

# The Effect of Nucleus Tractus Solitarius Nitric Oxidergic Neurons on Blood Pressure in Diabetic Rats

Masoomeh Kourosh Arami<sup>\*1</sup>, Abdolrahman Sarihi<sup>2</sup>, Seyyed Mansour Malacoti<sup>2</sup>, Gila Behzadi<sup>3</sup>, Mehrangiz Vahabian<sup>4</sup> and Iraj Amiri<sup>5</sup>

<sup>1</sup>Dept. of Physiology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran; <sup>2</sup>Dept. of Physiology, Hamedan University of Medical Sciences, Hamadan; <sup>3</sup>Dept. of Physiology, Shaheed Beheshti University of Medical Sciences, Tehran; <sup>4</sup>Dept. of Language, Hamedan University of Medical Sciences, Hamedan; <sup>5</sup>Dept. of Anatomy, Hamedan University of Medical Sciences, Hamadan, Iran

Received 1 March 2005; revised 24 May 2005; accepted 21 September 2005

## ABSTRACT

It has been shown that nitric oxide is synthesized in the central nervous system as well as in vascular endothelial cells. Recently, it was reported that nitric oxide was involved in central cardiovascular regulation, baroreflex modulation, and involved in a reciprocal release with excitatory amino acids in the nucleus tractus solitarii of rats. The purpose of the present study was to investigate the possible interaction of nitric oxide and glucose in the nucleus tractus solitarii on blood pressure regulation. Male Wistar streptozotocin induced diabetic rats were anesthetized with urethane. A cannula was inserted above the nucleus tractus solitarii and blood pressure was monitored intra-arterially. Unilateral microinjection of L-glutamate (2.3 nmol/60 nL) into the nucleus produced a decrease in blood pressure in diabetic rats. Microinjection of lidocaine (0.5  $\mu$ l %2) increased blood pressure. Unilateral microinjection of sodium nitroprusside (100 mmol/60 nL) into the nucleus increased blood pressure in diabetic rats. After microinjection of sodium nitroprusside, the depressive responses to glutamate were significantly attenuated. These results demonstrated the probable role of glucose on blood pressure regulation in diabetic animals affecting on nitric oxidergic neurons and so it implicates an interaction between nitric oxide and glucose in central cardiovascular regulation. *Iran. Biomed. J. 10 (1): 15-19, 2006*

**Keywords:** Nitric oxide (NO), Glutamate (Glu), Sodium nitroprusside, Lidocaine, Nucleus tractus solitarii, Diabetes, Rat

## INTRODUCTION

Previous studies have shown that nitric oxide (NO), as endothelium-derived relaxing factor, is synthesized from L-arginine in the central nervous system (CNS) by NOS (NO synthase) and acts through cGMP formation [1]. NOS is distributed in brain regions related to the regulation of cardiovascular functions [2].

The caudal zone of the nucleus tractus solitarii (NTS) is the site of the first synapse of baroreceptor fibers where NTS interacts with the nucleus reticularis lateralis (NRL) located in the rosteroventrolateral part of the medulla (RVLM) via a nitric oxidergic pathway [3, 4]. The basal production of NO in the NTS is 153 nmol/L [5], its sources within the NTS are peripheral afferent inputs to the nucleus, local interneuron within the

NTS, and inputs from other central sites to the NTS [6, 7]. It is revealed that NO plays a key role in glucose and cardiovascular homeostasis and drugs releasing NO may represent potential new treatment for insulin resistance [8].

