Impulse Flow in Sympathetic Efferents of the Abdominal Aortic Nerve after Intrathecal Administration of the Substrate and Inhibitor of Nitric Oxide Synthase

S. A. Rutkevich[1], S. A. Polenov[2], A. G. Chumak[1,3], and V. A. Kulchitsky[3,1]

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 147, No. 3, pp. 249-253, March, 2009
Original article submitted August 19, 2008

Experiments were performed on rats anesthetized with urethane and nembutal. Intrathecal administration of a nitric oxide inhibitor L-NAME (60 mg) into the cerebrospinal fluid of the thoracic spinal cord was followed by a 40-45% decrease in tonic activity of efferent fibers of the abdominal aortic nerves. L-NAME reduced a reflex increase in the rate of efferent impulses, which was induced by tetanic stimulation of afferent C-fibers in the mesenteric nerve. Administration of L-arginine into the cerebrospinal fluid of the spinal cord (80 mg/20 ml) was accompanied a long-term increase in tonic activity of efferent fibers of the abdominal aortic nerves (by 15-20%). These changes reflect a prolonged activating effect of L-arginine on sympathetic structures.

Key Words: *nitric oxide; intrathecal administration; tetanic and posttetanic potentiation; reflex activity*

Nitric oxide (NO) has a role in interneuronal communication in the brain and spinal cord, which is mediated by a variety of synaptic and extrasynaptic processes [1,5,6,7,11,12]. Published data show that exogenous NO has various effects on neurons in various regions of the spinal cord, as well as on the cells located in the same lamina [13]. Previous experiments revealed that perfusion of spinal cord slices with NO donors, including sodium nitroprusside, is accompanied by an increase in the activity of Rexed's lamina X neurons. However, this treatment was mainly accompanied by the inhibition of cells in the dorsal horns of the spinal cord [13]. Administration of NO synthase inhibitors was followed by an increase in spontaneous activity of neurons in the gelatinous substance [13]. Studying the effect of intrathecal treatment with sodium nitroprusside showed that NO can decrease or increase the activity of the sympathetic nervous system in the spinal cord, which has a role in the maintenance of blood pressure [9]. These data suggest that the depressor effect of NO is associated with stimulation of $GABA_A$ and $GABA_B$ receptors. By contrast, the pressor effect of NO is probably realized via glutamate receptors [9]. Much attention is paid to a polymodal effect of NO on various systems in the organism [2,10]. NO-containing drugs are of limited use in clinical practice due to the diverse or opposite effects of NO.

There is general agreement that NO has an important role in the regulation of various processes in the organism (*e.g.*, activity of sympathetic and parasympathetic efferents). However, the mechanisms for NO-mediated regulation of these processes remain unknown. This is related to methodical errors, which results in the induction of noci-

[1]Department of Human and Animal Physiology, Belarussian State University, Minsk, Belarus; [2]Laboratory for Physiology of Digestion, I. P. Pavlov Institute of Physiology, St. Petersburg, Russia; [3]Department of Physiology and General Pathology, Institute of Physiology, Belarussian National Academy of Sciences, Minsk, Belarus. ***Address for correspondence:*** rutkevitch@inbox.ru. S. A. Rutkevich

ceptive reflexes during laminectomy, exposure of the spinal cord, change in the fine mechanisms of interneuronal communication, and abnormal reaction to application of receptor ligands to the spinal cord [6,8,10,12].

This work was designed to minimize the traumatic effect on the spinal cord by means of intrathecal administration of a NO precursor or NO synthase inhibitor.

Here we studied the activity of efferent fibers of the abdominal aortic nerve after application of L-arginine or L-NAME to the thoracic spinal cord.

MATERIALS AND METHODS

Experiments were performed on 22 rats weighing 280-320 g and anesthetized with 500 mg/kg urethane and 30 mg/kg nembutal. The study was conducted in accordance with the principles of humane attitude toward laboratory animals. The animals were divided into 2 groups. Pharmaceutical compounds were administered intrathecally to evaluate their effect on the induction of tonic impulses from sympathetic efferent fibers in group 1 animals (n=11). The effect of these compounds on reflex reactions to electrostimulation of afferent fibers in the intestine and skin was studied in group 2 animals (n=11).

