

**EFFECTS OF NITRIC OXIDE DONORS ON NON-PREGNANT
AND PREGNANT RAT UTERINE AND
AORTIC SMOOTH MUSCLE**

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Abstract: OBJECTIVE: To compare the effects of nitric oxide (NO) donors, diethylamine/nitric oxide (DEA/NO) and nitroglycerin (NTG), on isolated uterine and aortic tissues from non-pregnant, mid and late pregnant rats.

METHODS: The uterus and thoracic aorta were obtained from non-pregnant (estrous cycle) and pregnant Sprague-Dawley rats on day 14 and day 21. The uterine and aortic rings were incubated in organ chambers filled with Krebs-Henseleit solution bubbled with 5% CO₂ in air for isometric tension recordings. Cumulative concentration-response relationships to DEA/NO and NTG were obtained in the aortic rings contracted with phenylephrine and in spontaneously contracting uterine rings.

RESULTS: The sensitivity and the maximal inhibitory effects of DEA/NO did not differ in aortic tissues of any group. DEA/NO-induced Maximal inhibition of spontaneous contractions of uterine tissues from mid-pregnant rats was greater (although not significantly) than in the tissues from non-pregnant animals (with similar sensitivity), but it was significantly depressed in tissues from late pregnant rats. The sensitivity to and maximal inhibitory effects of NTG were less in aortic tissues from late pregnant versus mid-pregnant and non-pregnant rats. In uterine tissues from late pregnant rats the effect of NTG was negligible. The inhibitory action of both NO donors was much more pronounced in aortic versus uterine tissues.

CONCLUSIONS: Uterine smooth muscle is less sensitive than vascular smooth muscle to NO. Uterine smooth muscle from late pregnant animals demonstrates refractoriness to both DEA/NO and NTG, while vascular smooth muscle from late

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pregnant animals demonstrates refractoriness to NTG, but not to DEA/NO.

Keywords: myometrium, aorta, nitric oxide, pregnancy, rat.

INTRODUCTION

Nitric oxide (NO), a free radical produced during conversion of L-arginine to L-citrulline by NO synthase is a unique and ubiquitous biologic messenger playing a major role in many physiological and pathophysiological processes, including smooth muscle relaxation, neurotransmission, host defense and inflammation¹.

NO contributes to the circulatory changes and vasodilatory responses in pregnancy². Chronic inhibition of NO production in rats induced the condition resembling human pre-eclampsia³. It was demonstrated that NO plays a role in the blunted responsiveness to vasopressor agents during pregnancy indicating that NO plays a major role in regulation of vascular tone and blood pressure and that inefficiency of NO production/release may lead to hypertension and preeclampsia^{3,4}.

Accumulating evidence also points to a role for NO in the regulation of uterine contractions during pregnancy⁵⁻⁷. NO is produced by the uterine tissues and NO donors inhibit uterine spontaneous contractions. The NO system is up-regulated during pregnancy maintaining relative uterine quiescence until term^{6,7,8}. However, the role of NO in the control of uterine contractility during pregnancy is still not completely defined.

NO donors, mainly NTG, are used in clinical obstetrics in patients with severe pre-eclampsia⁹ and preterm labor^{10,11}. There have been a few data comparing the effects of NO on the uterine and vascular smooth muscle in pregnancy⁷. The uterine and aortic smooth muscles are functionally different. The former are characterized by phasic spontaneous contractions with spike-like changes in membrane potential, while the latter develops tonic contraction associated with gradual changes in membrane potential. In the present study, we investigate the effects of two NO donors, diethylamine/nitric oxide (DEA/NO) and nitroglycerin (NTG), releasing NO spontaneously or after tissue metabolic transformation, respectively on isolated uterine and aortic rings from non-pregnant and pregnant rats at different gestational age.

MATERIAL AND METHODS

The experimental protocol was approved by the Animal Care and Use Committee at the University of Texas Medical Branch at Galveston, Texas.

Tissue preparation. Non-pregnant (estrous cycle) and pregnant Sprague-Dawley rats (180-250 gm) from Charles River Laboratories (Wilmington, MA) on day 14 (midpregnant) and day 21 (late pregnant) of gestation were used in the experiments.

Rat was sacrificed by carbon dioxide inhalation, thoracic aorta and uterine horns were excised and placed in cold Krebs-Henseleit solution saturated with 5% CO₂ in air. After cleaning of the tissues from surrounding tissues, ring segments (4 mm in width) of aorta and uterus, with the fetal and placental tissues gently removed, were cut. The vascular endothelium was removed by gentle rolling of the aortic rings over a twisted stainless steel wire covered with cotton.

