiovascular actions of this molecule are probably not confined to its direct effects on blood vessels and blood cells but comprise, in addition, effects on all neural substrates that contribute to the generation of the activity of autonomic nerves in the central and peripheral nervous system. In the absence of hemoglobin, its major sink in vivo [3], NO is a relatively stable molecule that diffuses over relatively large distances (>500 μm) in brain tissue [4,5], and S-Nitrosothiols generated in reactions of NO with thiol-containing peptides or proteins such as glutathione or albumin can store NO-equivalents and transport them even with the blood stream [6,7]. This means that, in principle, almost every tissue is continuously influenced by changing concentrations of NO and its metabolites. Effects of NO are prominent in brain regions that control sympathetic and vagal activities and, in addition, NO modulates the transmission of autonomic neural activity to target organs by actions within the spinal cord, ganglia and neuromuscular junctions. The resultant complex interaction of NO with autonomic functions implies that pathophysiological changes in the synthesis and metabolism of NO may have direct consequences for the neural control of circulation. Cardiovascular diseases in which altered neuronal effects of NO are probably important include essential hypertension, atherosclerosis, heart failure, septic shock and even diabetes mellitus. This review

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2. Synthesis and cellular functions of NO within the brain

The activity of preganglionic autonomic nerves is integrated by specialized neuronal cell populations that are located in the lower brain stem in close contact to each other and with the regions involved in the control of respiration [8–10]. The functional organization of the sympathetic and parasympathetic chains and the potential targets for NO are shown in Fig. 1. When neuronal NOS-density is taken as the indicator, it appears that endogenous NO-formation within the brain stem is relatively low when compared to other regions such as the hippocampus, the cortex or the cerebellum [11–14] but there are considerable differences in the NOS-density within the brain stem between species [15]. This is important because the actions of NO in neuronal cells are largely dependent on the effective concentration over time. The complexity and variability of NO-actions on the cellular level can be deduced from the variety of different biochemical reactions triggered by this molecule. Some of the most important ones are listed in Table 1. NO can produce both excitatory as well as inhibitory effects in neurons so that whether NO acts as an excitatory or inhibitory neuromodulator in a specific brain region may depend not only on the neuronal cell type but directly and importantly also on the spatio-temporal relationship of the concentrations of NO and its metabolites within tissues.

2.1. Central regulation of sympathetic activity

In 1991 Shapoval et al. [16] published the first study that proposed a direct effect of NO within the RVLM on sympathetic nerve activity (SNA). Microinjections of the NOS-inhibitor L-NAME into this area increased and the

Fig. 1. Functional organization of the neural control of circulation. Abbreviations: RVLM: rostral ventrolateral medulla; AMB: Nucleus ambiguous; DMV: Dorsal motor nucleus of the vagus; IML: Intermediolateral cell column; SPG: Sympathetic ganglion.

Table 1
Intracellular targets and mechanisms of action of NO in neuronal cells

<table>
<thead>
<tr>
<th>Targets</th>
<th>Reaction</th>
<th>Functional consequences</th>
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<tr>
<td>Guanylyl cyclase</td>
<td>Increased cGMP</td>
<td>Decrease in intracellular Calcium</td>
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<td>Modulation of transmitter release</td>
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<td>Activation of protein kinases</td>
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<td>Inhibition of Phospholipase C</td>
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<td>Superoxide anions</td>
<td>Formation of peroxynitrite</td>
<td>Cytotoxicity, oxidative stress</td>
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<td>Protein / Peptides</td>
<td>Nitrations</td>
<td>Tyrosine nitration</td>
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<td>Metals</td>
<td>Nitroxylation</td>
<td>Modulation of ion channel activities</td>
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<td>DNA</td>
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<td>Inhibition of glycolytic enzymes</td>
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<td>NO-storage and NO-transport</td>
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* Adapted and modified from [3,5,181–183].
NO-donor sodium nitroprusside inhibited renal SNA in cats. Most subsequent studies [17–21] supported the concept that endogenously released NO has inhibitory effects in RVLM-neurons. The underlying mechanisms probably consist of an attenuation of glutamategic excitation [22] in presynaptic neurons within the RVLM and, in addition, a reduction of similarly excitatory effects of angiotensin II in that region [23]. The predominant stimulus for NO-release is probably the activation of the calcium dependent neuronal NO-synthase via calcium influx through NMDA glutamate receptors [24,25]. Endothelial NO-sources seem to be less important [22].