Laparotomy was performed to gain access to the abdominal nerves and organs. The intestinal loops were thoroughly removed and placed in a temperature-controlled chamber (37°C). The main trunk of the mesenteric nerves (*n. mesentericus*) and internal branch of the femoral nerve (*n. saphenus*) were prepared, cut, and put on stimulating electrodes to stimulate the afferent fibers. Stimulation with rectangular pulses (duration 1 msec, voltage 5 V, frequency 10 Hz) was applied before and after administration of pharmaceutical compounds. The AgCl bipolar reference electrodes were put on thick branches of the abdominal aortic plexus (*pl. aorticus abdominalis*). The nerves were covered with Vaseline oil. Core body temperature was measured with an electronic thermometer (37°C). The general state of animals was evaluated from the respiratory rate, depth of breathing, and ECG (standard lead II).

The role of NO in tonic and phasic activity of sympathetic efferent neurons was studied by a modified method [14]. This approach suggested the intrathecal administration of pharmaceutical compounds to modulate NO production (L-arginine, 80 mg/20 ml; and methyl ester L-NAME, 60 mg/20 ml, Sigma) [8]. Soft tissues of the neck were consecutively prepared (minimal trauma in the animal). The skin was cut. The cervical muscles were moved apart. The trachea and esophagus were drawn aside using a rasp. The ventral surface of the skull and vertebral column was exposed. A hole was made in the ventral surface of the dura mater of the spinal cord. The atlanto-occipital membrane was punctured with an injection needle (diameter 0.8 mm). A silicon catheter (diameter 0.5 mm) was inserted in the caudal direction. This catheter was moved in the subarachnoid space to the lower thoracic segments. By the end of each experiment, a solution of methylene blue was introduced through the catheter. Staining of nervous tissue was visualized near the catheter tip and 2-3 adjacent segments of the spinal cord. The solutions were injected using a Hamilton syringe. An isotonic solution of NaCl and artificial cerebrospinal fluid (20 ml) served as the control [10].

Electrical signals were recorded and processed on a computerized electrophysiological device [4]. The results were analyzed by Student's t test and one-way analysis of variance (ANOVA). The differences were significant at $p<0.05$.

RESULTS

Our previous experiments showed that tonic sympathetic efferent impulses in the abdominal aortic plexus of rats [3] and rabbits [5] is maintained at a constant level and does not depend on the depth of anesthesia under a stable body temperature and air temperature. Tonic impulses from the efferent nerves remained unchanged after administration of artificial cerebrospinal fluid or apyrogenic isotonic solution of NaCl into the liquor system of the spinal cord. A short-term increase in efferent activity of the branches of the abdominal aortic plexus was observed only in the 1st minute of infusion of test solutions (Figs. 1 and 2). The observed changes were probably related to a transient increase in cerebrospinal fluid pressure.

Administration of a NO precursor of NO synthase inhibitor into the cerebrospinal fluid was followed by other changes in efferent impulses in branches of the abdominal aortic plexus. Tonic activity was shown to decrease 10-11 min after intrathecal administration of a NO synthase inhibitor L-NAME (n=5, Fig. 1). A decrease in the pulse rate was most pronounced by the 40th minute. The changes were statistically significant (17.5±1.1 *vs.* 27.0±3.2 cps before infusion, $p<0.001$) and persisted for several hours. By contrast, administration of L-arginine was accompanied by an increase in the pulse rate in efferent fibers (Fig. 1). These changes were observed by the 11th minute, reached maximum

