Recent trends in the nitrergic nervous system

P. Roy, G. Venkat Ramana, M.U.R. Naidu, P. Usha Rani

ABSTRACT

The late 20th century witnessed the novel discovery of “Nitrergic” or “Nitroxidergic” innervation of the vascular smooth muscles, their role as a vasodilator in cerebral, ocular and penile vasculature, as well as their reciprocal action to adrenergic vasoconstriction. The identification of this nerve as a postganglionic parasympathetic nerve, the discovery of autonomic efferent nerves where Nitric oxide (NO) is the neurotransmitter (NTM) to blood vessels, its physiological role in the control of smooth muscle tone, and the pharmacological implications of NO have been reviewed. This will aid an in-depth analysis of vascular dysfunctions and the development of strategic pharmacotherapeutic interventions with time.

KEY WORDS: Erectile dysfunction, nitrergic neurotransmission, nitroxidergic, neurotransmitter, vasodilatation

Organic nitrates (Nitroglycerine) and sodium nitroprusside release NO which facilitates vascular smooth muscle relaxation and thus, act as NO donors. These NO donors substitute for endogenous NO deficiency, known as “Endothelial Replacement” or “Prodrug” which are effective in different forms of vasospastic disorders.[1]

The non-adrenergic non-cholinergic (NANC) inhibitory neurons in various smooth muscles were discovered in the 1970s. Burnstock (1972), Lundberg (1981), Ousman (1988) reported the neurotransmitter role of endogenous relaxants i.e. Substance P, VIP (vasoactive intestinal peptide), CGRP (calcitonin gene related peptide), ATP etc. The role of NO as a neurotransmitter (NTM) is known since 1989. But unlike the then existing definition of neurotransmitters the NO radical is an inorganic, labile, gaseous molecule with low molecular weight.[2]

The nerve whose transmitter function depends on the release of NO is called “nitroxidergic” (Toda et al., 1991b; Toda and Okamura, 1991a, 1992c) or “nitrergic” (Rand 1992). However, the NO Nomenclature Committee of the International Union of Pharmacology (chairman: Paul M. Vanhoutte) has chosen “nitrergic” as the official name.[3,4]

Neurogenic NO released from the autonomic efferent nerve fibers plays a key role as NTM in blood vessels i.e. it maintains smooth muscle tone of the cerebral and ocular blood vessels leading to their vasodilatation, and smooth muscle relaxation of corpus cavernosum leading to initiation and maintenance of penile erection.[5]

Discovery and hypothesis

The NANC vasodilator nerves were first discovered in dog cerebral arteries using nicotine. Then various smooth muscle tissues in different animals were tested by different methods, using agonists and antagonists also. After excluding the effects of other autonomic nerves and NTMs, the hypothesis of NO being a NTM in the vasodilator nerve to blood vessels was made.[3,4]

Process of nitric oxide synthesis

Electrical impulse (1-20 Hz) or nicotinic stimulation (10^-6-10^-4 M) generates an action potential in the nerve cell membrane via the pterygopalatine ganglion or presynaptic nicotinic receptor respectively. Thus, depolarization of the nerve terminal leads to opening of the slow channels and calcium (Ca++) influx along the concentration gradient. Further, release of Ca++ from intracellular storage sites results in increased levels of intracellular Ca++. This Ca++ binds with calmodulin (CaM) to form a complex which activates protein kinases. The latter phosphorylates a protein that activates neuronal Nitric Oxide Synthase (nNOS) in the presence of the calcium-calmodulin (Ca-CaM) complex and co-factors.[5]