It was reported that microinjection of L-arginine and sodium nitroprusside (SNP) into the NTS decreased blood pressure (BP) but L-NAME (NO synthase inhibitor) increased BP [9-12]. Many neurons in the caudal NTS recorded *in vivo* respond to moderate glycemic fluctuations [13]. The NO plasma levels and soluble guanylyl cyclase (SGC) levels in diabetes are controversial. In some studies, the endothelial form of NOS was upregulated in diabetes mellitus and also plasma concentration of NO in diabetes increased [14, 15], whereas in other studies activity of vascular SGC diminished in diabetes [16]. Since glucose infusion would yield a

\*Corresponding Author; Tel: (+98-811) 826 5540; Fax: (+98-811) 827 6299; E-mail: masoomeh\_k@hotmail.com

remarkably greater increase in arterial pressure during NO synthesis blocking [13], in this study, we decided to determine the effect of hyperglycemia on BP in diabetes during NO synthesis inhibiting or exciting in NTS, and then to find a way for hypertension treatment in diabetes.

## MATERIALS AND METHODS

Male Wistar rats ( $n = 18$ ), weighing 250-300 g, were obtained from the Pasteur Institute of Iran, Tehran. The rats were kept in individual cage in a room with controlled light 12 h on/12 h off, and the temperature was maintained at 23°C-24°C with free access to water and food for one week. After 24 hours fasting, diabetes was induced by a single intraperitoneal injection of 0.2 to 0.3 ml 50 mM of sodium citrate solution (pH 4.5) containing streptozotocin (65 mg/kg body weight.) After injection, animals were fasted for a further 24 h, after which plasma glucose levels were checked and diabetes was confirmed (glucose level > 250 mg/dl) [17].

After about one week, rats were anesthetized with urethane (1.0 g/kg i.p. and 300 mg/kg i.v.), and then were placed in a stereotaxic instrument. For NTS microinjection, a 22-gauge stainless steel guide cannula was implanted stereotaxically into the NTS with the anterior-posterior coordinates 13.3 mm; medio-lateral, 0.6 mm; and dorsoventral, 8 mm [18], through which a 28-gauge stainless steel injection cannula was inserted into the NTS. BP was measured directly through a cannula placed in the femoral artery and connected to a pressure transducer (Gould p23 ID) and polygraph (NARCO) [19]. BP (mean, systolic and diastolic BP) was measured before every injection. Then, the injection cannula of NTS was connected to a Hamilton microsyringe by polyvinyl tubing and was filled with L-glutamate (L-Glu, 78 pmol/60 nL) to functionally identify the NTS. After decrement of BP due to Glu injection, and >60 minutes recovery, we divide 18 rats into two groups:

In the first group of diabetic rats, lidocaine (78 pmol/60 nL) was injected to functionally inhibit the NTS and in the second group, SNP was injected (100 mM, as a NO-donor). In this group, >60 minutes after SNP microinjection, Glu was injected for second time and BP change was compared with response to the first Glu injection.

At the beginning of experiments, the NTS injection sites were confirmed by responsiveness to L-Glu administration. A specific decrease in BP (at least -35 mm Hg) has been demonstrated after microinjection of 2.3 nmol L-Glu in the NTS. The response is restricted to the intermediate one third of the NTS, and the administration of the same dose of L-Glu in adjacent areas to the NTS fails to elicit the response [9]. Injections were given over 10 seconds by air pressure generated by a hand-held syringe while the pipette tip was positioned in the NTS. For NTS microinjection, the drugs were dissolved in a sterile saline to the final concentrations in a volume not exceeding 60 nL. For each drug, only 60 nL was pressure-microinjected into the NTS.

After the completion of the experiment, ink was injected through the cannula, and the rats were perfused with saline, followed by a solution of 4% formaldehyde, and finally with 30% sucrose solution. Section (40  $\mu$ m) of the brainstem was stained with cresyl violet, and proper placement of the pipette tip in the NTS was verified with histological sections under the microscope.

