GENERAL REVIEW

UDC: 612.015::[616.853-092.9+612.337
DOI: 10.2298/VSP131025051S

Multidisciplinary approach to nitric oxide signaling: Focus on the gastrointestinal and the central nervous system

Multidisciplinarni pristup signalizaciji posredovanoj azot-monoksidom: gastrointestinalni i centralni nervni sistem u fokusu

Marija Stojanović, Ljiljana Šćepanović, Dragan Hrnčić, Aleksandra Rašić-Marković, Dragan Djuric, Olivera Stanojlović

Institute of Medical Physiology "Richard Burian", Faculty of Medicine, University of Belgrade, Belgrade, Serbia

Key words:
nitric oxide; synaptic transmission; duodenum; epilepsy; homocysteine.

Ključne reči:
azot monoksid; transmisija, sinaptička; duodenum; epilepsija; homocistein.

Introduction

Nitric oxide (NO), with close historical ties to cardiovascular physiology, is an important endogenous mediator, the most potent natural vasorelaxant known, involved in many biological functions [1]. NO acts as an intra/intercellular signalling molecule and is a mediator in almost all organ systems [2]. NO is known to occur in many cells types, including vascular endothelial cells, neurons, and epithelial cells [3]. The transmitter of nonadrenergic, noncholinergic (NANC) inhibitory neurons has been the subject of hundreds of investigations over the past 3 decades. Recent evidence suggests that NO may serve as a NANC inhibitory signaling molecule in the gastrointestinal (GI) tract. NO serves as the primary enteric inhibitory neurotransmitter in GI muscles, and nitrergic neurons regulate gut tone, phasic contractile amplitude and frequency, and inhibitory reflexes [4].

In the central nervous system (CNS) NO is involved in some major processes such as memory through long-term potentiation (LTP) and learning [5]. This gasotransmitter also contributes to a pathogenesis of epilepsy. The role of NO in the generation of epilepsy is contradictory since there is evidence of its proconvulsive and anticonvulsive effects [6]. In this review, we will discuss about the possible role of NO as neurotransmitter in the GI and CNS, with focus on the contribution of NO-mediated signaling pathways in the GI motility and CNS excitability.

Physiological functions of nitric oxide

In 1978, Furchgott [7], discovered a substance in endothelial cells that relaxes blood vessels, calling it endothelium-derived relaxing factor (EDRF), then he had worked out EDRF's nature and mechanism of action. It has been identified to be NO, an important compound in many aspects in both physiological and pathological conditions. NO is biosynthesied from L-arginine by the NO synthase. NO synthesis and release is mediated through the activation of NO synthase (3 isoforms of NO synthase, NOS enzymes) by an elevation of cytoplasmic Ca^{2+}, conversion of L-arginine to NO and L-citrulline, NO diffuses passively into the extracellular fluid. Although NO is made by a cytosolic enzyme, it is a highly permeable molecule that can easily diffuse out of the cell that makes it [8]. NO is a tasteless, colourless gas. It is rapidly absorbed *via* the pulmonary vasculature directly into the bloodstream. The mechanism of NO action is not fully understood, but many of its actions are mediated by the activation of guanylate cyclase, which results in an increase in the concentration of cyclic guanosine 3',5'-monophosphatate (GMP) in smooth muscle. The enhanced production of cyclic GMP that results from activation of guanylate cyclase may result in: activation of GMP-dependent protein kinase or direct actions of cyclic GMP on ion channels or other second messenger systems may also be activated by NO (non-cGMP dependent NO effects). NO displaces nitrogen and increase the volume of

Correspondence to: Olivera Stanojlović, Institute of Medical Physiology "Richard Burian", Faculty of Medicine, University of Belgrade, Višegradska 26/II, 11 000 Belgrade, Serbia. Phone/Fax: +381 11 3607 106. E-mail: ostanoj@gmail.com

gas in body cavities such as the middle ear, sinuses, pleural space, and GI tract [9]. NO and NO donors (e.g., sodium nitroprusside) cause relaxation of vascular smooth muscle through the accumulation of cyclic GMP or through the direct activation of K^+ channels. Several studies have suggested that NO might decrease the intracellular Ca^{2+} level or reduce the Ca^{2+} sensitivity of the contractile elements, which results in smooth muscle relaxation. Furthermore, the relaxing action of NO has been indicated indirectly by inhibiting the release of the neurotransmitters acetylcholine and substance P [10].

We shall list a number of physiological functions of NO and we can distinguish the following: it is involved in the regulation of blood flow, maintenance of vascular tone, control of platelet aggregation, modulation of the activity of the mastocytes, as a neurotransmitter in the CNS and peripheral nervous system (NANC, neurons), in the nervous control of the cerebral blood flow and in the neuroendocrine regulation or synaptic plasticity [11–15].