Meanwhile, L-Arginine is obtained from extracellular space via cationic amino acid transporter (CAT) in the cell membrane, from intracellular sources of protein degradation or by endogenous synthesis i.e. in the liver through the urea cycle, and is also recycled from L-citrulline in neuronal cells and the kidneys. This L-arginine is the substrate for nNOS which helps in the synthesis of NO and L-citrulline in the presence of oxygen. Thereafter, the signal transduction system for smooth muscle relaxation involves activation of soluble guanylyl cyclase (GC) by NO. Activated GC catalyses conversion of GTP to cGMP. cGMP activates protein kinase G while it inhibits L-type Ca++ channels and thus, calcium entry into cells. Activated protein kinase G ultimately plays a vital role in the reduction of intracellular free Ca++ levels and Ca++ sensitivity.
Thus, Ca\textsuperscript{2+}-dependent activation of myosin light-chain kinase is prevented resulting in muscle relaxation (Figures 1, 2).

The mechanism of release of NO as a NTM is differently modulated by arginine as well as calcium. Calcium influx plays a vital role in nitrergic neurotransmission as evidenced by the attenuation of exocytic NTM release with low Ca\textsuperscript{2+}. Besides inhibition of Ca\textsuperscript{2+} influx through N-type channels by \(\omega\)-conotoxin, effectively reduces nitrergic transmission (Figure 1).

The rate of NO production is determined by the enzyme activity as well as by availability of substrate and cofactors. The isoforms of nitric oxide synthase (NOS) are inducible (iNOS/NOS-2, induced by cytokines) and constitutive (cNOS, activated by Ca\textsuperscript{2+} influx and Ca-CaM complex). iNOS present in inflammatory cells, vascular smooth muscle cells and endothelial cells responds to pathological states. cNOS is present under physiological conditions mainly in the endothelium and neurons known as eNOS (NOS-3) and nNOS (NOS-1) respectively.
L-arginine, the only endogenous substrate of NOS, plays an important role in NO production during nervous system development and in disease conditions e.g. stroke, multiple sclerosis, Parkinson’s disease, and HIV dementia. Further, cellular NO production depends on exogenous L-arginine concentration despite theoretical saturation of NOS enzymes with intracellular L-arginine. This phenomenon is known as “Arginine paradox” which may be explained by the following: (1) L-arginine-induced insulin, which has vasodilatory actions. (2) Neither extracellular nor intracellular concentration determines the NOS activity but rather the L-arginine amount transported across the plasma membrane may do so. (3) Endogenous NOS inhibitors (NG, NG-dimethyl-L-arginine, L-citrulline, argininosuccinic acid and agmatine) reduce the enzyme sensitivity to L-arginine. (4) Intracellular L-citrulline is a potent NOS inhibitor so that the cells may need extra L-arginine to compete with L-citrulline inhibition.

Various agents like oxyhemoglobin, superoxide etc. which affect the release and/or function of NO as a NTM (Figure 1) are discussed in subsequent paragraphs.

**Confirmation of hypothesis**

The above hypothesis that NO is a NTM holds good because electrical or nicotinic stimulation of perivascular nerves in animal cerebral arteries causes vasodilation. And this vasodilatation is inhibited by NOS inhibitors like L-NMMA (N\(^{\omega}\)-monomethyl-L-arginine) (which is reversed by L-arginine), L-NA, L-NAME and N-iminoethyl-L-ornithine, Mg\(^{2+}\), Cd\(^{2+}\), calmodulin inhibitors (o-7, calmidazolium) and on removal of external Ca\(^{2+}\).[10]

On removing the endothelium, the actions of NOS inhibitors are abolished. Further, with exogenous application of NO, i.e. acidified NaNO\(_2\) or NO donors (nitroglycerine, sodium nitroprusside), dose-dependent cerebral vasodilatation occurs which is abolished by oxy Hb (NO scavenger), methylene blue and [1,2,4]oxadiazole[4,3-a]quinoxalin-1-one (ODQ) (inhibitors of soluble GC). But stimulation of superfused cerebral artery strips sans endothelium by electrical pulses or nicotine causes release of nitrates and nitrites also.[36]

