Role of Nitric Oxide in Buffering Short-Term Blood Pressure Fluctuations

Harald M. Stauss and Pontus B. Persson

Blood pressure instability may promote cardiovascular morbidity. Recent data suggest a role of nitric oxide in stabilizing arterial blood pressure. A rise in blood pressure enhances endothelial shear stress and nitric oxide release. The resulting vasodilation antagonizes the initial increase in blood pressure. This system can respond within 2–10 seconds.

Cardiovascular morbidity and mortality depend on a variety of well-known risk factors, including obesity, hypercholesterolemia, cigarette smoking, physical inactivity, and hypertension. In addition, enhanced blood pressure variability, even in the absence of hypertension, has been identified as an independent cardiovascular risk factor. For example, the risk for coronary heart disease was found to be fivefold higher in untreated subjects with large systolic blood pressure variability compared with subjects with low systolic blood pressure variability (5). Moreover, the severeness of Goldblatt hypertension may rely on variations in renal arterial pressure (10).

How does the human body protect itself against these deleterious blood pressure fluctuations? The most intensively studied mechanism to buffer blood pressure fluctuations is the arterial baroreceptor reflex. Changes in arterial pressure are detected by receptors located at the aortic arch and the carotid sinuses. The afferent pathway projects to the brain stem, where sympathetic and parasympathetic efferent signals are generated. This response antagonizes the initial changes in arterial blood pressure by adjustments in heart rate and vascular tone. After an initial drop in blood pressure, an increase in heart rate can usually be observed within a few heartbeats and the adjustment of blood pressure is usually complete within 1–2 min. Thus the baroreceptor reflex is sufficiently rapid to prevent the occurrence of orthostatic hypotension with dizziness or fainting. However, there are pathophysiological conditions associated with impaired baroreceptor reflex function. For example, the sensitivity of the reflex is markedly reduced in atherosclerosis, heart failure, autonomic neuropathy, or pure autonomic failure. Because the requirement to stabilize arterial blood pressure is such a fundamental necessity for cardiovascular function, it is surprising that there should be only one short-term blood pressure control mechanism that can respond within less than a minute.

A vascular blood pressure control system

A considerable amount of evidence suggests that a second short-term blood pressure control mechanism exists besides the baroreceptor reflex. This rapidly responding system acts at the site of the vasculature by the following mechanism (Fig. 1): changes in arterial blood pressure lead to corresponding changes in vascular shear stress. This mechanical stimulus causes an increase in cytosolic Ca^{2+} content in the endothelial cells. The resulting Ca^{2+}-calmodulin complex enhances the activity of the endothelial isoform of nitric oxide synthase (eNOS). The subsequently formed nitric oxide (NO) diffuses into the adjacent vascular smooth muscle cells, where it activates the enzyme guanylyl cyclase. This leads to a rise in cGMP that acts by reducing the intracellular free

H. M. Stauss is Privatdozent and P. B. Persson is Professor at the Department at Johannes-Müller-Institut für Physiologie, Humboldt-Universität, (Charite), Tucholskystrasse 2, 10117 Berlin, Germany.

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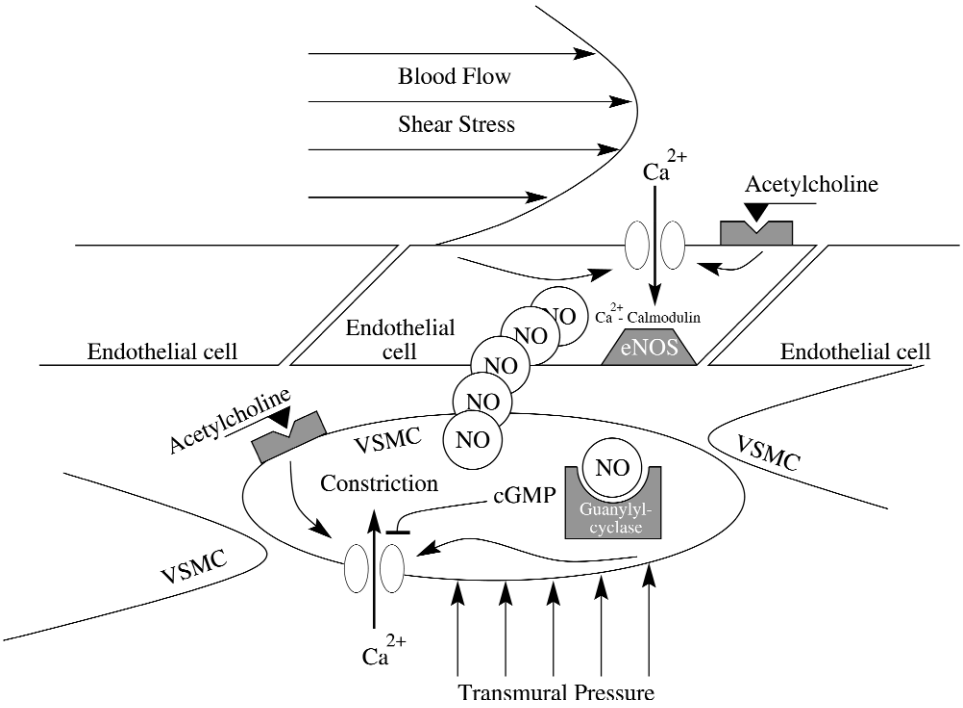

