Contribution of nitric oxide and mitochondrial permeability transition to the cardiovascular control in hypertension and experimental hyperglycemia

L.N.SHAPOVAL
Department of Circulation Physiology
Bogomolets Institute of Physiology, National Academy of Sciences of Ukraine
4, Bogomolets street, 01024 Kyiv,
UKRAINE.
shapoval@biph.kiev.ua

Abstract: This lecture summarises our experience demonstrating the crucial role of nitric oxide (NO) in the medullary cardiovascular control, the importance of the mitochondrial permeability transition in the neuron functioning, and peculiarities of the contribution of both NO and mitochondrial permeability transition to this control in hypertension and experimental hyperglycemia.

Key words: nitric oxide, mitochondrial permeability transition, hypertension, experimental hyperglycemia

1 Nitric oxide and cardiovascular control

Identification of NO as a neurotransmitter in the central nervous system (CNS) stimulated those studies aimed at learning about the roles of NO in regulating the activity of specific physiological systems (including the cardiovascular system) that are coordinated by the brain. We were the first [18] to show that NO influences mechanisms of the neuronal vasomotor control, realized by neurons within the rostral (RVLM) and the caudal (CVLM) ventrolateral medulla in cats via activation of the guanylate cyclase. In our experiments, injections of either sodium nitroprusside which produces NO spontaneously, or L-arginine as a physiological precursor for NO formation into the RVLM resulted in attenuation of the renal nerve sympathetic activity together with the systemic arterial pressure (SAP) reduction. Based on the fact that the RVLM preferentially contains sympathoexciting neurons, which, to a large extent, are the medullary origin of the descending pathways and provide tonic sympathetic drive to the vessels, our results gave evidence that attenuation in the renal nerve sympathetic activity was the result of inhibiting NO-producing neurons. Later, NO-producing neurons were detected in the RVLM of different species using NADPH-diaphorase staining [25], and those neurons were reported to be of great importance in maintaining the optimum level of the arterial pressure [3, 14, 26, for review]. Our study demonstrated that both injections of an exogenous NO donor and stimulation of endogenous NO production modified the functional activity of the medullary neurons involved in the cardiovascular control in a similar way: the integral parameter characterizing the state of the cardiovascular system, the systemic arterial pressure (SAP), decreased in a dose-depended manner in most experiments. Some ambivalence of the responses observed attracted our attention: in a part of experiments, injections of sodium nitroprusside into the nuclei tested induced hypertensive responses. The analysis of the structure of hemodynamic responses resulting from an activation of endogenous NO production in the nuclei within the medulla oblongata [23] indicated that left-side injections of L-arginine induced the SAP lowering mainly due a decrease in the peripheral vascular resistance, while changes in the heart rate and cardiac pump function (stroke volume and cardiac output) were insignificant. On the contrary, its right-side injections resulted in the SAP drop mainly due to a decrease in the cardiac activity. Prevalence of the vascular component of a hemodynamic response induced by left-side injections of a test agent in the medulla oblongata seems to be brought about with asymmetry of the neurons responsible for different aspects of nervous control of the heart and vessels. From three known isoforms of NOS (neuronal, NOS-1; inducible, NOS-2; and endothelial, NOS-3) production of NO in neurons

from L-arginine is mainly catalized by NOS-1, although NOS-3 is also available. In our experiments, inhibiting of NOS-1 in the medullary neurons with injections of either L-N^G monomethyl-L-arginine (L-NMMA), a competitive inhibitor of all NOS isoforms, or N^G nitro-L-arginine (L-NNA), a specific inhibitor of NOS-1, was in most experiments accompanied by a considerable increase in the SAP and enhancement of the renal nerve sympathetic activity [18, 23]. Effects of injections of L-arginine were also inhibited with 7-nitroindasol, another specific NOS-1 inhibitor. Taken together, these data confirm a viewpoint on the important role of L-arginine- NO-synthase pathway of metabolism in the control of the vascular tone and cardiac activity. Based upon a study [12] showing that NO induces a release of γ-aminobutyric acid (GABA) within the RVLM, we hypothesized that NO might modulate an activity of GABA in neurons within both dorsomedial and ventrolateral medulla to maintain optimum level of the SAP. The major findings of that study were the following: after preliminary inhibition of NOS-1, GABA injection into the nuclei tested resulted in a hypotensive responses reduced as compared with those in control rats, and preliminary activation of NOS-1 with L-arginine enhanced effects of GABA injections. From these results, we would expect that NO, at least partly, could act via activation of GABA neurons within the medulla oblongata to maintain optimum level of the SAP [22].

