The Effects of Nitroglycerin on the Contraction of Rat Uterine Smooth Muscle

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Background: It has been reported that nitroglycerin relaxes not only vascular smooth muscle but also uterine smooth muscle. The aim of the present study was to investigate the effect of nitroglycerin on rat uterine contractile activity in vitro. The effects of nitroglycerin on myometrial spontaneous activity and oxytocin-induced contractions were also observed.

Methods: Uterine smooth muscle tissues were obtained from non-pregnant female rats (n = 21). The uterine segments were mounted in tissue baths. After spontaneous or oxytocin-induced activity had been accomplished, nitroglycerin in various concentrations was added to the bath and the effects were continuously registered.

Results: Nitroglycerin induced a dose-dependent inhibition of spontaneous as well as oxytocin-induced myometrial contractile activity. Complete muscular relaxation on spontaneous contractility was obtained at a concentration of 50μg/ml. Complete muscular relaxation on oxytocin-induced contractility was obtained at a concentration of 75μg/ml.

Conclusions: Nitroglycerin inhibited the uterine contractile response to exogenous oxytocin as well as spontaneous in the estrous rat. (Korean J Anesthesiol 2006; 50: S 57–60)

Key Words: nitroglycerin, oxytocin, rat uterine smooth muscle, uterine relaxation.

INTRODUCTION

Nitroglycerin is a well-known smooth muscle relaxant that reduces the preload of ventricle by dilating vascular smooth muscle. Consequently, it reduces the heart size and ventricular vascular tone, increases cardiac output and also dilates coronary artery. With this effect, it has been used for myocardial ischemia, systemic or pulmonary hypertension, acute ventricular failure, and coronary artery spasm.1,2

At the same time, it has been shown to have the relaxation of the uterine smooth muscle.3 Some case reports have shown the value of nitroglycerin for manual removal of placenta in awake patients4,5 and for treating uterine inversion during elective cesarean section under spinal anesthesia.6 It has also been used to facilitate cesarean delivery under spinal anesthesia at twin extraction7 and during intrapartum external cephalic version.8

Hence, we investigated and compared the effects of nitroglycerin on the spontaneous uterine activity and on the oxytocin-induced uterine activity in non-pregnant white rats.

MATERIALS AND METHODS

The study was approved by our institutional review board for research involving animal subjects. As experimental animals, 21 female Sprague-Dawley rats weighing 200-250 g were used. All rats were anesthetized by the intraperitoneal injection of thiopental (250 mg/kg), the abdomen was opened immediately, and the uterus was extracted. The myometrial tissue specimens were dissected into strips of myometrium (approximately 2 × 2 × 10 mm) in a Petri dish filled with Krebs solution; the muscle fibers of these strips were oriented parallel to the longest dimension. These myometrial strips were mounted in 25 ml-tissue baths containing Krebs solution. One end of the longest dimension of a muscle strip was connected to a hook that was fixed to the base of the bath. The other
end of the strip was connected to another hook fixed to an extension of the lever arm of a force displacement transducer. The bath solution was maintained at 37°C by circulating the heated water in the space between the double walls, and continuously aerated with a gas mixture of 95% oxygen and 5% carbon dioxide. The Krebs solution was composed of 118.3 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl₂, 25 mM NaHCO₃, 1.2 mM KH₂PO₄, 1.2 mM MgCl₂, and 11.1 mM glucose and the pH of the solution ranged from 7.35 to 7.45.

The isometric tension of the myometrial strip was measured using a force displacement transducer (FTO3®; Grass Instruments Co., MASS, USA) and the recordings of traces were made on a computer (PowerLab® data recording system; ADInstruments Pty Ltd., Castle Hill, Australia). To establish an optimal length, the resting tension was adjusted to 0.5 g. The bath solution was flushed with fresh solution every 20 min. Approximately 90 min into the study (about 1.5 to 2 h from the time of muscle harvesting), muscle preparations developed rhythmic spontaneous contraction. After spontaneous uterine activity had been accomplished, nitroglycerin in various concentrations was added to the baths with a micropipette, and the change of the contraction pattern was examined (Group S.A). In addition, after the treatment with the oxytocin (100μU/ml) nitroglycerin at the same concentrations (1-75μg/ml) was added and the change of uterine contraction was examined (Group OT). At that time, the drug concentration was added cumulatively every 20 minutes by increasing to 1, 10, 25, 50, and 75μg/ml.