Mechanical measurement. Both aortic and uterine rings were positioned between two tungsten wire (250 μ m) stirrups in the organ chambers containing 10 ml of Krebs-Henseleit solution continuously bubbled with 5%CO₂ in air (pH \sim 7.40 at 37°C). The composition of the Krebs-Henseleit solution (in mM/L): sodium chloride 119, potassium chloride 4.7, potassium phosphate 1.2, magnesium sulfate 1.2, sodium bicarbonate 25.0, calcium chloride 2.5, sodium ethylenediaminetetraacetic acid 0.026 and glucose 11.1. The passive tension was gradually increased to 2 gm, which is optimal for both tissues, during the equilibration period of more than 1.5 hours.

Isometric tension recording was made with Harvard isometric transducers (Harvard Apparatus, South Natick, MA, USA) connected to an eight-channel model RPS 7C Grass polygraph recorder (Grass instruments, Quincy, MA, USA) and stored and analyzed with a help of computer software Assistant Plus (Software Technology, Rochester, NY, USA) and computer-linked digitizing system (SPUC, Schering, Berlin, Germany) as described^{12,13}. Integral activity, as an area under the curve, for each uterine ring was analyzed for 10 min before (basal integral activity) and for 10 min after the addition of each concentration of the agents.

Aortic rings equilibrated at 2 gm passive tension were contracted with potassium chloride (60 mM), and tension developed was determined at 30 min. Thirty minutes after washing out and rest, aortic rings were contracted with phenylephrine (10⁻⁶ M/L) and after tension reached the plateau, concentration-relaxation relationships to DEA/NO and NTG were obtained.

Agents used. Diethylamine/nitric oxide (DEA/NO) was obtained from Research Biochemicals International (Natick, Mass, USA). Nitroglycerin (NTG) was obtained from Nipponkayaku Inc. (Tokyo Japan). Stock solution of DEA/NO (100 mM) was prepared with deionized water, aliquoted and kept frozen (-20° C) until used. The desired concentration of DEA/NO and NTG were made with deionized water at the day of experiment and kept on ice during the experiment.

DEA/NO and NTG were cumulatively added to the organ chamber solution in 0.5 log units increments in the volumes 10-23 μ l, except the final concentration of NTG (10⁻⁴ M), which was 454 μ l.

Data analysis. Results are expressed as means \pm S.E.M. The change in integral activity of uterine rings after 10 min exposure to each concentration of the agent was expressed as percentage change from the basal integral activity for similar period of time. Effective concentrations ($-\log$ M) of the agents causing 50% inhibition of phenylephrine-induced tension in aortic ring or 50% decrease in spontaneous

contractile activity in uterine rings (IC_{50} 's) were calculated from each concentration-response curve. The statistical analysis was performed by means of unpaired *Student's t*-test with Welch's post-test and one way ANOVA with Newman-Keuls post-test when two or more means were compared, respectively. A probability of 0.05 or less was considered statistically significant.

RESULTS

Effect of DEA/NO and NTG on the uterine spontaneous contractile activity

Mean data of the concentration-response relationships of DEA/NO and NTG in uterine rings from non-pregnant, mid-pregnant and late pregnant rats are presented in Fig. 1 and 2, respectively. Both NO donors inhibited spontaneous contractions of the uterine rings from non-pregnant and midpregnant rats in a concentration-dependent manner. However, both NO donors did not significantly inhibit the spontaneous contractile activity of the uterine rings from late pregnant rats. Inhibition of spontaneous contractions of the uterine rings from non-pregnant and pregnant rats at midgestation was significant ($p < 0.05$) at a DEA/NO concentration of 10^{-5} M and 10^{-4} M (Fig. 1). At maximal concentration of DEA/NO (10^{-4} M), the inhibition of spontaneous contractions of the uterine rings from late pregnant rats was significantly less as compared to the rings from non-pregnant and mid-pregnant animals (Table 1).