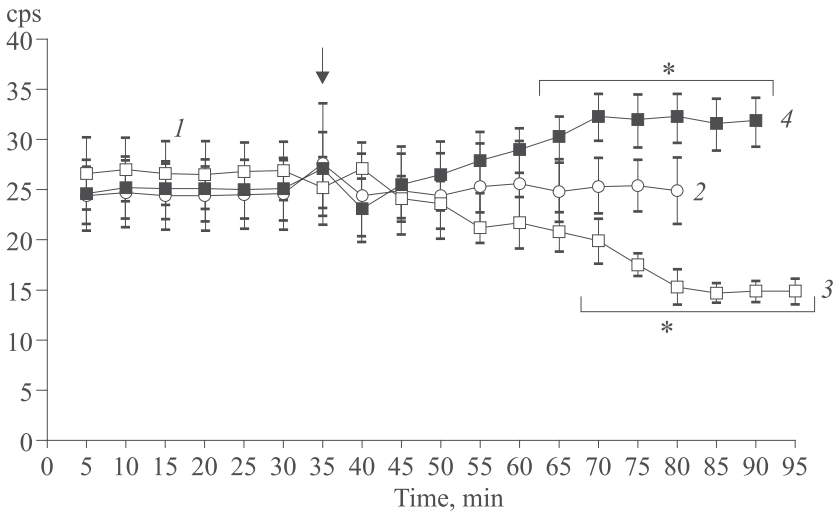

Fig. 1. Rate of tonic impulses in the abdominal aortic sympathetic efferent fibers before (*1*) and after intrathecal administration of artificial cerebrospinal fluid (*2*), NO synthase inhibitor (*3*), and biological substrate of NO synthase (*4*). Arrow: administration of test solutions. *$p<0.05$ compared to *1* and *2*.

after 40 min (up to 32.3±2.3 *vs*. 25.1±2.0 cps under basal conditions, $p<0.01$), and persisted until the end of the study (not less than 2 h).

The induced activity of efferent fibers in the abdominal aortic nerve was modified by intrathecal administration of NO modulators. Stimulation of afferent C-fibers in *n. mesentericus* (duration 1 msec, voltage 5 V, frequency 0.2-20 Hz) or Agd,C-fibers in *n. saphenus* was followed by an increase in impulse activity of sympathetic efferents in the abdominal aortic nerve. Tetanic and posttetanic potentiation of the reflex response was induced by tetanization of afferent fibers (Fig. 2, *a*). A quantitative study showed that reflex phasic efferent impulses exceed the basal tonic activity in frequency (by 2-2.5 times, Fig. 3) and returns to the control level not less than 5 min after cessation of stimulation of afferent fibers. Our results are consistent with current concepts of visceral pain [3]. Under these conditions, the activation of "silent" efferent fibers is mediated by the viscerovisceral and somatovisceral reflexes.

The effect of stimulation of visceral and somatic afferent fibers was less pronounced in L-NAME-receiving rats. A reflex increase in the rate was significantly reduced 40 min after application of L-NAME (as compared to electrostimulation-induced activation before drug treatment; Fig. 2, *b*). This