On the other hand, NO plays a role in memory formation. NO is a retrograde messenger at N-methyl-D-aspartate (NMDA) receptors mediated synapses. Inhibitors of NO synthesis in any case, block initiation of LTP which involves an NMDA receptor mediated intracellular cascade finishing in lasting modulation of synaptic morphology [16, 17]. Inversely proportional relationship between NO and glutamate, is also described. *In vivo* and *in vitro* studies with NO donors, NO synthase inhibitors and glutamate receptor antagonists have shown that NO increases the release of glutamate in several regions of the brain (hippocampus, striatum, hypothalamus and locus ceruleus) and spinal cord [18, 19].

To maintain the postsynaptic activation, a retrograde communication with the presynaptic component must exist. It has been suggested that the retrograde NO molecule triggers the release of glutamate *via* cyclic GMP-dependent way. The effects of NO on glutamate release depends on the level of NO. Thus, when the concentration is low, NO reduces the release of glutamate in spite of elevated levels of cyclic GMP. But when NO increases the levels of cyclic GMP, an inhibitory effect on glutamate release reverses, indicating that cyclic GMP showed biphasic effects [20]. Morphine-induced impairment of memory formation can be prevented by NO donor [21].

Nitric oxide in gastrointestinal smooth muscle

Opinions concerned with GI system is polarised as to whether or not NO causes nausea and vomiting. It can certainly increase intestinal and middle ear volumes, which may in turn lead to nausea [9]. On the other hand, ghrelin, a gastric peptide, which possesses orexigenic effects, is the endogenous ligand for the growth hormone with stimulating effects on growth hormone and GI motility. Gaskin et al. [22], demonstrated that a sub-threshold dose of N(omega)-nitro-L-arginine methyl ester (L-NAME) significantly blocked the ghrelin-induced increase in food intake. Ghrelin increased NO synthase levels in the hypothalamus-supporting the hypothesis that ghrelin's effects are NO dependent.

In GI smooth muscle, NO or NO donors evoke different responses, including relaxing, contractile effects, relaxations followed by contractions or contractions followed by relaxations, which depend on the compound, tissue and species [23–25]. The nerves whose transmitter function depends on the NO release are called "nitrergic" and such nerves are recognized to play major roles in the control of smooth muscle tone and motility [26]. NO is likely an inhibitory neurotransmitter in the human jejunal longitudinal smooth muscle, acting *via* mechanism mediated by guanylyl cyclase [27]. It was suggested that basal release of NO caused an oscillatory patterns of electrical and mechanical activities. NO is a vasodilator and mediates gastric blood flow and it is responsible for helping to maintain the integrity of the gastric epithelium and the mucus barrier [28].

There are numerous data supporting the hypothesis that NO plays a pivotal role in NANC relaxation or inhibitory junction potential associated with electrical field stimulation or nicotinic agonists in the duodenum, jejunum, and ileum from a variety of mammals, including the human and rat [27, 29]. It was reported that the ability of the nitrergic neurotransmitter to induce relaxation of the rat gastric fundus was influenced by the mechanism used to induce tone, and sarcoplasmic/endoplasmic reticulum Ca^{+2} ATPase appeared to play a role in nitrergic relaxation [30].

Giant migrating contractions of the rat colon, possibly mediated by neuronal release of acetylcholine, appear to be partially suppressed by constitutive release of NO [31]. Nitrergic innervation also contributes to the regulation of the smooth muscle tone in the rat rectum [32].

At the GI level, 2 constitutively expressed isoforms, namely endothelial NO synthase (eNOS) and neuronal NO synthase (nNOS), are expressed basally at the vascular endothelium and the enteric nervous system of the GI tract [33].

However, NO shows a dual behavior: at physiological concentrations, released through the constitutive synthase (cNOS), it regulates house-keeping functions and is responsible for production of NO in physiological context. In physiological conditions, NO acts as an endogenous mediator modulating both, the repairing and integrity of the tissues, and exhibits gastroprotective properties against different types of aggressive agents. In contrast, its overproduction by the inducible isoenzyme (iNOS) exhibits cytotoxic activity because interacting with reactive species producing peroxinitrites and other compounds, which are highly damaging for the tissues. iNOS produces NO in pathophysiological circumstances. High concentrations of NO are related to numerous pathological processes of GI tract including peptic ulcer, chronic gastritis, GI cancer, bacterial gastroenteritis, and celiac or chronic inflammatory bowel diseases. Indeed the adverse action of cigarette smoking on ulcer healing is largely dependent on the deficiency of cNOS and a subsequent depression of gastric blood flow and angiogenesis. To this end, NO may act as a crucial signal to promote endothelial cell differentiation into vascular tubes [34].