NTG induced significant ($p < 0.05$) inhibition of spontaneous contractions of uterine rings from non-pregnant and midpregnant rats at a concentration of 3×10^{-5}

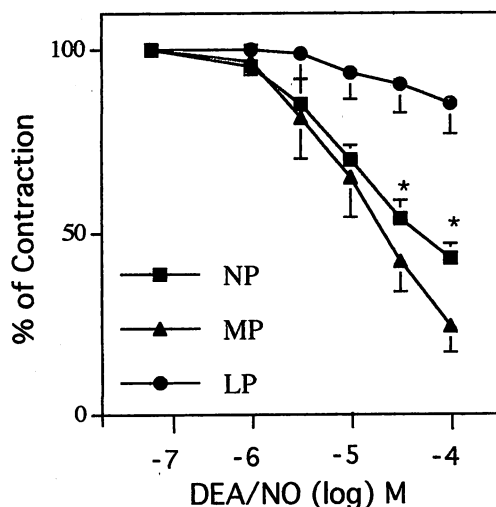


Fig. 1. Concentration-response relationships to diethylamine/nitric oxide (DEA/NO) in uterine rings from non-pregnant (NP), mid-pregnant (MP) and late pregnant (LP) rats. Abscissa - concentration (log M), ordinate - percent of basal integral contractile activity. Data presented as means \pm SEM. $N = 6$ per group. * $p < 0.05$, vs MP.

Table 1. IC_{50} 's and maximal inhibitory effects (Mx) of diethylamine/nitric oxide (DEA/NO) and nitroglycerin (NTG) on spontaneous contractions of uterine rings and phenylephrine (10^{-6} M)-induced tension of aortic rings without endothelium from non-pregnant, mid-pregnant and late pregnant rats. $n=6$ per group

| | Non-pregnant | | Mid pregnant | | Late pregnant | |
|------------------|--------------|-----------------------|--------------|-----------------------|-------------------------|-------------------------|
| DEA/NO | | | | | | |
| IC ₅₀ | Mx (%) | IC ₅₀ | Mx (%) | IC ₅₀ | Mx (%) | |
| Uterus | 4.99±0.13 | 56.9±4.4 | 4.94±0.13 | 75.6±5.0 | n.d. | 14.8±8.6 ^a |
| Aorta | 7.03±0.1 | 86.4±1.9 ^b | 7.06±0.10 | 97.5±1.6 | 6.91±0.1 | 93.8±2.1 |
| NTG | | | | | | |
| Uterus | 4.86±0.1 | 47.1±5.7 | 4.66±0.1 | 33.6±4.8 ^c | n.d. | 12.0±4.9 ^a |
| Aorta | 7.34±0.1 | 92.2±1.9 | 7.03±0.1 | 83.4±5.0 ^c | 6.26±0.2 ^{a,c} | 65.5±2.8 ^{a,c} |

Values are the means \pm SEM

^a-statistically significant ($p < 0.05$) difference VS mid-pregnant and non-pregnant

^b-statistically significant ($p < 0.05$) difference VS mid-pregnant and late pregnant

^c-statistically significant ($p < 0.05$) difference VS DEA/NO

n.d.- not determined.

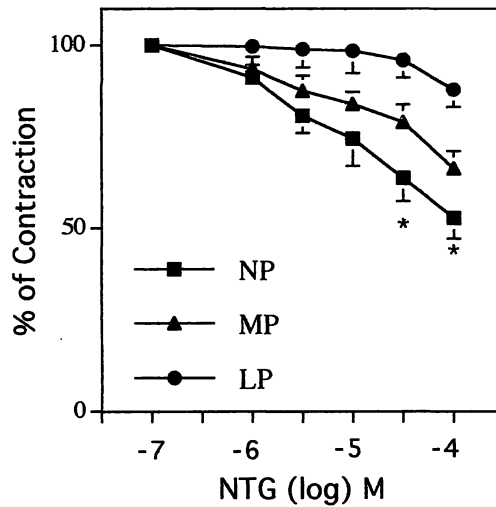


Fig. 2. Concentration-response relationships to nitroglycerin (NTG) in uterine rings from non-pregnant (NP), mid-pregnant (MP) and late pregnant (LP) rats. Abscissa - concentration (log M), ordinate - percent of basal integral contractile activity. Data presented as means \pm SEM. $N=6$ per group. * $p < 0.05$, vs MP.

M and 10^{-4} M, respectively (Fig. 2). At maximal concentration of NTG (10^{-4} M) the inhibition of spontaneous contractions of the uterine ring from late pregnant rats was significantly less as compared to the rings from non-pregnant and mid-pregnant animals (Table 1). The sensitivity to both NO donors did not differ in uterine rings

from non-pregnant and mid-pregnant rats and it was not determined in rings from late pregnant animals due to negligible inhibitory effects. There was no significant difference between the IC_{50} 's of the NO donors in uterine tissues from non-pregnant and midpregnant rats (Table 1). The inhibitory effects of maximal concentrations of DEA/NO was significantly greater as compared to those of NTG in rings from mid-pregnant rats (Table 1).

