Прилози, Одд. биол. мед. науки, МАНУ, XXVI, 1, с. 13–24 (2005)
Contributions, Sec. Biol. Med. Sci., MASA, XXVI, 1, p. 13–24 (2005)
ISSN 0351–3254
УДК:616.12-008.331.1:616-089.5

LEVEL OF NITRIC OXIDE IN HYPERTENSIVE PATIENTS SCHEDULED ON GENERAL ANAESTHESIA

B. Shirgoska[1], T. Trajkovska[2], M. Šoljakova[2], L. Simjanovska[3], R. Isijanovska[4], J. Netkovski[1], G. D. Efremov[3]

[1]Clinic for Ear, Nose and Throat Surgery, Clinical Centre, Skopje, R. Macedonia
[2] Clinic for Anaesthesia, Reanimation and Intensive Care, Clinical Centre, Skopje, R. Macedonia
[3] Research Centre for Genetic Engineering and Biotechnology, MANU, Skopje, R. Macedonia
[4]Institute for Epidemiology; Medical Faculty, Skopje, R. Macedonia

A b s t r a c t: In this prospective study we have analysed the level of nitric oxide in hypertensive patients scheduled for general anaesthesia. In the study were included thirty-four patients with chronicle inflammatory disease of the middle ear who have undergone surgical treatment at the Clinic for Ear, Nose and Throat Surgery.

The aim of our study was to determine the plasma level of nitric oxide (NO) and its effects on the circulatory system in hypertensive patients during the general anaesthesia maintained with inhalation of oxygen and nitrous oxide (O_2/N_2O) mixture.

Patients were divided in two groups. During the maintenance of general anaesthesia the patients from the first group were ventilated with O_2/N_2O, while patients from the second group were ventilated with oxygen and air (O_2 / air) mixture. The other principles during the general anaesthesia were equal for both groups.

For determination of the NO plasma levels we have used the enzymatic method according to Conrad *et al.*, 1993.

Our results showed that there is a statistically significant difference of NO plasma level between the two groups. The level of NO was higher in the first group (ventilated with O_2/N_2O) compared to the second group (ventilated with O_2/air). The mean arterial pressure and systemic vascular resistance were significantly decreased in the first group, as well.

Our results suggest that nitrous oxide (N_2O) most probably plays the role of NO donor in hypertensive patients during the maintenance of the general anaesthesia with N_2O/O_2 mixture.

Key words: nitric oxide, nitrous oxide, essential hypertension, general anaesthesia.

Introduction

Nitrous oxide was discovered by Joseph Priestley, 1773, about one year after he had described oxygen as a chemical compound of air. For the first time in medicine N_2O was used by the American dentist Horace Wells during tooth extraction under N_2O inhalation.

Since 1844, nitrous oxide has been one of the most often used anaesthetics in the operating theatre, due to many positive effects, such as: basal sedation, analgesic, cognitive, psychometric, respiratory and cardiovascular effects. We have examined the vascular effects of nitrous oxide during general anaesthesia of patients with essential hypertension who have undergone middle ear surgery. In general, the essential hypertension is caused by endothelial dysfunction that leads to disequilibrium between vasoconstriction and vasodilatation agents (Fitzerald *et al*., 1983).

Most of the patients with essential hypertension have low levels of endothelial nitric oxide synthase (eNOS) (Heller, 1996), which is the enzyme for synthesis of NO in vascular cells (Anggard, 1994; Bredt and Snyder, 1994, Henrich, 1991, Murad, 1999). The bloodless operative field which is a requirement for the successful performance of microsurgical procedures on the ear is hard to achieve in hypertensive patients (Barash *et al.*, 1996). The use of N_2O for maintenance of anaesthesia in hypertensive patients can avoid this problem. Since N_2O is approximately 34 times more soluble in blood than nitrogen, a probem of accumulation of N_2O in the closed space in the body might be expected. However this is not the case with cavum tympani during surgery of the middle ear.