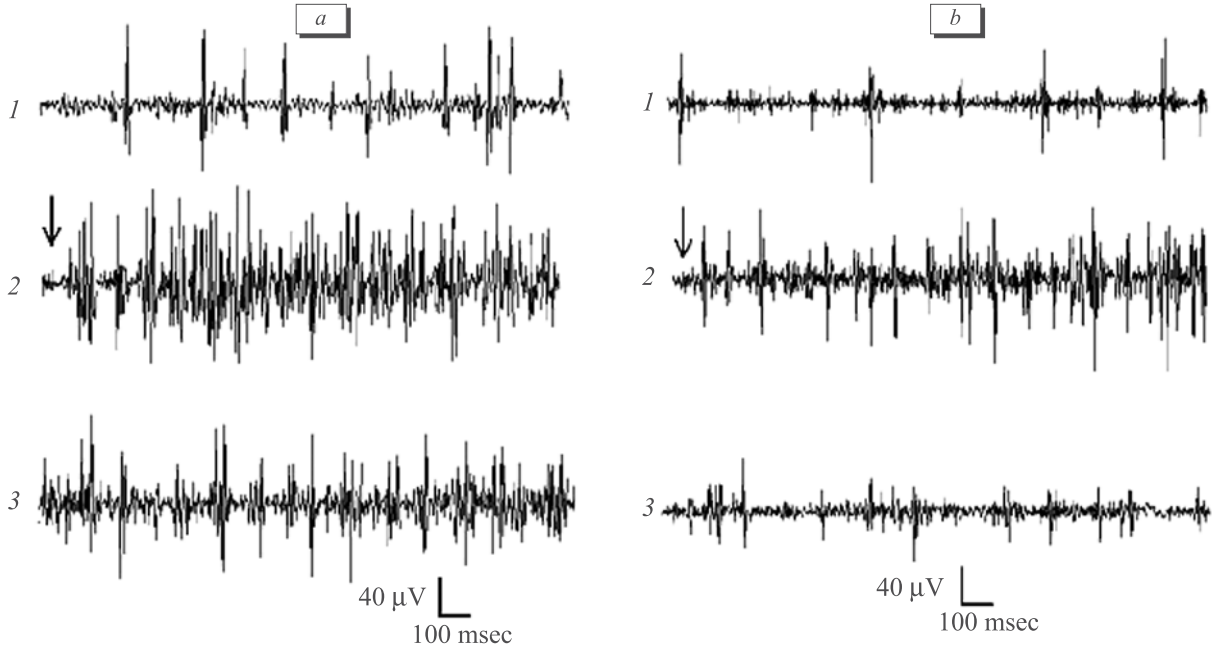

Fig. 2. Reflex potentiation in nerves of the abdominal aortic plexus during stimulation of the common mesenteric nerve (*n. mesentericus*, 10 Hz): (*a*) neurograms before administration of a nonselective NO synthase inhibitor; and (*b*) 40 min after administration of L-NAME. Baseline (*1*); start of electrostimulation (arrow, *2*); and 10 sec after cessation of stimulation.

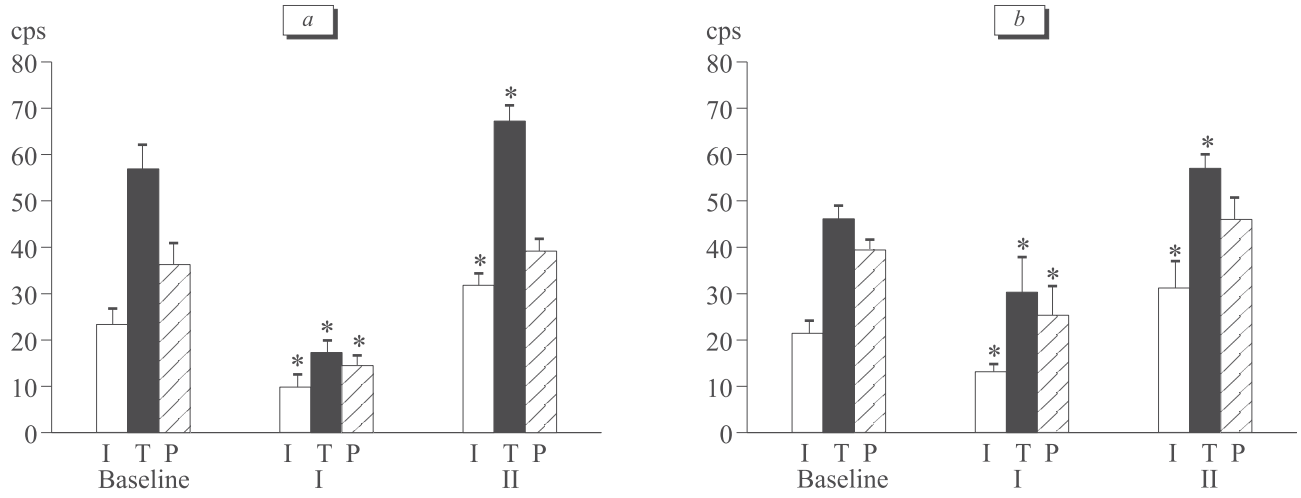

Fig. 3. Reflex response in branches of the *pl. aorticus abdominalis* to electrostimulation of efferent fibers of the *n. mesentericus (a*) and *n. saphenus (b*) before (baseline) and 40 min after intrathecal administration of NO modulators L-NAME (60 mg/20 ml, I) and L-arginine (80 mg/20 ml, II) at the level of the lower thoracic segments of the spinal cord. I, initial rate of tonic impulses; T, tetanic reflex activation of efferent fibers during stimulation; P, posttetanic reflex activation of efferent fibers over the 1st minute after cessation of stimulation. *$p<0.05$ compared to the pretreatment level.