FIGURE 1. Mechanism of the vascular blood pressure control system. Vascular shear stress promotes Ca^{2+} influx into endothelial cells. Subsequently, the Ca^{2+}-calmodulin complex activates endothelial nitric oxide (NO) synthase (eNOS). NO diffuses to the adjacent vascular smooth muscle cells (VSMC), where it activates the guanylyl cyclase. cGMP causes a reduction in intracellular Ca^{2+} concentration in VSMC and, therefore, leads to vasodilation.

Ca^{2+} concentration. Thus vascular resistance decreases and blood pressure is maintained at its initial level.

Functional significance of the vascular blood pressure control system

If this vascular blood pressure control system is of any hemodynamic significance, one would expect a stronger blood pressure variability when this control system is inhibited. Indeed, it has been demonstrated in conscious dogs and rats that pharmacological inhibition of NOS by application of false substrates causes a marked increase in blood pressure variability. This enhanced blood pressure instability persists when the hypertension associated with NOS inhibition is prevented by additional application of exogenous NO donors (11). Power spectral analysis revealed that the enhanced blood pressure variability is mainly manifested in a low frequency range similar to the frequency range generally ascribed to sympathetic modulation of vasomotor tone. In rats, this frequency range is located between 0.2 and 0.6 Hz and in dogs between 0.1 and 0.5 Hz. Thus the vascular NO system is most effective in dampening blood pressure fluctuations in a time range between 2 and 10 s. Taking into account that the NO molecule has a biological half-life time of ~6 s, it seems reasonable to assume that this system is able to buffer blood pressure fluctuation within 2–10 s. However, it should be mentioned that the blood pressure buffering effect of NO remains controversial. In humans (2) and rats (3), a reduced low-frequency spectral power was found after inhibition of NO synthase. However, in these studies hypertension due to NO inhibition was not counteracted, and thus sympathetic inhibition due to the baroreceptor reflex may have occurred. Since sympathetic modulation of vasomotor tone acts in the same frequency band as the vascular NO system, it seems that the reduction in low-frequency spectral power of arterial blood pressure in these two studies (2, 3) was due to sympathetic inhibition and was not related to the blockade of NOS.

Power spectral analysis of arterial blood pressure during NOS blockade in rats and dogs demonstrated that the NO system is most effective in buffering blood pressure fluctuations at ~0.4 Hz. However, this does not mean that the NO system cannot buffer slower blood pressure fluctuations as well. In a study by Gouedard et al. (4), blood pressure fluctuations related to the renin-angiotensin system were investigated in conscious rats. In this study, blood pressure fluctuations in a frequency range between 0.02 and 0.2 Hz were enhanced by NOS inhibition with the use of the false substrate N^G-nitro-L-arginine methyl ester (L-NAME). This effect was prevented by the angiotensin II-AT_1 receptor antagonist losartan. This finding can be interpreted in such a way that activation of the renin-angiotensin system is associated with an intrinsic instability of arterial blood pressure in the frequency range between 0.02 and 0.2 Hz. Under physiological conditions, these blood pressure fluctuations are buffered by the NO system and, therefore, are not apparent. However, if the NO system is blocked, blood pressure variability becomes unmasked. When losartan is added to L-NAME, the renin-angiotensin system that initially generated the blood pressure fluctuations is inhibited and blood pressure fluctuations diminish. This example demonstrates that the vascular blood pressure control