2 Contribution of NO to cardiovascular control in hypertension

There is growing body of evidence that cardiovascular diseases based to a large extent on neuronal effects of NO, for example, hypertension, are associated with impairments in the NOS pathway of metabolism of L-arginine. Since NO is an inhibitor transmitter in the system of medullary cardiovascular control, assumption that rats with genetically determined hypertension could feel the lack of NO seems to be logical. In our experiments [20, 21], we showed that injections of L-arginine into the medullary cardiovascular nuclei in spontaneously hypertensive rats, like in normotensive ones, resulted mainly in hypotensive responses. Comparative analysis of the data obtained did not reveal principle qualitative differences between those responses. However, in spontaneously hypertensive rats, activation of the synthesis of endogenous NO resulted in more intensive hypotensive responses as compared with those in normotensive rats. They were mostly due to a decrease in the peripheral vascular resistance. Injections of NOS-1 antagonist L-NNA into the medullary nuclei of rats with genetically determined hypertension mainly resulted in increasing the SAP which practically did not differ from that observed in normotensive rats. Those results confirmed an assumption that in spontaneously hypertensive rats NOS-1 was not impaired, but a substrate for the synthesis of NO was in insufficient amount, and a question arose about possible causes of such lack of NO. In that context, our interest was focused on arginase. It is an important regulatory enzyme which uses L-arginine for its metabolism like NOS isoforms. Since NO-synthases and arginases compete for L-arginine as a substrate for their metabolisms, a real probability of competitive relations between these groups of enzymes exists, as a whole, so a suggestion that arginase can affect NO synthesis via modulation of intracellular L-arginine seems to be logical. Of two known isoforms of arginase, arginase II is a mitochondrial enzyme which is expressed in both endothelial cells of the vessels [24] and in nervous cells of the brain [10], especially in the cortex, cerebellum, medulla oblongata and spinal cord. Arginase was shown to play an important role in endothelial dysfunction in hypertension [15, 27] but the role of this enzyme in the mechanisms of the medullary cardiovascular control has not been elucidated yet. In our experiments [10, 11], an analysis of hemodynamic effects of inhibiting an activity of arginase in medullary cardiovascular nuclei did not spot any essential differences between responses in spontaneously hypertensive and normotensive rats. This fact raised the possibility that a mentioned enzyme was potentially active both in the norm and in hypertension, but it was probably needed in different degree. Injections of norvaline, non-specific arginase antagonist, usually resulted in the SAP increase [20], while injections of a specific antagonist of arginase DFMO into the sites of localization of cardiovascular neurons induced an initial decrease in the SAP, which might have resulted from a compensatory activation of NOS-1. After an initial drop in the SAP, its level increased

significantly [21]. Taken together, these results are the evidence that arginase could contributes to medullary cardiovascular control in hypertension, competing for L-arginine as a substrate for synthesis of NO.