**Confirmation of the role of NO as neurotransmitter (NTM)**

Antioxidants i.e. pyrogallol, hydroquinone, duroquinone inhibit NO-mediated vasodilation whereas Superoxide dismutase (SOD) enhances such response. In bovine retractor penis muscle and mouse anococcygeus muscle, when the effect of SOD is inhibited by administering Di-ethyl-di-thio-carbamate, the antioxidants inhibit NO response. Thereafter SOD again reverses this inhibition. Thus, endogenous SOD protects neurons from superoxide anions and free radicals and NO is the NTM involved in the vasodilator response.[11,12]

**Localization of neurons containing NOS**

The nitrergic neurons are localized by reduced nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d) histochemistry, double-label immunohisto-chemistry and immunohistochemistry using antibodies against nNOS, neuropeptideY (NPY) and GRP.[13,14]

The nNOS immunoreactive nerve fibers are located in the proximal and distal portions of the middle cerebral artery (MCA), in the dog. They run irregularly along the arterial walls, at times forming fiber bundles and some times ramifying repeatedly. The thick fibers are located in the outer layer of adventitia while the thin fibers lie towards the lumen. The same is the case with the basilar artery. While fibers to the MCA originate from the ipsilateral pterygopalatine ganglion in dogs, in case of rats they originate from the otic ganglion. Similar nitrergic nerve supply is also found in human cerebral arteries.[15,16]

**Tracing the origin of nitrergic nerves**

With electrical stimulation of the sphenopalatine ganglion and facial nerve there is increased cortical blood flow in mammals which is not mediated by ACh (acetylcholine). And this flow decreases in the presence of L-NAME. Further, a nerve action potential generated in the superior salivatory nucleus delivers central information through the geniculate and pterygopalatine ganglion to the cerebral artery and its branches. This regulates their vascular tone under resting and stimulated conditions. Since the histologically confirmed superior salivatory nucleus is a known source of cholinergic pregangli-onic neuron, only the postganglionic nerve contains both nitrergic and cholinergic neurons.[17]

Histochemical studies also confirm the sites releasing NO i.e. from neurons and the endothelium. Moreover, after damaging the pterygopalatine ganglion, the NOS-containing neurons and the vasodilator response to nerve stimulation in the cerebral arterial wall disappears after one week.[18]

**Pharmacological basis**

*In vitro* studies in various mammals with electrical and chemical stimuli produce vascular smooth muscle relaxation in a frequency and concentration-dependent manner, respectively, in partially contracted cerebral arteries. The degree of response varies in different species, cerebral arteries and age groups depending upon the density of NADPH diaphorase/NOS in nerve fibers and the density of nicotinic receptors.

Further, the mechanism of vasodilation induced by elec-
trical and nicotinic stimuli seems to be different. This is because the response to electrical stimuli is potentiated by inhibitors of amine transporter while it is abolished by TTX, L-NA and ω-CT (N-type Ca⁺⁺ channel blocker) but not by C₆ (hexamethonium). On the other hand the response to nicotine is abolished by C₆ other ganglionic blockers and amine transporter inhibitors (e.g. cocaine, tricyclic antidepressants like imipramine and desipramine, propranolol, phenotlamine, bretylium, etc.) but is resistant to tetrodotoxin (TTX).

While muscarinic receptor activation evokes NO release from nNOS-expressing endothelium, at least two populations of presynaptic nicotinic receptors (TTX-sensitive and TTX-insensitive) are involved in nicotine-induced amine release. The acetylcholine-stimulated nicotinic receptors in nitrergic nerves are responsible for release of NO because atropine completely blocks muscarinic receptors and partially inhibits nicotinic receptors. But cerebral vasodilatation in the presence of high concentration of acetylcholine (ACh) (10⁻⁴ M) is resistant to atropine, blunted by cocaine and abolished by C₆.