The aim of our study was to examine the effects of nitrous oxide on vascular cells of hypertensive patients who were scheduled under general anaesthesia for middle ear surgery. Therefore we determined the plasma levels of nitric oxide (NO), hart rate (HR), systolic, mean and diastolic blood pressure (SAP, MAP and DAP) electrocardiogram (ECG), peripheral pulse oxymetri (SpO_2) and systemic vascular resistance (SVR), at five time intervals during the general anaesthesia.

Material and methods

Thirty four hypertensive patients, scheduled under general anaesthesia for middle ear surgery, were classified according to the American Society of

Anesthesiology (ASA). All patients belonged to ASA physical status II. The patients were divided in two groups: the first group of 17 patients, who were maintained in general anaesthesia with ventilation of O_2/N_2O = 35%/65%; the second group of 17 patients who were maintained in general anaesthesia with ventilation of O_2 /air = 35%/65%.

All patients were breathing pure oxygen for 3 minutes before intubation for denitrogenation of their lungs.

The following anaesthetic protocol was used: patients were premedicated with 5μg/kg body weight Fentanil i.v; intubation was facilitated by Propofol (2 mg/kg) and Rocuronium (0.5 mg/kg); bolus intravenous doses of Fentanil and Rocuronium were added for maintenance of the anaesthesia during the operation; neuromuscular block was reverted by Prostigmin and Atropin at skin closure.

Patients were selected randomly in two groups to receive O_2/N_2O or O_2/air; 35%/65%, for maintenance of the anaesthesia.

The following inclusion criteria were used for selection of the patients:

1. adult patients (older than 18),
2. ASA II, hypertensive patients,
3. without antihypertensive therapy last two weeks before surgery,
4. middle ear surgery.

Methods

Perioperative monitoring (Ebert 1990; Houltz *et al.*, 1995)

- Heart rate (HR)
- Systolic, mean and diastolic blood pressure (SAP, MAP and DAP)
- Electrocardiogram (ECG)
- Peripheral pulse oxymetri (SpO_2).

All parameters were measured by "Criticon" monitor (General Electronics, CA, USA). HR, ECG and SpO_2 were continuously measured during the operation. Blood pressure was measured every five minutes during the operation, using the indirect method (Lalevic, 1999).

Samples of venous blood were taken five times during the operation in vacuutainer tubes with EDTA.

All parameters were analysed at the following time intervals:

T1. 30 minutes before the operation

T2. 1 hour after induction of anaesthesia

T3. 2 hours after induction of anaesthesia

T4. after the extubation

T5. 2 hours after the extubation.

- Systemic vascular resistance (SVR) was calculated by the formula:

$$SVR = \frac{MAP - CVP}{CO} \times 79.9$$

SVR – Systemic vascular resistance, normal value = 900–1400 dyn x sec x cm-5

CVP – central venous pressure, normal value = 5.5±0.5 mmHg

CO – cardiac output = 3,5–5 L/min.

CO was measured 30 minutes before and 2 hours after the operation at the Institute for Cardiovascular Diseases. Transthoracic echocardiography was used to determine LVEDV and HR.

CO = LVEDV x HR

LVEDV – left ventricular end diastolic volume

HR – heart rate

MAP was measured every 5 minutes during the operation.

CVP was measured before, during and after the operation.

Blood samples were centrifuged at 3000 /10 min and the plasma was collected and kept frozen at -20^{o}C until NO determination.

Plasma level of NO was measured using the method by Conrad *et al*, 1993, which is an enzymatic measurement of the stable end-products of NO metabolism, nitrates and nitrites. All samples were analysed for nitrates and for both nitrites and nitrates after reduction by nitrate reductase in the presence of 10 μM NADPH. The absorbance of the reaction product was measured at 450nm. Reference values for plasma levels of NO are 40.33 ± 12μmol/l.

Statistical analyses were performed with the paired Student's t-test. All measures were analysed by ANOVA. Significance was considered at $p < 0.05$.

Results

In this study two groups of patients were included. Both groups contained adult male and female patients, aged between 30 and 50 years. Demographic data of both groups are presented in Table 1.