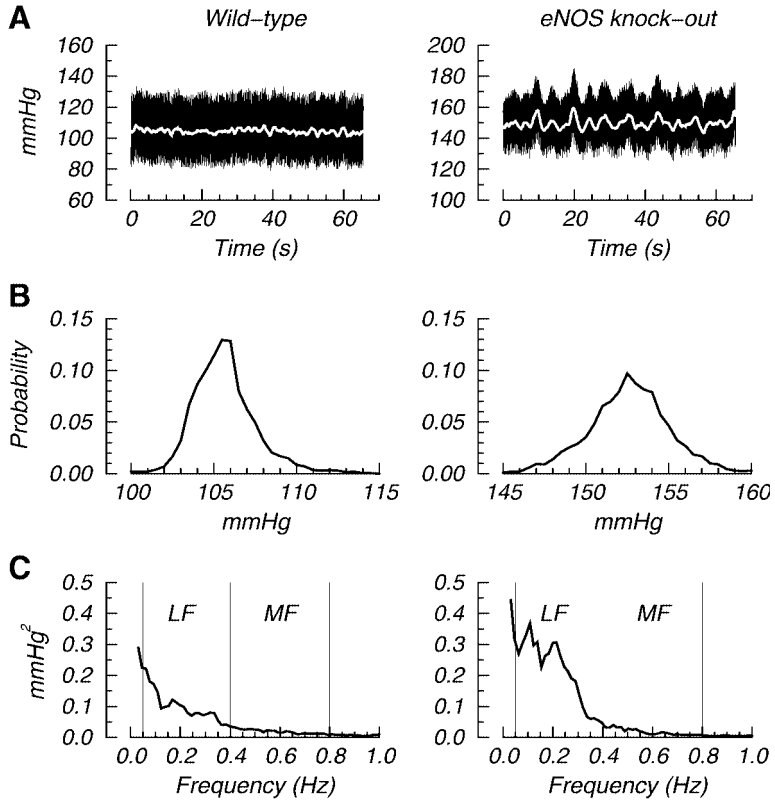

FIGURE 2. Blood pressure variability in wild-type mice and eNOS mutant animals. *A*: original blood pressure tracings in representative animals. White lines indicate mean blood pressure. Spontaneous blood pressure variability is markedly enhanced in conscious eNOS knockout mice. *B*: probability distributions of mean blood pressure values. After adjusting mean blood pressure of each strain, the probability distributions of all animals were averaged. A much broader distribution was found in eNOS knockout mice. *C*: arterial blood pressure power spectra. The spectra of all animals were averaged. The enhanced blood pressure variability in eNOS knockout mice is manifested in a frequency band between 0.05 and 0.4 Hz. (Figure was reproduced from *Hypertension* 33: 1359–1363, 1999, with permission.)

system can also stabilize arterial blood pressure below the frequency band between 0.2 and 0.6 Hz, where it appears to be most effective in rats and dogs.

As mentioned above, pharmacological blockade of NOS in rats and dogs is accompanied by an enhanced blood pressure variability (4, 11), indicating that endogenously generated NO can buffer blood pressure fluctuations. However, these studies were not able to ascribe the blood pressure stabilizing effect of endogenous NO to a specific NOS isoform. In a recent study (13), we therefore compared blood pressure variability in conscious knockout mice that specifically lack the gene for eNOS with their wild-type controls. Compared with their wild-type controls, blood pressure was higher and overall blood pressure variability was markedly enhanced (Fig. 2, *A* and *B*). Thus the blood pressure buffering effects of endogenous NO appear to be mediated by eNOS. The enhanced blood pressure variability in eNOS knockout mice was mainly manifested in a frequency band between 0.05 and 0.4 Hz, with a maximum at 0.2 Hz (Fig. 2*C*). Thus blood pressure in these mice reveals an intrinsic instability, with fluctuations of ~5 s duration. This frequency characteristic agrees fully with that found in other species after pharmacological NOS inhibition and with the biological half-life of NO, which is roughly 6 s.