So, it is hypothesized that ACh and nicotine probably act on nicotinic receptors in the ganglionic cell membrane increasing ion permeability. This leads to depolarization of cell membrane to a level of firing generated action potential. Further, with electrical stimulation Ca⁺⁺ influx leads to exocytosis of transmitter vesicles in adrenergic and cholinergic nerves or activates nNOS in nitrergic nerves.[31]

**Interaction of nitrergic, cholinergic and adrenergic nerves**

Often a neuronal network shows immunoreactivity to NOS, acetylcholinesesterase (AChE) / choline acetyl transferase, VIP, tyrosine hydroxylase and/or NADPH diaphorase. While in some neurons there is coexistence of NOS and VIP: some others have VIP and AChE coexisting. So while NO mainly maintains cerebral arterial tone, VIP etc. modulate the synthesis or release of neuronal NO.[19]

ACh and other muscarinic agonists inhibit nitrergic nerve-mediated cerebral vasodilatation which is abolished by muscarinic receptor antagonists (Figure 3). So the former is due to pre-junctional M₁ receptor activation by neurogenic ACh. This is not seen in dogs neither in vivo nor in the presence of exogenous NO. Further, the prejunctional α₁ adrenoceptors interfere with the synthesis or release of NO but the β-adrenoceptor-cAMP system plays no role. Effects of NO donors and NOS inhibitors on neurogenic NE in the peripheral vasculature in animal studies are controversial. This leads to the suspicion that NO, possibly from nitrergic nerves, prejunctionally inhibits/modulates adrenergic nerve function in some mammalian vasculature. And NOS inhibitors potentiate adrenergic vasoconstriction. This is because nitrergic nerves release NO which acts as physiological antagonist at postjunctional smooth muscle, thus opposing the contractile action of NE. Probably, cGMP is the inhibitory modulator of NE release from adrenergic nerves.[17,20]

The response to nitrergic stimulation seems to be similar to that of S-nitrosothiols i.e. S-nitroso-L-cysteine (NOCys), nitrosoglutathione (GSNO) and S-nitroso-L-acetyl-D,L-penicillamine (SNAP). But then the chemical nature of the nitrergic NTM is not identical in all tissues, probably depending on the local availability of the compounds that form NO adducts. The nitrosothiols like NOCys and GSNO release NO in some tissues.[21,22]

**Clinical significance**

The major effects of NO are mediated by the second messenger, cGMP, or by reactive nitrogen derivatives. The latter are produced by interaction of NO with molecular oxygen and superoxide radicals. While some conditions like hypertension, angina and impotence are due to deficiency of NO, some other conditions like circulatory shock, stroke and inflammation are due to excess of NO.[6,7]

**Role in the central nervous system (CNS)**

NO plays a major role as a NTM and/or a modulator of ligand-gated receptors in the CNS. It has short- and long-term potentiating effects on excitatory amino acids in brain development and memory. It has a protective role in neuronal degeneration e.g. NOS-2 is implicated in Alzheimer’s disease, multiple sclerosis and Huntington’s disease. But high NO levels can kill cells indiscriminately.

7-Nitroindazole (NOS-1 inhibitor) and L-NAME have significant anti-noceiceptive effects. The former also reduces signs of opioid withdrawal in animal models, reduces cerebral blood flow, and reduces size of cerebral infarcts in animal models. Ischaemic cerebral damage is associated with NOS-3 deficiency in mice. NO and cGMP might also play a role in epileptic seizures.[6,7]

**Nitric oxide in cerebral vasospasm after subarachnoid hemorrhage (SAH)**

Late cerebral vasospasm usually follows SAH in one to two weeks when oxyHb is formed from lysis of clots around the
Nitricergic nerves are found in the choroid plexus from the pterygopalatine ganglion, in retinal vessels, in ciliary arteries and in ophthalmic arteries. While the tone of the external ophthalmic artery is maintained by the nitricergic vasodilator nerve as well as the adrenergic vasoconstrictor nerve, the internal one is predominantly innervated by the nitricergic nerve. High NO levels cause destruction of photoreceptor cells in the retina.\[26\]