3 Mitochondrial permeability transition and cardiovascular control in hypertension

Within recent years, the mitochondria have attracted renewed attention due to their proposed roles in the release of cytochrome *c* and other pro-apoptotic factors in different models of apoptosis [13, 28]. Mitochondrial permeability transition (mPT) is thought to contribute substantially to the regulation of normal cell metabolism (regulation of oxidative phosphorylation and Ca^{2+} metabolism) and to regulatory processes leading the initiation to apoptosis and cell death [4, 6, 15]. Mitochondrial permeability transition pores (mPTPs) have been recognized as specific nonselective openings in mitochondrial membranes to allow the diffusion of solutes up to about 1500 Da from the mitochondrial matrix to the extra-mitochondrial space, and vice versa. The inner mitochondrial membrane is known to be usually impermeable to solutes having no specific carrier. If this impermeability is lost due to different causes, consequences for the cell can be rather far-reaching. The mitochondrial membranes contain ready-made large pores, namely those of the protein import system, and their opening might be whether accidental or regulated by certain cellular processes. The molecular mechanisms underlying metabolic regulation of the mPTP formation under both physiological and pathological conditions are poorly known. By now, two mechanisms have been described to explain mitochondrial membrane permeabilization [6]. The first deals with permeabilization of only the outer membrane without changes in the inner one, while the second involves both the outer and the inner membranes and corresponds to the opening of the mPTP. The factors facilitating membrane permeabilization lead to loss of electrochemical gradients, uncoupling of oxidative phosphorylation and promote the release of cytochrom *c* and other inter-membrane space components to the surrounding cytosol. Sustained opening of the mPTPs causes damage in mitochondrial metabolism. In the cardiovascular system, a growing body of evidence supports the concept that pharmacological inhibition of the mPTP opening is an effective strategy for protection of the heart against ischemia/reperfusion injury [8, 11]. The existing data indicate that at physiological concentrations NO inhibits the mPTP opening, whereas at high concentrations it may sensitize it [2]. NO is thought to affect the mitochondria by inducing the production of reactive oxygen (ROSs) and nitrogen (RNSs) species [1, 16, 17]. In our experiments [20], an increase in permeability of mitochondrial membranes in the medullary neurons with injections of an inductor of mitochondrial permeability transition pore phenylarsine oxide (PAO, 0.5 to 500 nmol) into the medullary cardiovascular nuclei usually induced the SAP drop in both normotensive and spontaneously hypertensive rats in a dose-dependent manner. In spontaneously hypertensive rats, the SAP shifts were less expressed as compared with those in normotensive rats. It does not seem that small amounts of PAO increased the permeability of the inner mitochondrial membranes and induced mPTP openings, but PAO was likely to increase the permeability of the outer membranes. In that case, a release of Ca^{2+} to the surrounding cytosol might be within physiological limits and could activate NOS-1. Since NO production is a Ca^{2+} dependent process, an increased synthesis of the latter in the medullary neurons might be the cause of the hypotensive responses following injections of a small dose of PAO. On the contrary, applying of high doses of PAO seemed to induce the mPTP opening, which resulted in a dramatic drop of the SAP and animal's death. It confirms the idea that a sustained increase in mitochondrial permeability transition with mPTP inductors results in damage to the neurons and impairs mechanisms of medullary cardiovascular control. In this situation, it becomes obvious that inhibiting of mPTP opening could be a suitable way to protect those neurons against their damage. We tested cyclosporine A, melatonin and coenzyme Q as antagonists of mPTP opening. Injections of melatonin (0.7-70 nmol), which is recognized as an inhibitor of mitochondrial permeability transition and ROS scavenger, into the medullary nuclei induced mainly hypertensive responses with relatively short latencies of about 5sec; the maximum response was observed in 30-40 sec, on average, and in most cases the initial level of the

SAP recovered in 2-3 min. In rats with genetically determined hypertension, injections of 0.7 nmol of melatonin into the medullary nuclei under study were mainly accompanied with hypertensive responses which were more intensive than those in normotensive rats. These data suggest that inhibiting of the mitochondrial permeability transition in the medullary neurons can cause a temporal increase in their functional activity. Injections of cyclosporine A into the cardiovascular nuclei induced the SAP shifts which depended on its dose: it might either increase or reduce the SAP. Simultaneous injections of both melatonin (0.7 nmol) and PAO (0.5 nmol) into the medullary nuclei under study induced an initial short-term and statistically insignificant drop in the SAP, which was followed with an increase in the SAP. Effects of injections of small doses of PAO into nuclei under study were attenuated after preliminary administration of a substrate for synthesis of endogenous NO (L-arginine), suggesting neuroprotective effect of physiological concentrations of NO. The results reported herein demonstrate that NO and mPTP play an important role in the cardiovascular control in both the norm and hypertension.

4 Peculiarities of NO contribution to the nervous cardiovascular control in diabetes mellitus

A variety of studies demonstrated that vascular diseases are major complications in patients with diabetes mellitus. It is generally agreed that glucose is the principal regulator of the insulin gene. The mechanisms by which hyperglycemia injures vascular cells and leads to functional changes include a lot of different factors. Enhanced oxidative stress and impairments in nitric oxide synthesis are believed to be of considerable importance in the pathogenesis of diabetic vascular diseases. It is also generally accepted that diabetes contributes to variety of CNS complications, but peculiarities of NO contribution to the nervous cardiovascular control in this pathology, to a large extent, remain to be determined. In our experiments, injections of L-arginine into the medullary nuclei (NTS, AMB, LRN and PMn) of the rats with streptozotocin-induced hyperglycemia resulted in the hypotensive responses but diminished as compared with control rats with normal blood glucose levels; we also observed an increased number of hypertensive responses following L-arginine injections into the medullary nuclei. In those rats with experimental diabetes mellitus, inhibition of NOS-1 with injections of NOS-1 antagonist L-NNA resulted mainly in the hypertensive responses like in rats with normal blood glucose level and in rats with genetically determined hypertension, but it was reduced as compared with both hypertensive rats and those with the blood glucose levels within physiological limits. Taken together, these data indicate that NOS-1 in the neurons within medullary nuclei of the rats with experimental hyperglycemia was not inactivated like in hypertensive rats. NO-synthesizing neurons were likely to be partly damaged, as injections of either L-arginine or L-NNA into the medullary nuclei resulted in less expressed shifts of the SAP as compared with control rats. An increase in the mitochondrial permeability transition with injections of PAO into the cardiovascular nuclei induced the SAP shifts in a dose-dependent manner. Following the mPTP opening, induced by large dose of PAO, the SAP dropped dramatically leading to animal's death. Small doses of PAO did not seem to induce mPTP opening, while they increased the permeability of the outer mitochondrial membrane inducing the hypotensive response. Those responses were less expressed as compared with both normotensive rats and ones with normal blood glucose level. Inhibiting of mPTP opening with either melatonin, coenzyme Q_{10} seemed to have a protective effect on the medullary NO-producing neurons: inhibition of mPTP opening with preliminary administration of melatonin or coenzyme Q_{10} tended to increase the effect of L-arginine injections and significantly prolonged survival time of those animals.