Endogenous NO also contributes to the inhibition of acid secretion in the stomach. NO is implicated in mechanisms

maintaining the integrity of the gastric epithelium. In this connection, it regulates gastric blood flow and directly stimulates gastric mucus secretion by activating soluble guanylate cyclase [35]. A blockade of NO production resulted in an impairment of the vascular response and the subsequent alkaline flux in the lumen. This would impair the restitution process. Intragastric administration of hydrochloric acid stimulated a subpopulation of nitrergic, but not cholinergic, myenteric plexus neurons, which might play a role in secretion, vasodilatation, and muscle relaxation [36]. Expression of nNOS in parietal cells suggests a participation of endogenous NO in the regulation of gastric acid secretion [37]. Helicobacter pylori increased pepsinogen secretion from dispersed human peptic cells through a Ca^{2+}- and NO-mediated intracellular pathway [38].

In the GI tract NO participates in the modulation of the smooth musculature tone, such as the regulation of intestinal peristaltism, gastric emptying and antral motor activity. It also regulates acid and gastric mucus secretion, alkaline production, and is involved in the maintenance of mucosal blood flow. NO biology can influence nutrition and be nutritionally modulated to affect mammalian (patho)physiology. NO as modulator of feeding behavior and mediator of GI homeostasis could be used for supplementation as a therapeutic modality for preserving GI health [39]. Presumed mechanisms of relaxation of NO in GIT tract are:

– Cyclic GMP seems to be a key substance for nitrergic inhibitory responses in the most mammalian GI tracts, including that of humans. Cyclic GMP-dependent reduction of cellular free Ca^{2+} without changing the membrane potential;

– Reduce the Ca^{2+} sensitivity of the contractile element [40];

– Cyclic GMP-dependent opening of apamin-sensitive K^+ channels or other types of ion channels to produce hyperpolarization and relaxation;

– Indirectly by inhibiting the release of the neurotransmitters acetylcholine and substance P [41];

– Cyclic GMP-independent mechanisms, such as actions of NO on ion channels involved in muscle contractility, either directly or *via* membrane hyperpolarization.

Stimulation of these nerves, elicits hyperpolarization of postjunctional smooth muscle membranes referred to as inhibitory junction potentials and relaxation [42–44]. These neurons mediate the majority of inhibitory responses in the GI tract and regulate many important physiological reflexes, such as relaxation of the lower esophageal sphincter after swallowing, receptive relaxation of the proximal stomach during eating, and descending inhibition in response to distension [45].

Clear species variations in the functioning of nitrergic nerves were also seen in the distal colon [46].

L-NAME has been used by many investigators to determine the role of endogenous NO in various physiological and patophysiological conditions. In our experiments L-NAME shows increasing of the resting tone, amplitude and frequency of the contractions of the isolated duodenal segments. It has been shown that L-arginine reversed the action of L-NAME. These data confirm the evidence for the participation of the L-arginine-NO pathway in the relaxation of isolated rat duodenum [47].

Nitric oxide signaling modulation by homocysteine in the gastrointestinal system

We wanted to examine the effects of D,L-homocysteine thiolactone (HCT) on duodenal motility, proved to have a prokinetic effect. Homocysteine is a sulfhydryl amino acid derived from catabolism of methionine. As GI smooth musculature is similar to blood vessel's muscles, we investigated how elevated homocysteine levels affect NO mediated neurotransmission in the gut. HCT leads to immediate increase in tone, amplitude and frequency of spontaneous movements of isolated rat duodenum [47]. In the presence of L-NAME, treatment with HCT caused significant increase of resting tone, amplitude and the frequency of the contractions. These results suggest that mechanism of acting of HCT on the duodenal segments contractions are based on the modulation of nitrergic neurotransmission in the gut [47]. We found that NANC relaxations induced by low frequencies of electrical field stimulation were significantly changed in duodenal preparations obtained from duodenal segments treated with HCT. These findings suggest that homocysteine causes an impotrant impairment on NANC innervation of the rat duodenum [47]. Our results show that HCT increases the motility of isolated rat duodenum. They are consistent with the results of Park et al. [48] which suggest that sulfur-containing amino acids like D,L-homocysteine potentiates depolarisation of murine proximal colon cells. These effects include increasing the amplitude and frequency of spontaneous contractions of murine colonic stripes.

Choe et al. [49] in their study investigated the effects of methionine on the contractile activity of human colon smooth muscle *in vitro*. Methionine is a sulfur containing amino acid that is transformed into homocysteine during biometabolism. Their results indicate that methionine increases the amplitude contractions of colonic muscle strips, which supports our results [49].