However, in two studies the opposite (i.e. sympathoexcitation) was observed when NO-donors were injected into the RVLM at concentrations in the upper millimolar range [26,27]. Furthermore, there are no significant changes in baseline sympathetic nerve activity (SNA) when neuronal NOS is specifically blocked either systemically or within the whole brain stem or when baroreceptor denervated animals are studied in which BP-changes have no direct influence on SNA [22,28]. The most likely explanation for this is that NO produced by, or administered to, the lower brain stem similarly affects the caudal ventrolateral medulla (CVLM) that provides an GABA-ergic inhibitory projection to the RVLM [29,30]. Thereby, the actions of NO on both regions could cancel out each other. However, it is important to note that systemic or organ-wide blockade of NO-synthesis represents a rather artificial experimentally induced situation and that NO-synthesis and actions in both regions are probably seperately regulated in vivo. The complexity of NO-action becomes further apparent in the nucleus of the solitary tract (NTS). Increases in NO-concentrations in that region consistently produces sympathoinhibition [31–34] although it is not only a relay for the sympathoinhibitory baroreceptor-reflexes but also for a number of sympathoexcitary reflexes such as the chemoreflexes [35]. Most studies proposed that in the NTS, in contrast to the RVLM, the cellular effect of NO may be primarily excitatory through retrograde enhancement of presynaptic glutamate release as initially described for hippocampal neurons [36]. Thus, the available evidence suggests that NO can have both inhibitory as well as excitatory effects even in relatively nearby regions and this phenomenon has been similarly observed within the spinal cord [37].

Previous observations showing that glutamate receptors antagonists cause significant hypotension in most models [38–40] may favour a predominance of the inhibitory effects of NO. However, these compounds act primarily by prevention of direct glutamate effects (depolarization) and only secondarily through reduction of glutamate evoked NO-release. Nevertheless, glutamate antagonists probably reduce NO-release in general thereby preventing potentially both the inhibitory and the excitatory actions of NO in different brain regions.

On the basis of the findings described above it is not surprising that the numerous studies in which sympathetic activity was measured following systemic inhibition of all NOS-isoforms in whole animals or humans yielded completely heterogeneous results. Some authors reported increases [41–43], others found decreases [44–47] or no changes in SNA [48–50]. In a recent study on rats [51], even an increase in renal and a decrease in lumbar SNA was observed following systemic NOS-inhibition. The explanation for these confusing results must not only be sought in the diversity of NO-actions but also in the fact that the generation of sympathetic activity within the RVLM is a result of the similarly complex interaction many excitatory inputs that come from higher brain regions and bodily reflexes (Fig. 1) and from inhibitory inputs provided by baroreceptor afferents. One simple reason for divergent findings in intact animals is therefore the fact that blood pressure increases produced by systemic NOS-inhibition cause a baroreflex mediated inhibition of SNA [52,53], an effects which most important in acute experiments but much less in chronic studies because baroreceptor reflexes reset to altered BP-levels within relatively short periods [54–57].

Taken together it appears that at least under physiological conditions, endogenously released NO in effect reduces overall sympathetic excitability within the brain stem and, in addition, possibly through actions in higher brain regions such as the hypothalamus [58–60] or the limbic system.

2.2. Parasympathetic activity

A major cardiovascular effect of systemic NOS-inhibition is long-lasting bradycardia [61–63] caused by either direct central effects or by enhancement of the baroreflex-mediated activation of vagal tone. Several groups have suggested that centrally acting NO may modulate preganglionic parasympathethetic neurons within the nucleus ambiguus (AMB) and dorsal vagal motor nucleus (DMV) [64–66] an effect that may not only affect heart rate but significantly alter gastrointestinal functions as well. Furthermore, in the NTS, NO appears to excite neurons with connections to the DMV and AMB [67]. Experiments on conscious rabbits [50,68], however, show that NOS-inhibition increases baroreflex mediated bradycardia. Therefore, it seems that the modulation of central parasympathetic activity may be, in principle, similarly organized as described for the sympathetic chain i.e. the net effect of NO is an attenuation of the overall excitability resulting in a reduction of the efferent outflow.