Effect of DEA/NO and NTG in rat aortic rings

DEA/NO (Fig. 3) and NTG (Fig. 4) concentration-dependently relaxed phenylephrine (10^{-6} M)-contracted endothelium-denuded rat aortic rings. DEA/NO significantly decreased the tension of aortic rings at concentration of 10^{-8} M and nearly abolished phenylephrine-induced tension at concentration 10^{-6} M (Fig. 3). DEA/NO-induced relaxation of aortic rings from mid-pregnant and late pregnant rats were significantly greater than in rings from non-pregnant rats, although there was no significant changes in the sensitivity (Table 1). NTG relaxed phenylephrine-contracted rings from concentration 10^{-8} M or 3×10^{-8} M to a maximal concentration (10^{-5} M), the inhibition of the tension and the sensitivity to NTG of the aortic ring from late pregnant rats was significantly less ($p < 0.05$) than those in rings from non-pregnant or midpregnant animals (Fig. 4, Table 1). The sensitivity to DEA/NO and NTG was equal in the aortic rings from non-pregnant and mid-pregnant rats, although maximal relaxation induced by DEA/NO was significantly greater in the latter (Table 1). Relaxation and sensitivity to NTG were significantly less in aortic rings from late pregnant rats (Table 1).

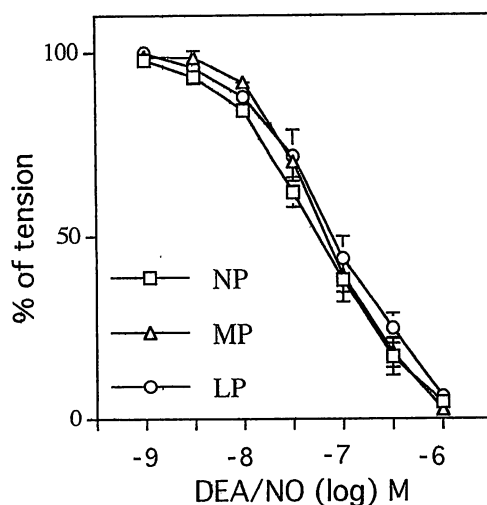


Fig. 3. Concentration-response relationships to diethylamine/nitric oxide (DEA/NO) in aortic rings from non-pregnant (NP), mid-pregnant (MP) and late pregnant (LP) rats. Abscissa - concentration (log M), ordinate - percent of phenylephrine (10^{-6} M)-induced tension. Data presented as means \pm SEM. $N=6$ per group.

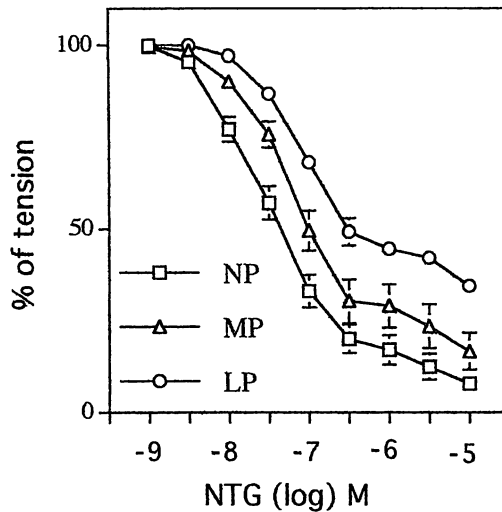


Fig. 4. Concentration-response relationships to nitroglycerin (NTG) in aortic rings from non-pregnant (NP), mid-pregnant (MP) and late pregnant (LP) rats. Abscissa - concentration (log M), ordinate - percent of phenylephrine (10^{-6} M)-induced tension. Data presented as means \pm SEM. N=6 per group.

DISCUSSION

The data presented demonstrate that (1) DEA/NO and NTG inhibit spontaneous contractions of uterine tissues from non-pregnant and mid-pregnant, but not late pregnant rats. (2) The inhibitory effect of DEA/NO in uterine tissues from mid-pregnant animals is significantly greater than those of NTG. (3) The relaxant effect of and sensitivity to NTG, but not to DEA/NO, in aortic rings with removed endothelium is gestational age dependent. (4) The rat myometrium is less sensitive than rat aortic smooth muscle to NO.