For a long time, insulin signaling was thought to be involved in controlling the arterial pressure via an activation of only peripheral mechanisms. The central nervous system was not generally considered to be an insulin-dependent tissue, because the blood-brain barrier was thought to be impermeable to insulin. The situation changed after evidence had been presented that insulin could cross the blood-brain barrier and under certain circumstances act on the central nervous system. Since insulin and its receptors are widely distributed in the brain of the rat [9], it suggests the significant role of insulin in the CNS. Injections of insulin in the NTS were

shown to induce depressor responses and bradycardia [7]. To study if insulin is involved in controlling central cardiovascular effects, we provided its injections into the cardiovascular nuclei (NTS, AMB, PMn and LRN) of control rats, with blood glucose level within physiological levels, and rats with experimental hyperglycemia. In our experiments, injections of insulin (125 nl, 40 IU/ml) into the populations of the neurons within the cardiovascular nuclei of control rats resulted usually in the hypotensive responses, which suggests that insulin might be involved in the central cardiovascular control via activation of insulin receptors on the neurons within the medulla oblongata. In rats with experimental hyperglycemia, injections of the same amount of insulin into the cardiovascular nuclei induced shifts in the SAP diminished as compared with control rats, perhaps resulting from damage of some neurons in those rats, and in some cases we observed hypertensive responses. Preliminary inhibiting of NOS-1 with a specific NOS-1 antagonist 7-nitroindazol inhibited the effects of insulin injections into the cardiovascular nuclei. If we take into account the fact that in rats with experimental hyperglycemia haemodynamic responses on either activation or inhibiting of NOS reduced as compared to control ones, these results support an idea that effects of insulin on the medullary nuclei, to a large extent, depend on NO availability in the cardiovascular neurons.