Nitric oxide-mediated neurotransmission in the central nervous system: focus on hyperexcitability and epileptogenesis

Disorders of the CNS are one of the primary categories in health care system funds expenditures [50, 51]. With the prevalence of 1–2% in the general world population, epilepsy is among the leading neurological disorders [52]. Epilepsy is characterized by paroxysmal occurrence of motor seizures in behavior (different and specific semiology: from tonic-clonic *via* myoclonic to atonic seizurs) and ictal activity in electroencephalography (EEG) (different forms of spiking activity from isolated spikes to generalized bursts/trains of spikes or spike-and-wave discharges depending on the type of epileptic acitivity). Imbalance between excitatory and inhibitory phenomena within the CNS is thought to be the primary mechanisms involved in the process of epileptogenesis [53]. However, many other mechanisms are recognized as potential

parts of epileptogenesis mosaic. Recently, inflammation, as well as gasotransmitters-mediated signaling processes have been identified as one of the important pathways in modulation of epileptic activity [54–57]. Our understanding of the process of epileptogenesis relies on adequate experimental models of epileptic disorder. Therefore, experimental models are unequivocal tools for investigations in epileptogenesis. It should be pointed out that no single model system could be useful for all types of epilepsy [58]. Having that in mind, a number of very useful experimental models of epilepsy were developed up to now. Recently, Stanojlović et al. [59] showed that acute administration of HCT to adult rats significantly alters neuronal circuits, leading to epileptogenic activity in the EEG with characteristic spikes–and-wave discharges (SWD), and convulsive episodes (manifested through 5-grade descriptive rating scale addapted by Stanojlović et al. [59]) in animal behavior. HCT-induced seizures are accepted as a suitable model of generalized epileptic seizures in which coexistence of convulsive and absence-like seizures were proven [60, 61]. This is one of the advantages of this particular epilepsy model which allowed reliable investigations of variety seizure activity modifications, like paradoxical sleep deprivation [62].

NO displays pleiotropic effects in the CNS. All the three NO synthase isoforms have been expressed in the brain. Neurons produce NO mostly by activation of nNOS [63]. Constitutive isoforms of NO synthase are responsible for the synthesis of physiologically vital amounts of NO [64], while inducible NO synthase (iNOS) produces high amounts of NO lasting hours or days [65]. nNOS is found to be mostly expressed in the hippocampus, cerebral cortex, corpus striatum and cerebellum, as well as in some cells of the autonomic nervous system [66]. iNOS has been found to be a major contributor to initiation/exacerbation of the CNS inflammatory/degenerative conditions through the production of excessive NO [67]. iNOS is reported to be highly expressed in brains of humans with epilepsy. In some spontaneously epileptic mouse overexpression of iNOS is also found [68, 69].

The role of NO-mediated neurotransmission in the process of epileptogenesis is highly unpredictable and contradictor in the existing scientific literature, since numerous evidences exists for both its proconvulsive and anticonvulsive activity [6]. The results on the role of NO in epileptogenesis have been recognized to depend, among other factors, on the source of NO production [70–72]. Moreover, different NOS modulation upon generalized seizures along the anterioposterior axis of the brain have been recently proved, showing dependence on vicinity of original epileptic focus [73]. Recently, we showed that the systemic administration of increasing doses of L-arginine in a dose–dependent manner significantly decreased seizure incidence and the number of seizure episodes and the prolonged latency time to the first seizure elicited by the convulsive dose of HCT [74]. On the other hand, pretreatment with L-NAME, in a dose–dependent manner, increased seizure incidence and severity and shortened latency time to the first seizure following the injection with the subconvulsive dose of HCT. In the same study, L-arginine decreased, while L-NAME increased the median number of SWD *per* rat, while duration of individual SWD was not modified. These results showed the functional involvement of NO in the HCT-induced epileptic activity [74].

Further studies have been undertaken in order to investigate the involvement of nNOS in HCT – induced seizures. With this aim, pharmacological inhibition of nNOS by 7-nitroindazole has been applied [75]. Systemic administration of 7-nitroindazole showed tendency to increase seizure incidence, decrease latency time to first seizure, increase number of seizure episodes *per* rat and increase severity of seizures induced by HCT in rats in a dose dependet manner. Therefore, these results were congruent with those obtained using non-selective NOS inhibition. Contribution of iNOS-derived NO in the process of epileptogenesis elicited by HCT was recently demonstrated in this model of seizures using aminoguanidine, as selective iNOS inhibitor [57]. The results of that study showed that pretreatment with aminoguanidine (applied in the three doses) increased convulsive properties, i.e. seizure incidence, the number of seizure episodes *per* rat and severity of HCT-induced seizures, as well as the number and duration of SWDs in EEG. Also, aminoguanidine decreased the latency time to the first seizure episode induced by HCT in the same dose-dependent manner. Quantitative analysis of ictal activity in EEG showed congruent results with those from behavioral assessment in that study. Namely, aminoguanidine pretreatment significantly increased the number and duration of SWD induced by HCT in that study [57].