Table 1 – Табела 1

Demographic data of the patients
Демографски податоци на пациентите

Group	Gender	Age	n
I	9 M (53%) 8 F (47%)	40 ± 9,8	17
II	7 M (41%) 10 F (59%)	39 ± 8,9	17

The level of NO in the first group of hypertensive patients, ventilated with O_2/N_2O mixture, was 22 ± 1.24 μmol/l 30 min before the operation, (T1) and 2 hours after the extubation (T5), while during the T2, T3 and T4 intervals the NO level was between 40 and 43 μmol/l. In the second group of hypertensive patients, ventilated with O_2 / air mixture, the NO level varied between 20 and 23 μmol/l during all five time intervals (T1–T5).

The results of determination of NO plasma levels for both groups of patients are presented in Table 2 and Fig. 1.

Table 2 – Табела 2

Plasma NO concentration in group I and group II of hypertensive patients

Плазматска концентрација на NO кај I и II група на хипертензивни пациенти

Time interval	group I		group II	
	NO μmol/l	± SD	NO μmol/l	± SD
T1	22,4	1,2	21,6	1,2
T2	41,2	2,1	22,6	1,3
T3	43,2	1,6	22,7	1,1
T4	40,5	1,2	21,3	1,7
T5	22,7	0,7	20,9	1,2

The main arterial pressure in the first group of hypertensive patients decreased from 122 to 91 mmHg during the first three time intervals of measurement (T1–T3) and it increased to 120 mmHg in T4 and T5. The MAP values in the second group of patients were close to the values of the first time interval (from 110–130 mmHg).

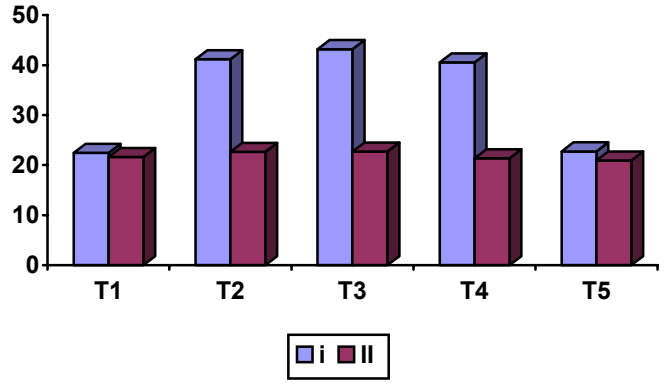

Figure 1 – *Middle values of NO (μmol/l) in both groups of hypertensive patients*

Фигура 1 – *Средни вредности на NO (μmol/l) кај двете групи хипертензивни пациенти*

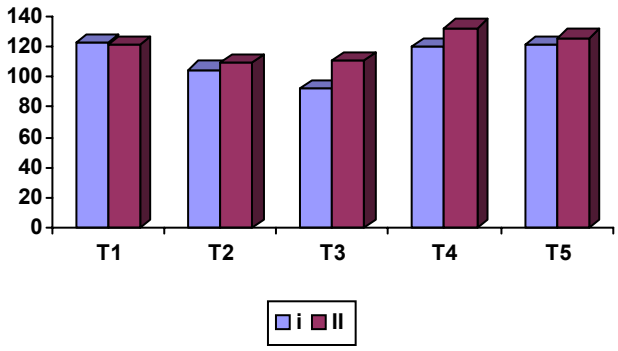

Figure 2 – *MAP values (mmHg) in both groups of hypertensive patients*

Фигура 2 – *Средни вредности на MAP (mmHg) кај двете групи хипертензивни пациенти*

The results from MAP measurements are presented in Table 3.

The systemic vascular resistance, measured during the third time interval (T3), in the first group of patients decreased from 1700 to 1200 dynxsecxcm^{-5}, while in the second group it decreased from 1700 to 1500 dynxsecxcm^{-5}. At the end of the operating treatment the SVR values in both groups of patients normalised to the level before the operation. The results of the SVR measurements are presented in Table 4.