parameter in 3 of 5 animals did not exceed the absolute level of tonic impulses in control specimens (Fig. 3, *a*). The pulse rate in abdominal aortic efferent fibers during electrostimulation was 2-fold lower that that observed before treatment (56.9±5.1 *vs.* 17.3±2.7 cps after NO synthase inhibition; $p<0.001$).

Another effect was detected after administration of a biological precursor for NO into the cerebrospinal fluid of the spinal cord. Increasing the concentration of L-arginine in the cerebrospinal fluid was accompanied by the enhanced response of phasic afferents to stimulation of the visceral and somatic nerves (Fig. 3, *a*, *b*). An increase in the pulse rate during electrostimulation was 15-20% higher than in the control (from 56.9±5.1 to 67.2± 3.3 cps for the common mesenteric nerve; from 46.1±2.8 to 57.0±3.1 cps for the saphenous nerve; $p<0.05$).

The observed effects of intrathecal administration of L-arginine or L-NAME indicate that NO has a role in the induction of tonic and reflex impulses from efferent fivers of the abdominal aortic nerve in rats.

Intrathecal administration of a NO synthase inhibitor and substrate had various effects on efferent fibers of the abdominal aortic nerve, which is consistent with published data on peripheral administration of test substances [2,5,6,8,10,14]. We conclude that a modified method for administration of test compounds into the cerebrospinal fluid of the spinal cord may be used to study the central regulation of various functions, including the pathogenetic mechanisms of visceral pain.

REFERENCES

1. V. A. Kul.'chitsky, S. A. Polenov, S. G. Pashkevich, *et al*., *Regulation of Functions during Activation of the Brain* [in Russian], Minsk (2004).
2. V. P. Reutov, E. G. Sorokina, V. E. Okhotin, and N. S. Kositsin, *Cyclic Transformations of Nitric Oxide in the Mammalian Organism* [in Russian], Moscow (1997).
3. S. A. Rutkevich and A. G. Chumak, *Vestn. Bel. Gos. Univer. Ser. 2*, No. 1, 53-59 (2008).
4. V. V. Soltanov and V. E. Burko, *Novosti Med.-Biol. Nauk*, No. 1, 90-96 (2005).
5. A. G. Chumak, V. V. Soltanov, V. A. Kul.'chitsky, *Modern Problems of Physiology of Autonomic Functions: Collection of Scientific Articles Devoted to the 75th Anniversary of N. A. Merkulova* [in Russian], Samara (2001), pp. 81-97.
6. K. E. Brack, N. Watkins, S. Pyner, and J. H. Coote, *Neuroscience*, **150**, No. 2, 487-497 (2007).
7. J. L. Cham, R. Klein, N. C. Owens, *et al*., *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, **291**, No. 1, R91-R101 (2006).
8. V. M. Gadotti, D. Tibola, A. F. Paszcuk, *et al*., *Brain Res*., **1093**, No. 1, 116-122 (2006).
9. M. A. Hakim, Y. Hirooka, M. J. Coleman, *et al*., *J. Physiol*., **482**, Pt. 2,. 401-407 (1995).
10. D. Levy and A. M. Strassman, *J. Neurophysiol*., **92**, No. 2, 766-772 (2004).
11. K. P. Patel, Y. F. Li, and Y. Hirooka, *Exp. Biol. Med*., **226**, No. 9, 814-824 (2001).
12. I. Perez-Neri, E. Castro, S. Montes, *et al*., *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci*., **85**, 250-256 (2007).
13. H. A. Schmid and U. Pehl, *J. Chem. Neuroanat*., **10**, No. 3, 197-201 (1996).
14. T. Yamamoto and N. Shimoyama, *Anesthesiology*, **82**, No. 5, 1266-1273 (1995).