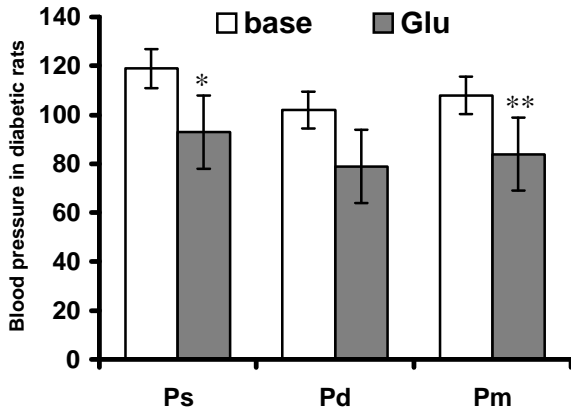
## RESULTS

Our results indicated that mean blood pressure (MBP) decreased from  $106 \pm 8$  mmHg (basal level) to  $86 \pm 12$  mmHg ( $P < 0.001$ ) and systolic BP decreased from  $119 \pm 8.02$  mmHg to  $93 \pm 14.989$  mmHg ( $P < 0.05$ ) by unilateral microinjection of L-Glu in NTS of diabetic rats (Table 1 and Fig. 1), and it was revealed that the injection site is appropriate. So, L-Glu injection in diabetic rats caused hypotension.

**Table 1.** Comparative effect of L-glutamate, sodium nitroprusside and lidocaine on systolic, diastolic and mean blood pressure.

|           | Before injection (base) |                |                | After injection |                 |                  |
|-----------|-------------------------|----------------|----------------|-----------------|-----------------|------------------|
|           | Ps                      | Pd             | Pm             | Ps              | Pd              | Pm               |
| Glu       | $119 \pm 8.02$          | $102 \pm 7.60$ | $108 \pm 7.60$ | $*93 \pm 14.98$ | $79 \pm 14.92$  | $**84 \pm 14.92$ |
| Lidocaine | $97 \pm 10.07$          | $85 \pm 12.25$ | $86 \pm 6.70$  | $99 \pm 8.71$   | $86 \pm 10.29$  | $*97 \pm 6.90$   |
| SNP       | $102 \pm 7.52$          | $83 \pm 8.60$  | $89 \pm 8.21$  | $86 \pm 10.29$  | $*67 \pm 10.07$ | $*73 \pm 10.11$  |

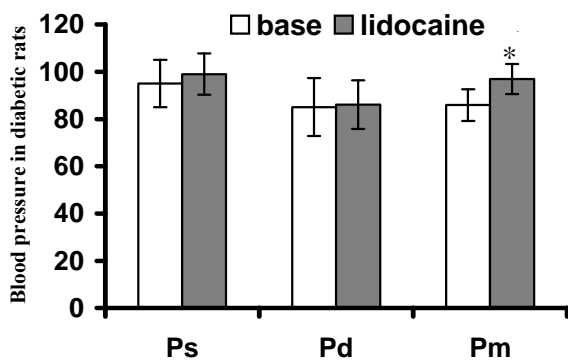
Ps, systolic pressure; Pd, diastolic pressure; Pm, mean blood pressure; SNP, sodium nitroprusside; Glu, L-glutamate; \* $P < 0.05$  significantly different from baseline value, \*\* $P < 0.001$  significantly different from baseline value.



**Fig. 1.** Effect of unilateral injection of L-glutamate (Glu, 2.3 nmol) into the NTS on blood pressure in anesthetized diabetic rats. Data are represented by mean  $\pm$  SEM. Vertical bars represent SEM change from baseline values. Each bar represents the average data from 18 rats. Ps, systolic pressure; Pd, diastolic pressure; Pm, mean blood pressure; \*,  $P < 0.05$  and \*\*,  $P < 0.001$  significantly different from baseline value.

In the first group, ten minutes after unilateral lidocaine microinjection in NTS, MBP increased from  $86 \pm 6.7$  (basal level) to  $97 \pm 6.9$  mmHg significantly ( $P < 0.01$ , Table 1). At times of 20, 40 and 60 minutes after lidocaine injection, it was no significant difference with respect to basal level. Then, NTS inhibition by lidocaine in diabetic rats caused hypertension (Fig. 2).