The interaction between NO and HCT is supposed to involve NMDA receptor complex, as well as interaction of both NO and HCT with glutamate and gamma-aminobutyric acid. These relationships, discussed in details in Hrnčić et al. [74], could contribute to obtained results on NO effects in HCT-induced seizures.

Conclusion

Nitric oxide in low concentration derived from constitutive nitric oxide synthase is cytoprotective by directly acting as an inducer of defense responses in the gastrointestinal tract. However, higher concentrations of nitric oxide from inducible nitric oxide synthase exhibit toxic effects through nitrosative and oxidative stress. These findings suggest that the cholinergic and nonadrenergic noncholinergic inhibitory nerves play important roles in regulating contraction and relaxation of the gut, and nitric oxide plays an important role in nonadrenergic noncholinergic inhibitory nerves of the digestive tract. In addition, a decrease of the action of cholinergic nerves and an increase of the action of nonadrenergic noncholinergic inhibitory nerves by nitric oxide may be largely related to the low pressure in some part of the gut. However, the findings related to the nitrergic innervation may provide us a new way of understanding gastrointestinal tract physiology and pathophysiology and might result in the development of new therapies of gastrointestinal diseases.

We have shown that nitric oxide causes anticonvulsive effects in the experimental model of epilepsy induced by D, L-homocysteine thiolactone.

Further studies are needed to elucidate all nitric oxide effects on the central nervous system, since new clues for dissolving the role of nitric oxide in the central nervous system and especially its disorders, like epilepsy, is of significant importance from physiological, pathophysiological and pharmacological viewpoint.

Acknowledgement

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Grant No. 175032.