Nitricergic NO and impaired ocular circulation and glaucoma

Application of topical nitroglycerin and other NO donors, nifedipine (new drug liberating NO, also has beta- and alpha-adrenoceptor blocking actions) results in fall of intraocular tension, enhanced ocular blood flow and protection of ocular fundus from neuronal damage. Similar, neuronal and endothelial NO also help in the prevention of atherosclerosis and thrombosis. Thus, the mediation of neurogenic and endothelial NO could also impart neuroprotection to glaucomatous eyes and delay the development of the disease.\[3,26,27\]

Role in the cardiovascular system (CVS)

Nitricergic NO and control of blood pressure (BP)

Nitricergic NO in migraine and cluster headache

Primary headache comprises migraine, cluster headache and tension-type headache. Of these the origin of migraine could be due to genetic, hormonal, dietary factors, chemicals, stress, lack of sleep and bright light. These factors seem to trigger vasoconstriction followed by reactionary overfilling of some blood vessels in the brain. Or they stimulate hyper-reactive nerve cells in the brain leading to vasoconstriction followed by vasodilation. Or they may trigger release of pro-inflammatory chemicals in the brain causing vasodilation. The release of NO from blood vessels, perivascular nerve endings or from brain tissue is an important molecular trigger mechanism evidenced during such vasodilation which is also associated with demonstrable calcium channelopathy (pathology of the calcium channels). So while some cases are successfully treated with NOS inhibitors, some others are relieved by flunarizine (Ca\(^{2+}\) channel blocker) or sumatriptan (smooth muscle constrictor).\[23-25\]

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The vascular tone in mammals is reciprocally regulated by sympathetic vasoconstrictor and nitricergic vasodilator nerves. Further, predominant innervations differ among different vessels in different organs and tissues as well as in different mammals.

NO plays a significant role in the central regulation of BP. With NO donors or NOS inhibitors injected microscopically at different sites of the brain i.e. rostral ventral-lateral medulla (attenuates renal nerve sympathetic activity), nucleus tractus solitarius (stimulation of NMDA/ N-Methyl-D-aspartate receptors), and paraventricular nucleus (mediation of GABA/ gamma amino butyric acid) there is a fall in systemic BP, Whereas, with injection in the caudal ventrolateral medulla the opposite response is seen.

The NOS-containing nerves are located in the afferent nerves, several nuclei in the brainstem, preganglionic parasympathetic and sympathetic nerves. Neurogenic NO acts mainly as neuromodulator in the CNS (Central nervous system) and as NM in the peripheral nervous system, particularly in the postganglionic parasympathetic neurons. The sympathetic nerve function, mainly postjunctionally and also prejunctionally is blunted by nerve-derived NO.

Apart from the central role of NO in the regulation of BP, NO is also involved in the baro reflex mechanism and control of vagal activity. NO interacts with angiotensin II in the sympathetic nervous system. In rat kidneys, basal release of nNOS-derived NO attenuates the proximal fluid uptake, which is probably responsible for the depressor action of NO. Further, this attenuation is abolished by acute renal denervation, thus, confirming the involvement of renal sympathetic nerves. Whether the tubular epithelia are innervated by nitricergic nerves or not is yet to be determined. Moreover, how nNOS-derived NO is involved in the adaptive and compensatory mechanisms under chronic hypertension remains to be elucidated.\[3,28\]

NO donors and cGMP analogs are indicated in hyperlipidemic patients with atherosclerosis who have reduced endogenous NO and have endothelial dysfunction. Further, NO being an antioxidant, it prevents oxidation of low density lipoproteins (LDL) and thus prevents foam cell formation in the vascular wall.\[3\]