Table 3 – Табела 3

MAP values in group I and group II of hypertensive patients
Вредности на MAP кај I и II група хипертензивни пациенти

Time interval	group I		group II	
	MAP mmHg	± SD	MAP mmHg	± SD
T1	122,3	11,4	121,2	8,8
T2	104,8	8,8	109,3	8,8
T3	91,8	6,8	110,7	7,9
T4	120,2	11,4	132,2	8,7
T5	120,6	10,2	125,7	9,5

Table 4 – Табела 4

SVR values in group I and group II of hypertensive patients
Вредности на SVR кај I и II група хипертензивни пациенти

Time interval	group I		group II	
	SVR dynxsecxcm^{-5}	± SD	SVR dynxsecxcm^{-5}	± SD
T1	1709,1	230,7	1708,1	217,6
T2	1444,2	150,8	1521,8	195,2
T3	1276,7	157,5	1542,2	176,8
T4	1674,2	203,7	1868,8	213,0
T5	1658,1	206,7	1769,9	191,4

The statistical analyses showed that there is a statistically significant difference between the two groups of patients for the three parameters: NO, MAP and SVR. The other parameters such as: heart rate (HR), electrocardiogram (ECG), peripheral pulse oxymetri (SpO_2) did not show any statistically

significant difference. The analysis of variance (F) and p-value for the NO, MAP and SVR are presented in Table 5.

Table 5 – Табела 5

Analysis of variance (F) and p value (p) for NO, MAP and SVR
Анализа на варијанса (F) и p вредности на NO, MAP и SVR

Parameter	group I		group II	
	F	p-value	F	p-value
NO	895,9032	0,00	6,093158	0,000248
MAP	30,856	0,0000	21,26028	0,00000
SVR	15,672	0,0000	9,462871	0,000003

The results of the Pearson-s coefficient analyses showed a strong negative correlation between the NO concentration and MAP in the patients ventilated with O_2 / N_2O mixture, during the T2 and T3 time intervals (Table 6). However, during the T4 and T5 time intervals the correlation remained weak negative. The Pearson-s coefficient in the second group of patients, ventilated with O_2 / air mixture, showed a weak negative correlation between NO concentration and MAP during all time intervals except T1 (before the operation).

Table 6 – Табела 6

Pearson-s coefficient of correlation between NO/ MAP and NO/SVR
Pearson-ов коефициент на корелација помегу NO/MAP и NO/SVR

Time intervals	Pearson-s coefficient			
	group I		group II	
	NO/MAP	NO/SVR	NO/MAP	NO/SVR
T1	-0.36*	-0.34*	+0.21	+0.29
T2	-0.47**	-0.53**	-0.23*	-0.29*
T3	-0.54**	-0.47**	-0.20*	-0.28*
T4	-0.23*	-0.27*	-0.31*	-0.15*
T5	-0.26*	-0.36*	-0.10*	-0.25*

* weak negative correlation, coef. < 0.36
** strong negative correlation, coef. > 0.36
* слаба негативна корелација, коеф. < 0.36
** силна негативна корелација, коеф. > 0.36

The Pearson-s coefficient analyses showed a strong negative correlation between the NO concentration and SVR in the patients ventilated with O_2/N_2O mixture only during the T2 time interval. During the other time intervals (T3, T4 and T5) the correlation remained weak negative with a gradual decrease. The Pearson-s coefficient in the second group of patients, ventilated with O_2/air mixture, showed a weak negative correlation between NO concentration and SVR during all time intervals.

Discussion

In order to examine the effects of nitrous oxide (N_2O) on vascular cells of hypertensive patients, who were scheduled under general anaesthesia for middle ear surgery, we have analysed the plasma levels of nitric oxide (NO). Our results from the NO determination of the patients ventilated with O_2/N_2O, showed higher NO concentrations compared to the patients ventilated with O_2/air. The NO concentrations were increased during T2, T3 and T4 time intervals in the first group of patients, while there were no increases of NO levels in all five time intervals of measurement in the second group of patients. These results suggest that a substantial amount of endothelial NO is produced when anaesthesia is maintained by O_2/N_2O mixture.