R E F E R E N C E S

1. *Ignarro LJ*. Nitric oxide: a novel signal transduction mechanism for transcellular communication. Hypertension 1990; 16(5): 477−83.
2. *Stark ME, Szurszewski JH*. Role of nitric oxide in gastrointestinal and hepatic function and disease. Gastroenterology 1992; 103(6): 1928−49.
3. *Moncada S, Palmer RM, Higgs EA*. Nitric oxide: Physiology, pathophysiology, and pharmacology. Pharmacol Rev 1991; 43(2): 109−42.
4. *Sanders KM, Ward SM*. Nitric oxide as a mediator of nonadrenergic noncholinergic neurotransmission. Am J Physiol 1992; 262(3 Pt 1): 379−92.
5. *Guix FX, Uribesalgo I, Coma M, Muñoz FJ*. The physiology and pathophysiology of nitric oxide in the brain. Prog Neurobiol 2005; 76(2): 126−52.
6. *Hrnčić D, Rašić-Marković A, Bjekić-Macut J, Sušić V, Mladenović D, Djuric D*, et al. Gaseous neurotansmitter nitric oxide: its role in experimental models of epilepsy. Arch Biol Sci 2012; 64(3): 1207−16.
7. *Furchgott RF*. Role of endothelium in responses of vascular smooth muscle. Circ Res 1983; 53(5): 557−73.
8. *Förstermann U, Closs EI, Pollock JS, Nakane M, Schwarz P, Gath I*, et al. Nitric oxide synthase isozymes. Characterization, purification, molecular cloning, and functions. Hypertension 1994; 23(6 Pt 2): 1121−31.
9. *O'Sullivan I, Benger J*. Nitrous oxide in emergency medicine. Emerg Med J 2003; 20(3): 214−7.
10. *Bolotina VM, Najibi S, Palacino JJ, Pagano PJ, Cohen RA*. Nitric oxide directly activates calcium-dependent potassium channels in vascular smooth muscle. Nature 1994; 368(6474): 850−3.
11. *Murad F*. Nitric oxide signaling: Would you believe that a simple free radical could be a second messenger, autacoid, paracrine substance, neurotransmitter, and hormone. Recent Prog Horm Res 1998; 53: 43−59, discussion 59-60.
12. *Kanada A, Hata F, Suthamnatpong N, Maehara T, Ishii T, Takeuchi T*, et al. Key roles of nitric oxide and cyclic GMP in nonadrenergic and noncholinergic inhibition in rat ileum. Eur J Pharmacol 1992; 216(2): 287−92.
13. *Palmer RM, Ferrige AG, Moncada S*. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 1987; 327(6122): 524−6.
14. *Rand MJ*. Nitrergic transmission: nitric oxide as a mediator of non-adrenergic, non-cholinergic neuro-effector transmission. Clin Exp Pharmacol Physiol 1992; 19(3): 147−69.
15. *Rand MJ*. New perspectives in neurotransmission: nitric oxide, the mediator of nitrergic transmission. Proc Aust Physiol Pharmacol Soc 1992; 23: 1−6.
16. *Hölscher C, Rose SP*. An inhibitor of nitric oxide synthesis prevents memory formation in the chick. Neurosci Lett 1992; 145(2): 165−7.
17. *Lange MD, Doengi M, Lesting J, Pape HC, Jüngling K*. Heterosynaptic long-term potentiation at interneuron-principal neuron synapses in the amygdala requires nitric oxide signalling. J Physiol 2012; 590(Pt 1): 131−43.
18. *Prast H, Tran MH, Fischer H, Philippu A*. Nitric oxide-induced release of acetylcholine in the nucleus accumbens: role of cyclic GMP, glutamate, and GABA. J Neurochem 1998; 71(1): 266−73.
19. *Trabace L, Cassano T, Tucci P, Steardo L, Kendrick KM, Cuomo V*. The effects of nitric oxide on striatal serotoninergic transmission involve multiple targets: an in vivo microdialysis study in the awake rat. Brain Res 2004; 1008(2): 293−8.
20. *Sequeira SM, Ambrósio AF, Malva JO, Carvalho AP, Carvalho CM*. Modulation of glutamate release from rat hippocampal synaptosomes by nitric oxide. Nitric Oxide 1997; 1(4): 315−29.
21. *Rezayof A, Amini R, Rassouli Y, Zarrindast M*. Influence of nitric oxide on morphine-induced amnesia and interactions with dopaminergic receptor agents. Physiol Behav 2006; 88(1−2): 124−31.
22. *Gaskin FS, Farr SA, Banks WA, Kumar VB, Morley JE*. Ghrelin-induced feeding is dependent on nitric oxide. Peptides 2003; 24(6): 913−8.
23. *Martinez-Cuesta MA, Esplugues JV, Whittle BJ*. Modulation by nitric oxide of spontaneous motility of the rat isolated duodenum: role of tachykinins. Br J Pharmacol 1996; 118(6): 1335−40.
24. *Izzo AA, Mascolo N, Maiolino P, Capasso F*. Nitric oxide-donating compounds and cyclic GMP depress the spontaneous contractile activity of the isolated rabbit jejunum. Pharmacology 1996; 53(2): 109−13.
25. *Tanovic A, Jimenez M, Fernandez E*. Actions of NO donors and endogenous nitrergic transmitter on the longitudinal muscle of rat ileum in vitro: Mechanisms involved. Life Sci 2001; 69(10): 1143−54.
26. *Abrahamsson H*. Non-adrenergic non-cholinergic nervous control of gastrointestinal motility patterns. Arch Int Pharmacodyn Ther 1986; 280(2 Suppl): 50−61.
27. *Zyromski NJ, Duenes JA, Kendrick ML, Balsiger BM, Farrugia G, Sarr MG*. Mechanism mediating nitric oxide-induced inhibition in human jejunal longitudinal smooth muscle. Surgery 2001; 130(3): 489−96.
28. *Martín MJ, Jiménez MD, Motilva V*. New issues about nitric oxide and its effects on the gastrointestinal tract. Curr Pharm Des 2001; 7(10): 881−908.
29. *Yamaji M, Ohta M, Yamazaki Y, Fujinami K, Fujita A, Takeuchi T*, et al. A possible role of neurotensin in NANC relaxation of longitudinal muscle of the jejunum and ileum of Wistar rats. Br J Pharmacol 2002; 137(5): 629−36.
30. *Van Geldre LA, Lefebvre* RA. Nitrergic relaxation in rat gastric fundus: influence of mechanism of induced tone and possible role of sarcoplasmic/endoplasmic reticulum Ca2+ ATPase. Life Sci 2004; 74(26): 3259−74.
31. *Li M, Johnson CP, Adams MB, Sama SK*. Cholinergic and nitrergic regulation of in vivo giant migrating contractions in rat colon. Am J Physiol Gastrointest Liver Physiol 2002; 283(3): G544−52.
32. *Takeuchi T, Niioka S, Kishi M, Ishii T, Nishio H, Hata F*, et al. Nonadrenergic, noncholinergic relaxation mediated by nitric oxide with concomitant change in Ca2+ level in rectal circular muscle of rats. Eur J Pharmacol 1998; 353(1): 67−74.
33. *Bredt DS, Snyder SH*. Isolation of nitric oxide synthetase, a calmodulin-requiring enzyme. Proc Natl Acad Sci USA 1990; 87(2): 682−5.
34. *Cho CH*. Current roles of nitric oxide in gastrointestinal disorders. J Physiol Paris 2001; 95(1−6): 253−6.
35. *Kato S, Kitamura M, Korolkiewicz RP, Takeuchi K*. Role of nitric oxide in regulation of gastric acid secretion in rats: effects of NO donors and NO synthase inhibitor. Br J Pharmacol 1998; 123(5): 839−46.