The question then arises as to why the other short-term blood pressure control system, i.e., the baroreceptor reflex, does not fully compensate for the higher blood pressure variability in eNOS knockout mice. To shed light on this issue, we investigated baroreceptor-heart rate reflex sensitivity in eNOS knockout mice by the so-called sequence method. Briefly, spontaneously occurring sequences of three or more consecutive heartbeats were detected during which both arterial blood pressure and interbeat interval simultaneously increased or decreased. Linear regressions between blood pressure and interbeat interval values of these sequences reflect baroreceptor-heart rate reflex sensitivity. We did not find any significant differences in baroreceptor-heart rate reflex sensitivity between the strains. However, the sequences were roughly twice as frequent in blood pressure time series from eNOS knockouts than in those from wild-type controls (13). The greater number of sequences in the eNOS knockout mice suggest that the larger blood pressure variability in eNOS knockout mice was partly compensated by the baroreceptor reflex. Thus the actual blood pressure buffering capacity of the vascular eNOS system was underestimated in this study.

NO that is released from the endothelium in response to vascular shear stress may also exert nonvascular effects. For example, it has been demonstrated that NO attenuates baroreflex function at the site of the carotid sinuses (8) and at specific sites within the central nervous system. However, this

action of NO is unlikely to participate in the NO-dependent blood pressure buffering system, since NO-mediated suppression of the baroreflex would enhance rather than buffer blood pressure variability. In addition, NO-mediated baroreflex inhibition predominantly concerns the baroreceptor-heart rate reflex, whereas sympathetic baroreflex function is largely preserved (6). Furthermore, since baroreflex function was not altered in eNOS knockout mice (13), and since baroreflex function was enhanced by specifically blocking the neuronal isoform of NOS with 7-nitroindazole (9), it is unlikely that NO-dependent suppression of baroreflex function is mediated by eNOS.

On the other hand, it has also been demonstrated that inhibition of NO synthesis by L-NAME reduces the excitability in nucleus tractus solitarius (NTS) neurons (7). On the other hand, NO can enhance the excitability within the NTS, the site of the first central relay station of afferent pathways of cardiovascular reflexes. Activation of the NTS (e.g., by baroreceptor activation) in turn causes inhibition of the neurons in the rostral ventrolateral medulla (RVLM), the origin of the central portion of the preganglionic sympathetic pathway. Indeed, it has been demonstrated that NO exerts an inhibitory effect on sympathetic activity in the RVLM (15). However, there is evidence that these central effects of NO are mediated by the neuronal rather than the endothelial isoform of NOS. Thus it is reasonable to assume that the central actions of NO do not participate in the blood pressure buffering effects of the vascular blood pressure control system discussed here.

Implications for blood pressure variability as a diagnostic tool

It has been suggested that investigation of blood pressure variability and calculation of its frequency distribution by power spectral analysis can provide useful information for various pathophysiological conditions. The underlying concept is based on the idea that an activated regulatory system causes oscillations at a specific frequency, i.e., at the resonance frequency. If all of these regulatory systems have different resonance frequencies, it would be possible to determine the activity of each of the different regulatory systems by frequency analysis of blood pressure variability. For example, the resonance frequency of the arterial baroreceptor reflex was found near 0.4 Hz in rats (1). In the same species, blockade of NOS caused a strong increase in blood pressure variability in the frequency range between 0.2 and 0.6 Hz (11). Hence it is difficult to distinguish between blood pressure regulation via NO and the arterial baroreceptor reflex by spectral analysis of arterial blood pressure. The frequency ranges overlap.

Nevertheless, in a clinical application, frequency analysis of blood pressure variability may still be useful. For example, in patients with autonomic neuropathy, endothelial NO production may not be disturbed, whereas baroreceptor reflex function is impaired due to the neuropathy. Thus frequency analysis of blood pressure variability can be used to quantify the impairment of baroreceptor reflex function and to follow the progression of the autonomic neuropathy. Another example is atherosclerosis. In atherosclerosis it has been demonstrated that arterial baroreceptor reflex function is impaired (14). In addition, it has been repeatedly shown that endothelial dysfunction accompanied by a reduced production of endothelial-derived NO is also present in this disease (12). Changes in blood pressure variability can, therefore, be expected from impaired baroreceptor reflex function and impaired endothelial NO production. Thus, in atherosclerosis, frequency analysis of blood pressure variability can be used to investigate the progression of the disease, but it is not possible to distinguish whether a deterioration of the disease is due to a progression in endothelial dysfunction or due to further impairment of baroreceptor reflex function.