Role in the respiratory system

In pulmonary vessels endogenous/ inhaled NO reaches pulmonary arterioles and reduces vascular resistance. So NO inhalation is useful in the treatment of PAH (Pulmonary artery hypertension) and RDS (Respiratory distress syndrome).\[3,6\]

Role in the reproductive system

In the uterine artery the nitricergic nerves arise from the pelvic paracervical ganglion. Pregnancy and estradiol therapy induce Ca\(^{2+}\) -dependent NOS, augmenting the release of NO from neurons and the endothelium.\[31\]

Abnormal cytotoxophiloblast invasion of spiral arterioles results in decreased uteroplacental perfusion. This causes widespread dysfunction of maternal vascular endothelium. And thus evolves the pathogenesis of pre-eclamptic toxemia (PET) of pregnancy. Long-term inhibition of NOS leads to syndromes like intrauterine growth retardation (IUGR), small-for-date babies etc., similar to those in PET, which respond well to high doses of L-arginine. So inhibition of NOS activity and/or NO
function could be a causative factor involved in the development of PET.

On the other hand, there can be an increase in NOS activity and NO metabolites in the placenta and plasma of patients with PET and eclampsia, which is directly related to the severity of PET. Thus, these parameters are the “diagnostic indicators” of eclampsia. Increase in these parameters is due to a physiological adaptive response in the patient in order to overcome the increased vascular resistance, and to minimize platelet and leucocyte adhesion to the surface of placental villi. PET could be a maladaptive state wherein the placenta inadequately responds to the demands for altered blood flow in pregnancy. Other than endothelial-derived NO, there is also neurogenic NO and nitrergic innervation of the human uterine vessels and female genital organs.[3][32]

Nitrergic NO and penile erection

The penis receives three different neuron groups i.e. from the thoracolumbar sympathetic, lumbosacral parasympathetic and lumbosacral somatic pathways. The nitrergic nerves are present in the postganglionic parasympathetic nerves, preganglionic sympathetic and parasympathetic nerves and in the afferent nerves projecting to the sacral parasympathetic nucleus in the penis. The density of such nerve fibers varies with developmental changes, age, hormonal changes and pathological states e.g. it is reduced in diabetics. The corpus cavernosum and penile vessels of both animals and humans are densely innervated by nitrergic nerves.

The functional state of the penis depends upon the balance between the contractile (anti-erectile) and dilating (erectile) factors. These factors are determined by the penile vascular tone and smooth muscle contractility of the corpora cavernosa. The increased NO levels in the corpus cavernosum by nerve stimulation leads to penile erection (see Figure 4). So impairment of the nitrergic nerve function leads to erectile dysfunction (ED) e.g. diabetic impotent men. Thus, there is reduced formation of NO metabolites and cGMP and hampered cavernosal relaxation in men with vascular impotence. The use of nitroglycerine ointment/patch have been tried in some cases.[3][32]

Advancing age, systemic diseases [diabetes, atherosclerosis, hypertension (HTN), renal failure and endocrine disorders], surgical procedures, trauma, drugs (psychotropics), and psychological problems are some of the causative factors of ED. So, any incoordination between the psychological, humoral, neuronal, and vascular factors lead to ED. Further, organic ED is of vascular, neurogenic and cavernosal type. Impaired neuronal relaxation of the corpus cavernosum under pathological states could be due to selective degeneration of nitrergic nerves, defective NO formation due to altered expression or activity of NOS, decreased sensitivity to NO, superoxide anion formation and increased extra-cellular inactivation of NO in the cavernosal smooth muscle or expression of dysfunctional soluble GC. There is improvement in nitrergic nerve function in diabetic rats after treatment with antioxidants and aldose reductase inhibitors. In addition to prophylaxis and reliable treatment of causative factors, ED responds well to treatment with PDE V inhibitor (oral sildenafil).[3][32]