To test whether NO system was involved in the cardiovascular effects of Glu, SNP was used in second group. SNP (NO donor) decreased MBP from  $89 \pm 8.2$  (basal level) to  $73 \pm 10.1$  mmHg

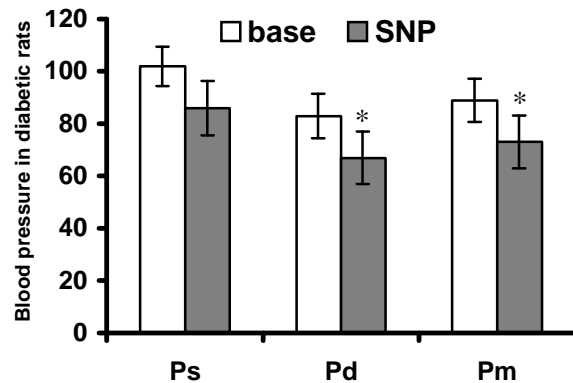


**Fig. 2.** Effect of unilateral injection of lidocaine (0.5l) into the NTS on blood pressure in anesthetized diabetic rats. Data are represented by mean  $\pm$  SEM. Vertical bars represent SEM change from baseline values. Each bar represents the average data from 9 rats. Ps, systolic pressure; Pd, diastolic pressure; Pm, mean blood pressure; \*,  $P < 0.01$  significantly different from baseline value.

and diastolic BP from  $83 \pm 8.6$  to  $67 \pm 10.07$  significantly ( $P < 0.05$ , Table 1). Therefore, SNP injection in NTS decreases BP in diabetic rats (Fig. 3).

Finally, we compared the effect of Glu and SNP on MBP of diabetic rats ( $88.6 \pm 9.7$  and  $100.66 \pm 18.31$ , respectively) that was significantly ( $P < 0.05$ , Fig. 4).

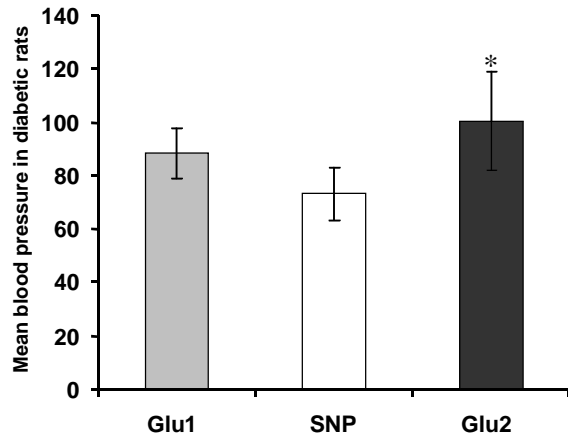
In all of the groups, in diabetic rats, BP was measured before (basal level) and after drug microinjection and so basal level of BP was as control group.



**Fig. 3.** Effect of unilateral injection of SNP (100 mM) into the NTS on blood pressure in anesthetized diabetic rats. Data are represented by Mean  $\pm$  SEM. Vertical bars represent SEM change from baseline values. Each bar represents the average data from 9 rats. Ps, systolic pressure; Pd, diastolic pressure; Pm, mean blood pressure; SNP, sodium nitroprusside; \*,  $P < 0.05$  significantly different from baseline value.

## DISCUSSION

The aforementioned studies support the potential in the CNS system for integration of Glu and NO. Direct and integrative effects of glutamatergic and nitroxidergic neurons on transmission of cardiovascular reflex signals within the NTS were observed [20, 21]. So, decreases of MBP by L-Glu microinjection demonstrated that the injection site was correctly selected. To confirm this finding, after lidocaine microinjection into this depressor area and inhibiting NO neurons, a significant, immediate and reversible decrease in MBP was seen. This fast acting local anesthetic agent blocks sodium channels, thus inhibiting neuronal electrical activity in the affected area. The 1- $\mu$ l injection of a 2% lidocaine solution has an effective duration of 10-15 min. This makes the microinjection of lidocaine an excellent technique for interrupting



**Fig. 4.** Comparative effect of L-glutamate (Glu, 2.3 nmol) and sodium nitroprusside (SNP) on mean blood pressure. Each bar represents the average data from 9 rats. \*,  $P < 0.05$  significantly different from baseline value.

local neuronal activity without permanently altering the system.