Summary and conclusions

Several lines of evidence suggest the existence of a vascular short-term blood pressure buffering system that relies on endothelial NO production in response to enhanced vascular shear stress. Inhibition of the vascular blood pressure control system by blockade of the NOS causes marked blood pressure instability. In addition, blood pressure variability in knockout mice lacking eNOS is dramatically enhanced compared with wild-type controls. Thus the vascular blood pressure buffering system seems to depend on a functional eNOS. Power spectral analysis of arterial blood pressure during inhibition of NOS in dogs and rats and experiments in eNOS knockout mice suggest that the vascular blood pressure control system is most effective in buffering blood pressure variability in a frequency range between 0.1 and 0.6 Hz. However, the NO system is also able to stabilize blood pressure at lower frequencies. This frequency characteristic partly overlaps with that of the arterial baroreceptor reflex. Frequency analysis of arterial blood pressure variability can, therefore, not only provide information about sympathetic modulation of vasomotor tone but also about endothelial function.

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References

1. Bertram D, Barres C, Cuisinaud G, and Julien C. The arterial baroreceptor reflex of the rat exhibits positive feedback properties at the frequency of Mayer waves. *J Physiol (Lond)* 513: 251–261, 1998.
2. Castellano M, Rizzoni D, Beschi M, Muiesan ML, Porteri E, Bettoni G, Salvetti M, Cinelli A, Zulli R, and Agabiti-Rosei E. Relationship between sympathetic nervous system activity, baroreflex and cardiovascular effects after acute nitric oxide synthesis inhibition in humans. *J Hypertens* 13: 1153–1161, 1995.
3. Cordero JJ, Gonzalez J, and Feria M. Effects of N^{ω}-monomethyl-L-arginine on short-term RR interval and systolic blood pressure oscillations. *J Cardiovasc Pharmacol* 24: 323–327, 1994.
4. Gouedard O, Blanc J, Gaudet E, Ponchon P, and Elghozi JL. Contribution of the renin-angiotensin system to short-term blood pressure variability during blockade of nitric oxide synthesis in the rat. *Br J Pharmacol* 119: 1085–1092, 1996.
5. Grove JS, Reed DM, Yano K, and Hwang LJ. Variability in systolic blood pressure--A risk factor for coronary heart disease? *Am J Epidemiol* 145:771–776, 1997.

6. Liu JL, Murakami H, and Zucker IH. Effects of NO on baroreflex control of heart rate and renal nerve activity in conscious rabbits. *Am J Physiol Regulatory Integrative Comp Physiol* 270: R1361–R1370, 1996.
7. Ma S, Abboud FM, and Felder RB. Effects of L-arginine-derived nitric oxide synthesis on neuronal activity in nucleus tractus solitarius. *Am J Physiol Regulatory Integrative Comp Physiol* 268: R487–R491, 1995.
8. Matsuda T, Bates JN, Lewis SJ, Abboud FM, and Chapleau MW. Modulation of baroreceptor activity by nitric oxide and *S*-nitrosocysteine. *Circ Res* 76: 426–433, 1995.
9. Murakami H, Liu JL, Yoneyama H, Nishida Y, Okada K, Kosaka H, Morita H, and Zucker IH. Blockade of neuronal nitric oxide synthase alters the baroreflex control of heart rate in the rabbit. *Am J Physiol Regulatory Integrative Comp Physiol* 274: R181–R186, 1998.
10. Nafz B, Stegemann J, Bestle MH, Richter N, Seeliger E, Schimke I, Reinhardt HW, and Persson PB. Antihypertensive effect of 0.1 Hz blood pressure oscillations to the kidney. *Circulation* 101: 553–557, 2000.
11. Nafz B, Wagner CD, and Persson PB. Endogenous nitric oxide buffers blood pressure variability between 0.2 Hz and 06 Hz in the conscious rat. *Am J Physiol Heart Circ Physiol* 272: H632–H637, 1997.
12. Shimokawa H. Primary endothelial dysfunction: atherosclerosis. *J Mol Cell Cardiol* 31: 23–37, 1999.
13. Stauss HM, Gödecke A, Mrowka R, Schrader J, and Persson PB. Enhanced blood pressure variability in eNOS knock-out mice. *Hypertension* 33: 1359–1363, 1999.
14. Wilfert K, Drischel K, Unbehaun A, Guski H, Persson PB, and Stauss HM. Vascular response to angiotensin II in atherosclerosis–role of the baroreflex. *Hypertension* 35: 685–690, 2000.
15. Zanzinger J, Czachurski J, and Seller H. Inhibition of basal and reflex-mediated sympathetic activity in the RVLM by nitric oxide. *Am J Physiol Regulatory Integrative Comp Physiol* 268: R958–R962, 1995.

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