2.3. Cardiovascular reflexes

Most studies concerned with cardiovascular reflexes such as the chemoreceptor reflex [69–74], the muscle
metabo- or exercise pressor reflex [64,75,76] and somatovisceral reflexes [17,22,77] equivocally reported direct or indirect inhibitory effects of NO on the sympathoexcitation produced by these reflexes. In addition to inhibitory actions of NO at the level of the peripheral receptors, there may be modulation of reflex transmission within the brain i.e. in the NTS and RVLM. Parasympathetic reflex components are similarly affected. The increase in parasympathetic tone (bradycardia) produced by activation of cardiopulmonary receptors for example (Bezold–Jarisch reflex), may be also reduced by central actions of NO primarily within the NTS and the nucleus ambiguus [78].

In contrast to the overall inhibitory modulation by NO of excitatory reflex inputs to the brain stem regions that regulate autonomic activities, is much less clear how NO modulates the sympathoinhibitory baroreceptor reflex in vivo. Baroreceptors have their first synapses in the NTS and are then further relayed to the caudal ventrolateral medulla (CVLM) which provides an GABA-ergic inhibition of the RVLM [52,53,79]. Activation of baroreceptor afferents by increases in blood pressure and/or pulse amplitudes thus reduces sympathetic outflow from the RVLM. Studies concerned with the role of NO in the sympathetic part of the baroreceptor reflex came up with rather heterogeneous results. Attenuation [21,80] and enhancement [81] of baroreceptor reflex fuctions as well as indifferent responses [46,49] to systemic variation of endogenous or exogenous NO-levels have been reported. Other studies that were more directly focused on the neurophysiological mechanisms underlying modulation of the reflex pathway similarly yielded more or less contradictory results. Matsuda and Coworkers [82] found inhibitory effects of NO on arterial baroreceptors in the carotid sinus which were later explained by direct inhibitory actions on sodium channels [83]. However, the physiological relevance of these effects remain to be determined since in experiments on cats [84] using a modified blind sack preparation of the carotid sinus that preserved pulsatile pressure stimuli, we could not find significant modulation of baroreceptor activity by NO. Furthermore, microinjections of NO or NOS-inhibitors into the central relays of the baroreflex (i.e. NTS, CVLM, RVLM) failed to alter baroreflex responses produced by electrical stimulation of the carotid sinus nerve [85]. Although at present a definitive conclusion cannot yet be drawn and despite the fact that there may be species differences as well, it seems that arterial baroreceptor reflexes may be much less affected by NO than the sympathoexcitatory pathways described above.

3. Neural control of the heart

Besides its actions on the activity of preganglionic autonomic nerves, NO directly influences the intrinsic cardiac nervous system which consists of efferent sympathetic and parasympathetic postganglionic neurons, chemodenervosensitive afferent neurons and local circuit neurons [86,87]. Between 30 and 40% of these neurons are NOS-positive in dogs [88]. Endogenous as well as exogenous NO excites these neurons leading to moderate positively inotropic and chronotropic responses as a net effect in vivo [88,89]. Furthermore, NO has additional effects at the level of parasympathetic postganglionic nerve endings that may be particularly important within the heart. Acetylcholine released by these fibers causes NO-release from the coronary endothelium which in turn cause vasodilation [90,91] and cholinergic inhibition of L-type calcium chan-

4. Neural control of blood vessels

4.1. General mechanisms

The vasodilating properties of NO are brought about by a reduction of vascular smooth muscle tone which is generated by either adrenergic, myogenic or humoral influences. Each constrictor mechanism contributes to a different extent to the regulation of organ blood flow but in general, the constrictor tone of blood vessels is a result of a combination of all. A number of studies both in vitro [102–106] and in vivo [28,107–110] have shown that NO very effectively counteracts sympathetic vasoconstrictor mechanisms. This interaction consists of at least two mechanisms: (1) The inhibition of NA-release from postganglionic nerve endings by NO and (2) intracellular antagonism of the noradrenergic signal transduction pathway that may be more effective than the inhibition of other vasoconstrictor mechanisms. Partially, the specificity is also caused by the widespread nitrooxidergic innervation of
the vasculature [111–114] which causes abluminal NO-release directly to the sympathetic varicosities.

The most important role of the interaction of NO with sympathetic vaso motor activity is probably to enable adequate perfusion of metabolically active organs upon general sympathetic activation during exercise or stressful situations [115,116]. NO primarily released by the endothelium when shear rates increase due to enhanced blood flow reduce both the release and the constrictor effects of noradrenaline thereby supporting flow dependent dilation. In rats, it has been shown that some postganglionic sympathetic nerves, in addition, contain preformed nitroso factors that are co-released upon increases in SNA contributing to the vasodilation in exercising organs in the presence of elevated α-adrenergic tone [117]. These mechanisms may be particularly important in feeding arteries and arterioles that are not under direct metabolic vasodilator influence.