Concurrent use of sildenafil with nitrates in patients of angina sometimes causes severe hypotension and myocardial infarction. In such cases a 6 h gap between the administration of both drugs is essential. Sildenafil may also affect color vision i.e. blue-green discrimination. Moreover, in patients with nerve injury and loss of potency or in those lacking libido, when ED does not respond to sildenafil, treatment with alprostadil, a PGE_1 analog, or with phentolamine injection into the cavernosa or with oral apomorphine (investigational drug which acts by releasing dopamine in the CNS) may prove useful. Patients developing adverse effects with sildenafil may be treated with newer PDE V inhibitors like tadalfil and vardenafil.[9]

Ageing-related attenuated erectile function also responds successfully to long-term oral L-Arginine. Further, in vivo gene transfer of eNOS, alone or with PDE-V inhibitor, improves erectile response to cavernosal nerve stimulation.[3][32]

Role in the immune system

Increased NO secretion by activated macrophages and neutrophils is a vital cytotoxic agent in the human immune defense system. Tumor cell lysis by NO is either due to DNA damage or inhibition of tumor cells’ ribonucleotide reductase or due to inactivation of cytoplasmic enzymes and proteins. L-arginine (NO precusor) enhances the natural killer (NK) and lymphokine-activated killer (LAK) cell activity. It is helpful in immuno-suppressive states like AIDS and cancer.

NO plays a role in tissue damage due to septic shock, heat stroke, ulcerative colitis, cerebral malaria and autoimmune diabetes mellitus. Administration of NOS inhibitors proves beneficial in such cases.[6][7]

Nitrergic NO in acupuncture, axon reflex and neurogenic inflammation

NO facilitates CGRP release from afferent neurons or probably acts as second messenger to CGRP in rat skin because NO plays a role in rat paw edema formation. But in larger mammals, NO from efferent vasodilator nerves and CGRP from sensory nerves mediate axon reflex, acupuncture response, inflammatory and immune response in the skin. Vasodilator response of NO is not seen here.[3]
Role of NO in hypercapnea, hypoxia, hypothermia and autoregulation

The vasodilator response to NO is enhanced by hypercapnic acidosis, possibly due to reduced degradation of NO in animals.\textsuperscript{[31]}

On the other hand, hypoxia causes cerebral vasodilation both by acting on non-vascular cerebral elements as well as by vascular effect in the endothelium, smooth muscles and perivascular nerves. So there is production of many vasodilating metabolites (potassium, H\textsuperscript{+}, prostaglandin, adenosine, NO, cytochrome P450 epoxygenase etc.), neuronal release of excitatory amino acids (leading to cerebral metabolism), direct vasodilator effect on cerebral arteries, decreased calcium influx, production of endothelium-derived cyclooxygenase (leading to the activation of ATP-sensitive K\textsuperscript{+} channels) and activation of ATP\textsuperscript{-} and Ca\textsuperscript{++} sensitive K\textsuperscript{+} channels.\textsuperscript{[34]}

The effects of hypothermia on neurogenic vascular relaxation in normoxia and hypoxia is mediated by neurogenic NO. But in some isolated arterial studies, there is augmentation of vasodilator nerve function during hypercapnea, whereas in others this function is impaired due to hypoxia. Of course, the latter is prevented by hypothermia, amiloride (Na\textsuperscript{+}-H\textsuperscript{+} exchange inhibitor) or by extracellular acidosis. Hypothermia leads to intracellular acidosis which can be beneficial by increasing blood supply to the ischemic sites of the brain.\textsuperscript{[35]}

Autoregulation is defined as the constant maintenance of blood flow to the vital organs in spite of rapid changes in BP. The myogenic theory of the smooth muscle cells which sense and evoke the response partly explains this mechanism. NO plays an important role as an endothelium derived relaxing factor (EDRF) and as NTM of vasodilator nerve.\textsuperscript{[36]}