***References*:**

1. G.C.Brown, V.Borutaite, Inhibition of mitochondrial respiratory complex 1 by nitric oxide, peroxynitrite and S-nitrosothiols, *Biochem. Biophys. Acta* Vol.1658, 2004, 44-49
2. Brookes P.S., E.P.Salimas, K.Darley-Usmar, et al., Concentration-dependent effects of nitric oxide on mitochondrial permeability transition and cytochrome c release, *J.Biol.Chem*., vol. 275, 2000, No.7, 20474- 20479
3. Chowdhary S and N.Townend, Role of nitric oxide in the regulation of cardiovascular autonomic control, *Clin. Sci*., Vol. 97, 1999, 5-17
4. Crompton M, The mitochondrial permeability transition pore and its role in cell death, *Biochem.J*., Vol. 34, 1999, 233-249
5. Demougeot C., Prigent-Tessier A., Marie C., A.Berthelot, Arginase inhibition reduces endothelial dysfunction and blood pressure rising in spontaneously hypertensive rats, *J.Hypertension*, Vol. 23, No 5, 2005, 933-934
6. Duchen M.R., Mitochondria in health and desease: perspectives on a new mitochondrial biology, *Mol. Aspects Med.*, Vol. 25, 2004, 365-451
7. Huang H-N., P.-J. Lu, W.Ch.Lo, et al. , In situ Akt Phosphorylation in the Nucleus Tractus Solitarii is involved in central control of blood pressure and heart rate, *Circulation*, Vol.110, 2004, 2476-2483
8. Halestrap A.P, S.J.Clarke and S.A.Javadov, Mitochondrial permeability transition pore opening during myocardial reperfusion – a target for cardioprotection, *Cardiovasc.Res.,* Vol. 61, 2004, 372-385
9. Havrankova J, J.Roth, M.Brownstein, Insulin receptors are widely distributed in the central nervous system of the rat, *Nature (Lond.*), Vol. 272, 1978, 827-829
10. Hong Yu., R.K.Iyer, RM, Kern, et al., Expression of arginase isozymes in mouse brain, *J.Neurosci. Res*., Vol. 66, No3, 2001, 406-422
11. Javadov S. and M.Karmazyn, Mitochondrial permeability transition pore opening as an endpoint to initiate cell death and as putative target for cardioprotection, *Cell. Physiol. Biochem*, Vol. 20**,** No. 1/4, 2007, 1-22
12. Kishi T., Hirooka Y., Sakai K. et al., Overexpression of eNOS in the RVLM causes hypertension and bradycardia via GABA release, *Hypertension*, Vol. 38, No 4, 2001, 896-904
13. Kroemer G., B.Dallsports, and M.Resce-Rigon, The mitochondrial death/life regulator in apoptosis and necrosis, *Annu. Rev. Physiol*., Vol. 60, 1998, 619-642.
14. Krukoff T.I, Central actions of nitric oxide in regulations of autonomic functions, *Brain.Res*., Vol. 30, 1999, 52-65
15. Mungrue I.N., D.S.Bredt, D.J.Stewart, and M.Hasain, From molecules to mammals: what's NOS got to do with it? *Acta Physiol.Scand*., Vol. 179, 2003, 123-135
16. Packer M.A., and M.P.Murphy, Peroxynitrite causes calcium efflux from mitochondria, which is prevented by cyclosporin A, *FEBS Lett*., Vol. 345, 1994, 237-240
17. Poderoso J.J., MC.Carreas, C.Lisdero et al., NO inhibits electron transfer and increases superoxide radical production in rat heart mitochondria and submitochondrial particles, *Arch. Biochem. Biophys*., Vol. 328, 1996, 83-92
18. Shapoval L.N, V.F.Sagach, and L.S.Pobegailo, Nitric oxide influences ventrolateral medullary mechanisms of vasomotor control in the cat, *Neurosci. Lett*., Vol. 132, 1991, 47-50
19. Shapoval L.N. Nitric oxide and nervous control of cardiovascular function, *In: Receptors,Channels and Messengers, Eds.P.G.Kostyuk, EA.Lukyanetz, DUS*, Kiev, 2005, 318-337

20. Shapoval L.N., Sagach V.F., Pobegailo L.S., Dmytrenko O.V., Contribution of NO-synthase and arginase to medullary cardiovascular control in rats, *Abstract book of the 3-d FEPS Meeting,* Nice, 2003, 37
21. Shapoval LN, OV.Dmytrenko, LS.Pobegailo et al., Hemodynamic responses induced by modulation of nitric oxide system and mitochondrial permeability in the medullary cardiovascular nuclei of rats, *Neurophysiology*, Vol. 39, No3, 2007, 232-244
22. Shapoval LN, Sagach VF, Pobegailo LS, et al., Role of nitric oxide in effects of intramedullary injected GABA on blood circulation, *Fiziol.zhurn*., Vol. 51, 2005, 43-50
23. Shapoval LN, VF.Sagach, Pobegailo L S., et al., Involvement of Nitric Oxide in the Medullary Control of Circulation in normotensive rats, *Neurophysiology*, Vol. 34, No. 4, 2002, 294-302
24. Topal G., Brunet L. Walch et al, Mitochondrial arginase II modulates nitric oxide synthesis through non-freely exchangeable L-arginine pools in human endothelial cells, *J.Pharmacol.Exp.Ther*., Vol. 318, No3, 2006, 1368-1374
25. Zanzinger J, H.Seller, Species differences in the distribution of nitric oxide synthase in brain stem regions that regulate sympathetic activity, *Brain Res*., Vol. 764, 1997, 265-268
26. Zanzinger J, Role of nitric oxide in neural control of cardiovascular functions, *Cardiovascul. Res*., Vol. 43, 1999, 639-649
27. Zhan C., Hein TW, Wang W et al., Upregulation of vascular arginase in hypertension decreases nitric oxide- mediated dilatation of coronary arteries, *Hypertension*, Vol. 44, No 6, 2005, 935-943
28. Zoratti M, I.Szabo, and U.De Marchi, Mitochondrial permeability transitions: how many doors to the house? *Biochem. Biophys. Acta*, Vol. 1706, 2005, 40-52