DEA/NO is an adduct of nitric oxide with diethylamine that spontaneously releases NO extracellularly by a non-enzymatic process with an estimated half-life of about 2.1 minutes in solution with pH 7.4 at 37°C ¹⁴. The compound has been previously shown to relax blood vessels¹⁴ and inhibit spontaneous contractions of human⁸ and rat⁷ myometrial tissues. NTG usually requires biological transformation by putative converting enzyme(s) to release NO¹⁵. NTG has been used in cardiology for more than a hundred years. NO generated by NTG dilates blood vessels by relaxation of arterial smooth muscles. That was the reason NTG was used in clinical obstetrics in patients with severe pre-eclampsia⁹. Moreover, NTG inhibits spontaneous contractions of human¹⁶ and rat¹⁷ uterine smooth muscle.

It has been demonstrated that NO-soluble guanylate cyclase-cyclic guanosine monophosphate (cGMP) pathway exists in myometrium that is up-regulated during pregnancy to maintain uterine relative quiescence until term⁵⁻⁸. It has also been demonstrated that NO synthase (NOS) activity in the rat myometrium peaked on

day 15 of gestation, then decreased progressively until day 21 of gestation, and did not decrease further till the spontaneous onset of labor on day 22⁶. DEA/NO has been shown to inhibit uterine spontaneous contractions, and the effect is elevated during pregnancy and decreased toward term^{7,8}. NTG and sodium nitroprusside (SNP) inhibited contraction of uterine strips from both pregnant and non-pregnant women¹⁸. Sensitivity to (SNP) did not differ in intact aortic rings from non-pregnant, midpregnant and late pregnant rats¹⁹. Similar to DEA/NO, SNP liberates NO spontaneously²⁰. The sensitivity to NTG, but not to DEA/NO in this study was significantly decreased only in aortic rings from late pregnant rats. The decrease in the sensitivity to NTG of aortic smooth muscle from late pregnant rats may result from the decrease in NTG-converting enzyme at late gestation.

The sensitivity to both DEA/NO and NTG of aortic smooth muscle was higher than that of myometrium. It is generally accepted that NO causes the inhibition of vascular tone via activation of soluble guanylate cyclase (sGC)-cGMP pathway. Although the existence of this pathway was documented in myometrial tissue^{6,7}, cGMP-independent effects of nitric oxide were also suggested²³⁻²⁵. The less sensitivity of uterine versus vascular smooth muscle to NO²⁶ and differential selectivity of NO to relax and to increase cGMP levels between vascular and non-vascular smooth muscle²⁷ suggests the differential pharmacological properties of NO. The action of NO may be realized via different pathways differently presented in different tissues and this may determine the difference in the effects observed. The uterine and aortic smooth muscles represent the tissues with different mechanisms of activation of contractile machinery, namely phasic spike-like and tonic mechanisms of activation, respectively. The tissues with spike-like mechanism of activation that is the tissues possessing spontaneous contractile activity generally demonstrated low sensitivity to NO²⁶⁻²⁸. None of the protein kinase G substrates detected during relaxation of rat aortic smooth muscle appeared to be phosphorylated in the rat myometrium after administration of cGMP activating agents²⁹.

NO donor, NTG has been used in patients with preterm labor^{10-11,30}. Clinical use of the NO donors for inhibition of labor may be limited by the cardiovascular side effects, unless preferential concentration of nitric oxide is achieved in the myometrium. NTG given intravenously caused hypotension in 67% of patients with preterm labor³⁰. However, transdermal application of NTG did not cause any side effects in the patients with preterm labor, and appeared to be useful¹⁰. The average length of gestation was 46 days in transdermal NTG treated patients compared to 27 days in ritodrine or placebo receiving patients¹¹. Two patients (7%) from the 30 patients receiving NTG had a single episode of hypotension. The difference in the side effect might result from the difference in the route of NTG administration. Therefore, we have to consider the effects of NO donors on uterine contractility and circulation when we using in patients with preterm labor.

In conclusion: (1) the rat myometrium is less sensitive to NO compared to the smooth muscle of the aorta, (2) DEA/NO is more potent tocolytic and vascular

relaxant than nitroglycerin at term, (3) the decrease in vasorelaxing effect of NTG with progression of pregnancy may result from the decreased biotransformation in vascular tissues, and (4) the decreased inhibitory effect of NO on uterine smooth muscle at term may be one of the factors the synchronizing of uterine contractions to initiate labor.

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