36. *Schicho R, Schemann M, Holzer P, Lippe IT*. Mucosal acid challenge activates nitrergic neurons in myenteric plexus of rat stomach. Am J Physiol Gastrointest Liver Physiol 2001; 281(5): G1316−21.
37. *Premaratne S, Xue C, Mccarty JM, Zai M, Mccuen RW, Johns RA,* et al. Neuronal nitric oxide synthase: expression in rat parietal cells. Am Am J Physiol Gastrointest Liver Physiol 2001; 280(2): G308−13.
38. *Lorente S, Doiz O, Trinidad SM, Castillo J, Lanas A*. Helicobacter pylori stimulates pepsinogen secretion from isolated human peptic cells. Gut 2002; 50(1): 13−8.
39. *Janero DR*. Nutritional aspects of nitric oxide: human health implications and therapeutic opportunities. Nutrition 2001; 17(10): 896−903.
40. *Karaki H, Ozaki H, Hori M, Mitsui-Saito M, Amano K, Harada K,* et al. Calcium movements, distribution, and functions in smooth muscle. Pharmacol Rev 1997; 49(2): 157−230.
41. *Garcia-Villar R, Dupuis C, Martinolle JP, Fioramonti J, Buéno L*. Functional evidence for NO-synthase activation by substance P through a mechanism not involving classical tachykinin receptors in guinea-pig ileum in vitro. Br J Pharmacol 1996; 118(5): 1253−61.
42. *Allescher HD, Lu S, Daniel EE, Classen M*. Nitric oxide as putative nonadrenergic noncholinergic inhibitory transmitter in the opossum sphincter of Oddi. Can J Physiol Pharmacol 1993; 71(7): 525−30.
43. *Mourelle M, Guarner F, Moncada S, Malagelada JR*. The arginine/nitric oxide pathway modulates sphincter of Oddi motor activity in guinea pigs and rabbits. Gastroenterology 1993; 105(5): 1299−305.
44. *Szilvassy Z, Nagy I, Szilvassy J, Jakab I, Csati S, Lonovics J*. Impaired nitrergic relaxation of the sphincter of Oddi of hyperlipidaemic rabbits. Eur J Pharmacol; 3996; 301(1−3): R17−8.
45. *Takahashi T*. Pathophysiological significance of neuronal nitric oxide synthase in the gastrointestinal tract. J Gastroenterol 2003; 38(5): 421−30.
46. *Venkova K, Krier J*. A nitric oxide- and prostaglandin-dependent component of NANC off-contractions in cat colon. Am J Physiol 1994; 266(1 Pt 1): G40−7.
47. *Stojanović M, Šćepanović LJ, Mitrović D, Šćepanović V, Stojanović T, Stojković M*, et al. Rat duodenal motility in vitro: procinetic effects of D, L- Homocysteine thiolactone and modulation of nitric oxide mediated inhibition. Arc Biol Sci 2013; 65(4): 1323−30.
48. *Park KJ, Baker SA, Cho SY, Sanders KM, Koh SD*. Sulfur-containing amino acids block stretch-dependent K+ channels and nitrergic responses in the murine colon. Br J Pharmacol 2005; 144(8): 1126−37.
49. *Choe EK, Moon JS, Park KJ*. Methionine enhances the contractile activity of human colon circular smooth muscle in vitro. J Korean Med Sci 2012; 27(7): 777−83.
50. *Ono T, Galanopoulou AS*. Epilepsy and epileptic syndrome. Adv Exp Med Biol 2012; 724: 99−113.
51. *Avoli M, Louvel J, Pumain R, Köhling R*. Cellular and molecular mechanisms of epilepsy in the human brain. Prog Neurobiol 2005; 77(3): 166−200.
52. *Duncan JS, Sander JW, Sisodiya SM, Walker MC*. Adult epilepsy. Lancet 2006; 367(9516): 1087−100.
53. *Badawy RA, Harvey AS, Macdonell RA*. Cortical hyperexcitability and epileptogenesis: understanding the mechanisms of epilepsy - part 1. J Clin Neurosci 2009; 16(3): 355−65.
54. *Vezzani A, Friedman A*. Brain inflammation as a biomarker in epilepsy. Biomark Med 2011; 5(5): 607−14.
55. *Wang R*. Two's company, three's a crowd: can H2S be the third endogenous gaseous transmitter. FASEB J 2002; 16(13): 1792−8.
56. *Hrnčić D, Rašić-Marković A, Djuric D, Sušić V, Stanojlović O*. The role of nitric oxide in convulsions induced by lindane in rats. Food Chem Toxicol 2011; 49(4): 947−54.
57. *Hrnčić D, Rašić-Marković A, Macut D, Sušic V, Djuric D, Stanojlovic O*. Homocysteine thiolactone-induced seizures in adult rats are aggravated by inhibition of inducible nitric oxide synthase. Hum Exp Toxicol 2014; 33(5): 496−503.