The relevance of the modulation of sympathetic constrictor mechanisms by NO has been questioned by some authors recently [46,47,118] because the vasodilator effects of NO were preserved in animal models with reduced or absent sympathetic vas constrictor activity. However, these results were not surprising as studies on isolated vessels clearly show. NO is still an effective dilator agent when the constrictor tone of blood vessels is not generated by SNA but by myogenic mechanisms or constrictor agonists. However, in the living organism sympathetic mechanisms play a predominant role in the regulation of organ blood flow through the precise adjustment of vascular resistance in the feeding vessels. And when vascular sympathetic activity is on a normal level in vivo, NO almost exclusively reduces sympathetic constriction in most organs [28]. The importance of the modulation of sympathetic vasoconstriction by NO is therefore directly dependent on the importance of sympathetic control of circulation itself.

4.2. Vaso-neuronal coupling in the CNS

Vascular tone and blood flow within the CNS are tightly coupled to the metabolic activity of neurons within specific brain regions. Neuronally released NO diffusing to adjacent blood vessels mediates, probably together with adenosine [119], activity-dependent vasodilation and local increases in cerebral blood flow [120]. Activation of neuronal NOS by the rise of intracellular calcium following binding of glutamate to NMDA-receptors may underly this phenomenon [25,121–123]. Furthermore, the above described release of NO from parasympathetic perivascular NANC-nerves may be also involved in the specific feed forward regulation of blood flow to active brain regions [114,124–126]. Finally, it has been suggested that NO may affect vaso-neuronal coupling by mediating the hypercapnic rise in cerebral blood flow [127], but subsequent studies did not support this hypothesis [128–130]. However, NO has probably a permissive role in the vasodilation caused by CO₂ [131] and this contribution of NO to basal vascular tone may influence other vaso motor mechanisms as well [132].

4.3. Neurogenic vasodilation in genital organs

The role of NO as a parasympathetic (NANC) transmitter is most important in the regulation of male sexual functions. In 1990 Ignarro and colleagues demonstrated the essential role of NO and cGMP as mediators of corpus cavernosum smooth muscle relaxation and thus erectile function [133]. Subsequently, it was shown that neuronal (parasympathetic) rather than endothelial sources account for the selective release of NO for erection [134–137] and that in females, the increase in blood flow through genital organs during sexual arousal may be similarly regulated [138,139]. How important this function of NO really is for many people may be gathered from the publicity that VIAGRA® (sildenafil, an inhibitor of phosphodiesterase V) received when it was launched worldwide as a drug for humans in 1998. This compound inhibits the breakdown of cGMP after NO-mediated activation of guanylate cyclase evoked by parasympathetic nerves in the genital tract during sexual stimulation [140]. A discussion of the variety of other functions of NO in the male and female reproductive systems has been subject of a recent review [141].

5. Human studies

Due to the methodological and ethical limitations of studies on volunteers and patients, only few studies on humans addressed the effects of NO on the neural control of circulation. These studies largely supported the evidence derived from animal experiments. NO-synthases have been detected in the human brain stem including the ventrolateral medulla [142,143] and in brain regions such as the hypothalamus which provide major synaptic inputs to the RVLM [144]. Nitroxidergic innervation of blood vessels seems to play an important role in the regulation of organ blood flow in humans particularly at arteriovenous anastomoses [145], in the brain [146], genital organs (see above) [147,148], and, at least to some extent, even in skeletal muscles [149]. Furthermore, the central role of NO derived from either the endothelium or perivascular nerves as a functional antagonist of the sympathetic vasoconstrictor tone has been repeatedly confirmed in studies on humans [150–152]. Due to the inevitable experimental restrictions, only very limited evidence exists on the role of NO in the central regulation of sympathetic activity in humans. Baseline sympathetic activity in humans decreases in response to systemic NOS-inhibition as a result of baroreflex-mediated inhibition of sympathetic activity when blood pressure increases due to the removal of...
dilatory effects of vascular NO [45,46]. This is in accordance to the findings in animals. However, to what extent the inhibitory modulation of sympathetic excitability by NO may play a role in humans, could not be determined by these studies and remains to be elucidated (see also 2.1, 4.1). Nevertheless, some of the pathophysiological findings described below suggest that NO may be a relevant ‘dampening factor’ in the central control of sympathetic functions in health and disease in humans as well.