In the previous studies, microinjection of L-Arg into the NTS elicited dose-dependent depressor and bradycardic effects. This suggests that L-Arg was transferred into NO by NOS present in the NTS [22, 23]. In this study, SNP was used for detection of nitric oxidergic neurons role in BP regulation. According to the results, it was observed that SNP injection into NTS decreases diabetic rats' MBP. The reason is that NO (released from SNP) diffused into presynaptic terminals to activate guanylate cyclase. Resultant CGMP can increase the firing rate of the calcium channels. Thus, increased calcium influx activates the neurons and releases excitatory amino acids including Glu [9].

The comparison of results of L-Glu and SNP microinjection demonstrated that the attenuation effect of SNP on MBP was more potent than that of L-Glu ( $P < 0.01$ ). Glu as an excitatory neurotransmitter causes excitation of all kinds of neurons including intrinsic nitric oxidergic neurons of NTS, but SNP increases only NO level in the nucleus. BP was similarly affected by both drugs but SNP was higher than the other, therefore it can be postulated that in diabetes, intrinsic nitric oxidergic neurons of NTS were somehow inhibited. With respect to sensitivity of many neurons in NTS to glycemic fluctuation and presence of hypertension in diabetes, it can be postulated that diabetes has probably inhibited either intrinsic nitric oxidergic neurons or has an excitatory effect on nitric oxidergic inhibitory neurons in NTS. It is concluded that in diabetes, hypertension is caused by nitric

oxidergic neurons inhibition which is caused by high level of glucose. The results of this study can be used for mechanism of BP control in diabetes.

## REFERENCES

- Lo, W.C., Jan, C.R., Wu, S.N. and Tseng, C.J. (1998) Cardiovascular effects of nitric oxide and adenosine in the nucleus tractus solitarii of rats. *Hypertension* 32: 1034-1038.
- Maeda, M., Inoue, M., Takao, S. and Nakai, M. (1999) Central control mechanisms of circulation in the medulla oblongata by nitric oxide. *Jpn. J. Physiol.* 49 (6): 467-478.
- Esteves, F.O., McWilliam, P.N. and Batten, T.F. (2000) Nitric oxide producing neurons in the rat medulla oblongata that project to nucleus tractus solitarii. *J. Chem. Neuroanat.* 20 (2): 185-197.
- Sya, G.Y., Beubana, V., Bousquet, P.A. and Feldmana, J. (2002) Nitric oxide discriminates the sites and mechanisms of action of centrally acting anti-hypertensive drugs in rabbits. *Neuropharmacology* 43 (8): 1330-1338.
- Dobrucki, L.W., Cabrera, C.L., Bohr, D.F. and Malinski, T. (2001) Central hypotensive action of clonidine requires nitric oxide. *Circulation* 104 (16): 1884-1886.
- Lo, W.C., Lin, H.C., Ger, L.P., Tung, C.S. and Tseng, C.J. (1997) Cardiovascular effects of nitric oxide and N-methyl- D- aspartate receptors in the nucleus tractus solitarii of rats. *Hypertension* 30: 1499-1503.
- Carolina, A., Dias1, R., Colombari, E. and Mifflin, S.W. (2003) Effect of nitric oxide on excitatory amino acid-evoked discharge of neurons in NTS. *Am. J. Physiol. Heart Circ. Physiol.* 284: H234-H240.
- Jayel, P.Y., Thalman, S., Cook, S., Duplain, H., Sartori, C., Vollenwelder, P. and Scherrer, U. (2004) Nitric oxide donors, a new treatment for insulin resistance, metabolic syndrome and diabetes. *Rev. Med. Suisse. Romande.* 124 (10): 642-644.
- Tseng, C.J., Liu, H.Y., Lin, H.C., Ger, L.P., Tung, C.S. and Yen, M.H. (1996) Cardiovascular effects of nitric oxide in the brainstem nuclei of rats. *Hypertension* 27: 36-42.
- Lo, W.J., Liu, H.W., Lin, H.C., Ger, L.P., Tung, C.S. and Tseng, C.J. (1996) Modulatory effects of nitric oxide on baroreflex activation in the brainstem nuclei of rats. *Chin. J. Physiol.* 39: 57-62.
- Wua, C.W., Wangb, Y., Kaoc, L.S., Tangc, F.I. and Chai, C.Y. (2002) Nitric oxide reduces blood pressure in the nucleus tractus solitarius: a real time electrochemical study. *Brain Res. Bull.* 15: 171-177.
- Vitagliano, S., Berrino, L., Damico, M., Maione, S., De Novellis, V. and Rossi, F. (1996) Involvement of nitric oxide in cardiorespiratory regulation in the