58. *Stanojlović OP, Živanović DP*. Experimental models of epilepsy. Med Pregl 2004; 57(7−8): 359−62. (Serbian)
59. *Stanojlović O, Rašić-Marković A, Hrnčić D, Sušić V, Macut D, Radosavljević T,* et al. Two types of Seizures in homo cysteine thiolactone-treated adult rats: Behavioral and electroencephalographic study. Cell Mol Neurobiol 2008; 29(3): 329−39.
60. *Rasić-Marković A, Djuric D, Hrncić D, Loncar-Stevanović H, Vucević D, Mladenović D,* et al. High dose of ethanol decreases total spectral power density in seizures induced by D,L-homocysteine thiolactone in adult rats. Gen Physiol Biophys 2009; 28: 25−32.
61. *Rašić-Marković A, Hrnčić D, Djurić D, Macut D, Lončar-Stevanović H, Stanojlović O*. The effect of N-methyl-D-aspartate receptor antagonists on D,L-homocysteine thiolactone induced seizures in adult rats. Acta Physiol Hung 2011; 98(1): 17−26.
62. *Hrnčić D, Rašić-Marković A, Bjekić-Macut J, Sušić V, Djuric D, Stanojlović O*. Paradoxical sleep deprivation potentiates epilepsy induced by homocysteine thiolactone in adult rats. Exp Biol Med Maywood 2013; 238(1): 77−83.
63. *Knowles RG, Moncada S*. Nitric oxide synthases in mammals. Biochem J 1994; 298(Pt 2): 249−58.
64. *Bredt DS, Hwang PM, Snyder SH*. Localization of nitric oxide synthase indicating a neural role for nitric oxide. Nature 1990; 347(6295): 768−70.
65. *Aktan F*. iNOS-mediated nitric oxide production and its regulation. Life Sci 2004; 75(6): 39−53.
66. *Zhou L, Zhu D*. Neuronal nitric oxide synthase: Structure, subcellular localization, regulation, and clinical implications. Niric Oxide 2009; 20(4): 223−30.
67. *Pannu R, Singh I*. Pharmacological strategies for the regulation of inducible nitric oxide synthase: neurodegenerative versus neuroprotective mechanisms. Neurochem Int 2006; 49(2): 170−82.
68. *González-Hernández T, García-Marín V, Pérez-Delgado MM, González-González ML, Rancel-Torres N, González-Feria L*. Nitric oxide synthase expression in the cerebral cortex of patients with epilepsy. Epilepsia 2000; 41(10): 1259−68.
69. *Murashima YL, Yoshii M, Suzuki J*. Role of nitric oxide in the epileptogenesis of EL mice. Epilepsia 2000; 41:195−9.
70. *Ferraro G, Sardo P, Di Giovanni G, Di Maio R, la Grutta V*. Involvement of nitric oxide in maximal dentate gyrus activation in the rat. Pflügers Arch - Eur J Physiol 2003; 102: 118.
71. *Royes LF, Fighera MR, Furian AF, Oliveira MS, Fiorenza NG, de Carvalho J,* et al. Involvement of NO in the convulsive behavior and oxidative damage induced by the intrastriatal injection of methylmalonate. Neurosci Lett 2005; 376(2): 116−20.
72. *Royes LF, Fighera MR, Furian AF, Oliveira MS, Fiorenza NG, Petry JC*, et al. The role of nitric oxide on the convulsive behavior and oxidative stress induced by methylmalonate: an electroencephalographic and neurochemical study. Epilepsy Res 2007; 73(3): 228−37.
73. *Prieto-Martín AI, Llorens S, Pardal-Fernández JM, Muñoz LJ, López DE, Escribano J*, et al. Opposite caudal versus rostral brain nitric oxide synthase response to generalized seizures in a novel rodent model of reflex epilepsy. Life Sci 2012; 90(13−14): 531−7.
74. *Hrnčić D, Rašić-Marković A, Krstić D, Macut D, Djuric D, Stanojlović O*. The role of nitric oxide in homocysteine thiolactone-induced seizures in adult rats. Cell Mol Neurobiol 2010; 30(2): 219−31.
75. *Hrnčić D, Rašić-Marković A, Krstić D, Macut D, Sušić V, Djuric D,* et al. Inhibition of the neuronal nitric oxide synthase potentiates homocysteine thiolactone-induced seizures in adult rats. Med Chem 2012; 8(1): 59−64.

Received on October 25, 2013.
Revised on March 14, 2014.
Accepted on April 14, 2014.
Online First August, 2014.