6. Pathophysiological implications

The discovery of various transport and storage forms of NO and its metabolites [6,153,154] made clear that the site of production and the sites of action of NO within the body can differ significantly when the absolute amount of produced NO is high. The most studied example of acutely increased NO-production with cardiovascular consequences is septic shock. Excess NO-release largely accounts for the failure of heart and blood vessels to respond to sympathetic activation or infusion of α-adrenergic agonists in this situation [155–159]. The hopes that NOS-inhibition would improve the clinical outcome of septic patients, however, have not been satisfied so far, possibly because hemodynamic stabilization is achieved by the price of impaired immune responses which need such high NO-concentrations to be effective.

Chronic inflammatory processes which do occur for example in atherosclerosis, heart failure, rheumatic diseases and subclinical infections do not cause hypotension or shock but may cause deleterious alterations of the NO-biochemistry in vivo. The key event on the cellular level is enhanced production of superoxide and its reaction with NO leading to oxidative stress both directly and through the generation of peroxynitrite. The complex events and biochemical and cellular consequences associated with NO-related oxidative stress have been addressed by several reviews recently [3,160,161]. In neurons oxidative stress has primarily excitatory and/or damaging effects [162]. Therefore, although the causal relationships and the cellular mechanisms remain to be elucidated, it is tempting to speculate that the reduction of NOS-activity within the lower brainstem and the increase in sympathetic outflow that occurs both in heart failure [58] and in response to long-term exposure to enhanced NO-levels (nitrate tolerance) [163] may be functional consequences of oxidative stress within the brain.

Another factor that may affect the balance between NO and the autonomic cardiovascular control is angiotensin II (AII). It is known that AII enhances sympathetic activity and vice versa (i.e. sympathetic activity increases AII-formation) [164–168]. In a study on conscious rabbits [23], for example, central sympathoinhibitory actions of NO were only significant when AII contributed to the excitation of the RVLM and the increase in sympathetic activity during heart failure may be, at least partly, caused by activation of a local renin–angiotensin system and concomitantly reduced NO-release within the brain stem [58,169,170]. Since AII, in addition, may enhance superoxide production and reduce constitutive NO-synthesis [171], a mutual reinforcement of the pathophysiological effects of oxidative stress and AII seems likely.

Altered constitutive neuronal NO-release may also contribute to the development of essential hypertension. In spontaneously hypertensive rats (SHR) neuronal NO-synthesis is different from that of control strains (WKY) both in hypothalamus and brainstem regions [172,173] as well as in preganglionic sympathetic neurons in the spinal cord [174]. This may be one explanation for the apparently paradoxical finding that hypertensive rats have impaired endothelial dependent vasodilator responses despite increased endothelial NOS-activity [175,176] an effect that was similarly observed in patients [177]. There are some hints that the key events in the NO-related pathogenesis of essential and renal hypertension may be, similarly, increased levels of AII and NO-related oxidative stress [172,178,179] but this has to be proved in detail. Recent observations showing that even endothelin-1 may have neuromodulatory effects within the brain stem [180] provide another example how closely and highly complex the interaction of vascular and neuronal factors in the pathogenesis of cardiovascular diseases may be.

7. Conclusions

The intensive research during the last decade revealed and repeatedly confirmed an important modulation of the autonomic nervous system by NO. Inhibitory actions of NO on several levels from the effector organs up to the brain regions that regulate sympathetic and parasympathetic activities constitute a functional antagonism between these systems. The primary effect of NO is probably an overall reduction of the excitability of autonomic regulation rather than a reduction of basal tonic activities and a number of local feedback control mechanisms allow highly differential regulation of the neural control of circulation by NO. However, due to the almost ubiquitous presence of NO and the tremendous diversity of cellular effects of NO at different concentrations in different tissues, there are severe hindrances for therapeutic interventions in the NO-system. Despite the intensive research within this field and despite lots of efforts to develop new NO-donors (and NOS-inhibitors) by the pharmaceutical industry, no significant extension of the use of NO-related drugs in cardiovascular medicine has been achieved until now. The goal of pharmacological research in this field should be therefore the development of more (organ-)specific drugs that activate specific cellular targets of NO or prevent the
deleterious effects of NO-related oxidative stress within the autonomic nervous system.

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