Pharmacological implications of neurogenic NO

PDE-V (Phosphodiesterase type V) Inhibitors

These inhibit a cGMP-degrading enzyme (PDE-V) e.g. sildenafil, tadalafl, vardenafil, an effective first line treatment for ED. A few cardiovascular side effects have been reported for which safety of its long-term use is still debatable. As observed in rats, if in humans also, the increased rate of cGMP hydrolysis by PDE-V proves a major contributing factor to the impairment of NO-mediated cerebral vasodilation following SAH, then PDE V inhibitors can be used for prophylaxis of cerebral vasospasm also.\textsuperscript{[3,6,37]}

Oral PDE-V inhibitor alone or with inhaled iloprost (stable analog of prostacyclin) is effective in the treatment of pulmonary hypertension. Dosage required is inadequate to lower systemic BP. Sildenafil also ameliorates the effects of inhaled NO withdrawal. PDE-V inhibition improves circulatory disturbances in the brain, eyes, and uterus by vasodilator nerve functions.\textsuperscript{[3,37]}

Free radical scavenger

Edaravone (3-methyl-1-phenyl-2-pyrazoline-5-one) is effective in the prevention and treatment of symptoms in patients with cerebral ischemia. Probably, it scavenges ischemia-generated hydroxyl radicals and thereby prevents damage to the brain and circulating system by restoring the actions of neurogenic and endothelial NO.\textsuperscript{[31]}

\textbf{\textit{Alpha\textsubscript{2}–adrenoceptor antagonists and antimuscarnic agents}}

In some cerebral, retinal, ciliary and uterine arteries and corpus cavernosum, where nitric innervation predominates over adrenergic system, the alpha2–adrenoceptor antagonists potentiate the release of NO and NO function. Non-selective and M\textsubscript{2}-selective muscarinic receptor antagonists also augment the response to nitricergic nerve stimulation.\textsuperscript{[17]}

Neuronal NOS inhibitors

The development of nNOS, eNOS, and iNOS selective inhibitors has been evolving over time. In spite of their limited role in cardiovascular diseases, nNOS-derived NO helps in the development of a hyperdynamic circulation consisting of low BP, low systemic vascular resistance and high cardiac output in liver cirrhosis.

Keeping NOS inhibitor in view, newer line of treatment of migraine, prevention of nerve cell damage in the brain and retina following ischemic insult, and action on tumor vascular resistance, besides others, are being evaluated.\textsuperscript{[39]}

Agents which suppress endogenous NOS inhibitors

Accumulated ADMA impairs NO synthesis which forms the basis for vascular and immune dysfunction in CRF patients. Increased plasma levels of endogenous NOS inhibitors are seen in cases of renal failure, DM, HTN, and hypercholesterolemia. While results with administration of exogenous ADMA is controversial, a novel protein inhibitor of nNOS (PIN) shows some promising outcome in the regulation of renal function. Human studies are essential to confirm the same.\textsuperscript{[31]}

Conclusion

The nitroglicer vasodilator nerves contribute mainly to regulate the tone in the cerebral blood vessels than in the peripheral ones. This stresses the fact that under physiological conditions in vivo, when cerebral arteries are constricted, blood flow is controlled mainly by the degree of vasodilation. Thus, the maintained vascular tone in the brain may be due to transmural pressure, circulating vasoconstrictors and weak adrenergic nerve activity. In contrast, in the peripheral arteries except for the coronary artery, it is well understood that the vascular tone is regulated mainly by adrenergic nerve activity. Vasodilation is induced mainly by a decrease in adrenergic nerve activity and an increase in nitricergic activity. Moreover about two-thirds of the vasodilation is due to neuronal NO that is activated by continuing impulses from the vasomotor center. The remaining one-third may be due to NO from the endothelium.\textsuperscript{[39]}

In the existing classification of the autonomic nervous system, the parasympathetic nervous system needs to include the nitrogic nervous system as well. More research is required to confirm the etiopathogenesis of diseases associated with depressed or excess NO formation and develop suitable pharmacotherapy for their treatment.

**References**