The correlation between NO and mean arterial blood pressure (MAP) in the first group of patients showed a strong negative correlation for the same time intervals. This is most probably due to the vasodilatation caused by the NO that lowers the MAP. Also, in the first group of patients there is a strong negative correlation between NO and systemic vascular resistance (SVR) for the same time intervals (T2 and T3), showing that the increased concentration of NO influence the decrease of SVR that is also caused by vasodilatation.

In contrast, the results for the second group of patients showed a minor variation of the NO level and a very weak correlation NO/MAP and NO/SVR, as well. In this group of patients the arterial pressure and the vascular resistance were not decreased since vasodilatation did not occur.

The hemodynamic effects of N_2O have been a subject of many studies in the past (Hohner *et al.*, 1994a; 1994b; Houltz *et al.*, 1995; Hahn *et al.*, 1997). A large prospective sturdy of 270 patients showing the hemodynamic effects of N_2O was published in 1990 by Eger *et al*. The patients in this study were randomized to groups with fresh gas flow with or without nitrous oxide. In the nitrous oxide group, the mean value for the systolic blood pressure was 108mmHg compared with 115mmHg for the group without N_2O. Comparable values for pulse were 66 beats/min for the nitrous oxide group and 70 beats/min for the other group.

Another study on the hemodynamic effects of anaesthesia is based on the use of O_2/nitrous oxide/sevoflurane, compared to O_2/air/sevoflurane (Forte *et al.*, 1997). In this study a reduction of blood pressure, systemic vascular resistance and "cardial index" in the first group was found in comparison with the second group of patients.

Although many studies have clearly shown the vascular effects of N_2O, nitric oxide (NO) as the most possible factor for these effects has not been determined, so far. Our study is the first study showing that NO released from N_2O, regulates vascular tone and it is the main reason for vasodilatation. In conclusion, the maintenance of the general anaesthesia by ventilation with O_2/N_2O mixture could be highly recommended for patients with essential hypertension.

REFERENCES

1. Anggard E. (1994): Nitric oxide: mediator, murderer and medicine, Lancet, 343: 1199–1206.

2. Barash P. G. (1996): with 98 contributors. Clinical anesthesia, Second Edition.

3. Bredt D., Snyder S. H. (1994): Nitric oxide: a physiologic messenger molecule. Annu Rev Biochem 3: 175–195.

4. Conrad K. P., Joffe G. M., Kruszyna H., Kruszyna R., Rochelle L. G., Smith R. P., Chavez J. E., Mosher M. D. (1993): Identification of increased nitric oxide biosynthesis during pregnancy in rats. FASEB J., 7(6): 566–71.

5. Ebert T. J. (1990): Differential effects of nitrous oxide on baroreflex control of heart rate and peripheral sympathetic nerve activity in humans. Anesthesiology, 72: 16–22.

6. Eger E. I., Lampe G. H., Wauk L. Z., Whitendale P., Cahalan M. K., Donegan J. H. (1990): Clinical pharmacology of nitrous oxide: an argument for its continued use. Anesth Analg., 71: 575–85.

7. Forte P., Copland M., Smith L., Milne E., Sutherland J., Benjamin N. (1997): Basal nitric oxide synthesis in essential hypertension. Lancet, 349; 837–841.

8. Fitzerald G. A., Pedersen A. K., Patronoi C. (1983): Analysis of prostacyclin and tromboxane biosynthesis in cardiovascular disease. Circulation, 67: 1174–1177.

9. Furchgott R. F., Vanhoutte P. M. (1989): Endothelium-derived relaxing and contracting factors. FASEB J., 3: 2007–18.

10. Hahn R. G., Riddez L., Brismar B., Strandberg A., Hedenstierna G. (1997): Haemodynamics during inhalation of a 50% nitrous-oxide-in-oxygen mixture with and without hypovolaemia. Acta Anaesthesiol Scand., 41: 485–91.

11. Henrich W. L. (1991): The edothelium-a key regulator to vascular tone. American Journal of Medical Science, 302: 319–328.