- nucleus tractus solitarii. *Neuropharmacology* 32 (5): 625-631.
13. Dallaporta, M., Himmi, T., Perrin, J. and Orsini, J.C. (1999) Solitarius tract nucleus sensitivity to moderate changes in glucose level. *Neuroreport* 10 (12): 2657-2660.
  14. Liorens, S. and Nava, E. (2003) Cardiovascular diseases and the nitric oxide pathway. *Crit. Vasc. Pharmacol.* (3): 3335-3346.
  15. Chien, W.Y., Yang, K.D., Eng, H.L., Hu, Y.H., Lee, R.Y., Wang, S.T. and Wang, P.W. (2005) Increased plasma concentration of nitric oxide in type 2 diabetes but not in nondiabetic individuals with insulin resistance. *Diabetes Metab.* 31 (1): 63-68.
  16. Witte, K., Hachenberger, J., Castell, M.F., Vahi, C.F. and Haller, C. (2004) Nitric oxide- sensitive soluble guanylyl cyclase activity is preserved in internal mammary artery of type 2 diabetic patients. *Diabetes* 53 (10): 2640-2444.
  17. Shimomura, I., Bashmakov, Y., Ikemoto, S., Horton, J.D., Brown, M.S. and Goldstein, J.L. (1999) Insuline selectively increases SREBP-1c m RAN in the livers of rats with stereptozotocin-induced diabetes. *Proc. Natl. Acad. Sci. USA* 96 (24): 13656-13661.
  18. Pexinos, G. and Watson, C. (1986) The rat brain in stereotaxi coordinates. Academic press, San Diego, USA.
  19. Rosa, F., Vasouez, J. and Lupi, J. (1997) Pharmacological modulation of the cardiovascular response to hypertonic NACL injection in the anteroventral area of the brain third ventricle. *Pharmacology* 45 (2): 98.
  20. Talman, W.T., Dragon, D.N., Otha, H. and Lin, L.H. (2001) Nitroxidergic influences on cardiovascular control by NTS: a link with glutamate. *Ann. NY Acad. Sci.* 940: 169-178.
  21. Dias, A.C., Vitela, M., Colombari, E. and Mifflin, S.W. (2005) Nitric oxide modulation of glutamatergic, baroreflex, and cardiopulmonary transmission in the nucleus of the solitary tract. *Am. J. Physiol. Heart Circ. Physiol.* 288 (1): H256-H262.
  22. Matsumura, K., Tsuchihashi, T., Kagiya, S., Abe, I. and Fujishima, M. (1998) Role of nitric oxide in the nucleus of the solitary tract of rats. *Brain Res.* 798 (1-2): 232-238.
  23. Ma, S., Abboud, F.M. and Felder, R.B. (1995) Effects of L-arginine-derived nitric oxide synthesis on neuronal activity in nucleus tractus solitarius. *Am. J. Physiol.* 268: R487-R491.