12. Heller Herman (1996): Hypertension, the endothelium, and the pathogenesis of chronic vascular disease. Kidney and blood pressure research, 19: 166–171.

13. Hohner P., Reiz S. (1994a): Nitrous oxide and the cardiovascular system. Acta Anesthesiol Scand., 38: 763–766.

14. Hohner P., Backman C., Diamond G., Friedman A., Haggmark S., Johansson G., Karp K., Reiz S. (1994b): Anaesthesia for abdominal aortic surgery in patients with coronary artery disease, Part II: Effects of nitrous oxide on systemic and coronary haemodynamics, regional ventricular function and incidence of myocardial ischaemia. Acta Anaesthesiol Scand., 38: 793–804.

15. Houltz E., Caidahl K., Hellstrom A., Gustavsson T., Milocco I., Ricksten S. E. (1995): The effects of nitrous oxide on left ventricular systolic and diastolic performance before and after cardiopulmonary bypass: evaluation by computer-assisted two-dimensional and Doppler echocardiography in patients undergoing coronary artery surgery. Anesth Analg., 81: 243–8.

16. Lalevic P. (1999): Anesteziologija, Zavod za udzbenike i nastavna sredstva, Beograd, Ch.13, 137–154.

17. Murad F. (1999): Discovery of Some of the Biological Effects of Nitric Oxide and its Role in Cell Signaling, Nobel Lecture, Bioscience Reports, 19: 133–153.

18. Pristley J. cytated by Lalevic P. (1999): Anesteziologija, Zavod za udzbenike i nastavna sredstva, Beograd, 1999; Ch. 6, 44–54.

19. Wells H cited by Lalevic P: Anesteziologija, Zavod za udzbenike i nastavna sredstva, Beograd, 1999; Ch. 6, 44–54.

Р е з и м е

АЗОТНИОТ ОКСИД КАЈ ХИПЕРТЕНЗИВНИ ПАЦИЕНТИ ВО ОПШТА АНЕСТЕЗИЈА

Б. Ширгоска[1], Т. Трајковска[2], М. Шољакова[2], Л. Симјановска[3], Р. Исијановска[4], Ј. Нетковски[1], Г. Д. Ефремов[3]

[1]Клиника за уво, нос и грло; Клинички центар, Скопје, Р. Македонија
[2]Клиника за анестезија, реанимација и интензивно лекување, Клинички центар, Скопје, Р. Македонија
[3]Истражувачки центар за генетско инженерство и биотехнологија, МАНУ, Скопје, Р. Македонија
[4]Институт за епидемиологија, Медицински факултет, Скопје, Р. Македонија

Триесет и четири пациенти со анамнеза за есенцијална хипертензија, хоспитализирани на Клиниката за уво, нос и грло беа подложени на општа ендотрахеална анестезија поради хронично воспаление на средното уво.

Цел на оваа проспективна студија е да се одреди плазматското ниво на азотниот оксид и неговите ефекти врз циркулацијата кај хипертензивни испитаници за време на општата анестезија.

Пациентите беа поделени во две групи: по вовед во анестезија кај првата група анестезијата беше одржувана со мешавина од кислород и азотен оксидул, а кај втората група со мешавина од кислород и воздух.

Методот применет за одредување на азотниот оксид (NO) во плазма е ензимскиот метод според Conrad и сор., 1993.

Резултатите покажаа дека кај групата со мешавина од кислород и азотен оксидул, плазматското ниво на NO е статистички значајно повисоко за разлика од групата каде што анестезијата беше одржувана без азотен оксидул. Исто така, и нивото на средниот артериски притисок и системскиот васкуларен отпор е значајно понизок кај групата чија анестезија беше одржувана со азотен оксидул.

Заклучок: Азотниот оксидул игра улога на NO донор даден во мешавина со кислород за одржување на општата анестезија кај пациенти со есенцијална хипертензија.

Клучни зборови: азотен оксид, азотен оксидул, есенцијална хипертензија, општа анестезија.