The amplitude of contractions before and after the application of nitroglycerin in various concentrations was calculated. The amplitude of the contraction before application of nitroglycerin represents controls expressed as 100%. The inhibitory effect of nitroglycerin was compared with the control and plotted as percentage of inhibition.

Percentage of inhibition = \( \frac{(1-T/T_0)}{1} \times 100 \)

To is the control tension measured before the application of nitroglycerin, and T is the tension measured after the application of nitroglycerin. Cumulative concentration-response curves for nitroglycerin on myometrial spontaneous activity and oxytocin-induced contractions were then determined.

All obtained results were expressed as mean ± standard deviation. For statistical analysis, the change before and after the application of drugs was analyzed by repeated measures ANOVA, and post hoc test was done using Tukey method within each group. The comparison between two groups was performed by Mann-Whitney U test. and P value less than 0.05 was considered statistically significant.

**RESULTS**

**The effect of nitroglycerin on the spontaneous uterine activity**

As the concentration of nitroglycerin was increased, the amplitude and frequency of uterine activity was decreased, and complete muscular relaxation was obtained at a concentration of 50μg/ml. In addition, the myometrial activity was immediately reestablished when the bath solution was replaced by fresh Krebs solution (Fig. 1).

**The effect of nitroglycerin on the oxytocin-induced uterine activity**

When the concentration of nitroglycerin was increased, the amplitude and frequency of uterine activity was reduced, and complete muscular relaxation was obtained at a concentration of 75μg/ml. At that time, when oxytocin was added again, uterine activity was restored immediately (Fig. 2).

Nitroglycerin induced a dose-dependent inhibition of spontaneous as well as oxytocin-induced myometrial contractile activity (Fig. 3, Table 1). In addition, There was a significant difference between two groups at a concentration of nitroglycerin 25~50μg/ml.
Fig. 2. The depressing effects of nitroglycerin (Ni) on contractions induced by oxytocin (OT) of rat uterine myometrium. S.A: Spontaneous activity.

Fig. 3. Effects of increasing concentration of nitroglycerin (Ni) on spontaneous activity (S.A) and oxytocin (OT) induced contractions of rat uterine myometrium. *P < 0.05 compared with S.A.

### Table 1. Effects of Nitroglycerin on Spontaneous Activity and Oxytocin Induced Contractions in the Uterine Smooth Muscle

<table>
<thead>
<tr>
<th>Nitroglycerin</th>
<th>Control 1μg/ml</th>
<th>10μg/ml</th>
<th>25μg/ml</th>
<th>50μg/ml</th>
<th>75μg/ml</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.A (%) (n = 9)</td>
<td>100</td>
<td>91.3 ± 3.1</td>
<td>78.4 ± 4.9</td>
<td>46.9 ± 2.8</td>
<td>1.0 ± 1.8</td>
<td>0.000</td>
</tr>
<tr>
<td>OT (%) (n = 12)</td>
<td>100</td>
<td>95.2 ± 2.2</td>
<td>85.3 ± 3.5</td>
<td>77.1 ± 4.9</td>
<td>54.8 ± 4.8</td>
<td>3.0 ± 2.3</td>
</tr>
<tr>
<td>P value*</td>
<td>0.094</td>
<td>0.126</td>
<td>0.002</td>
<td>0.000</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD. S.A: spontaneous activity, OT: oxytocin. *: statistical analysis was done by repeated measures ANOVA of within subjects, †: statistical analysis was done by Mann-Whitney U test.

### DISCUSSION

Nitroglycerin is an anti-hypertensive agent with effect of direct dilatation of vascular smooth muscle. It acts on both capacitance vessels and resistance vessels, and it is more potent on capacitance vessels. In addition, it renders the dilatation of coronary artery, hence, its use is increasing in patients with myocardial ischemia and angina pectoris. Nitroglycerin is nonflammable drug which can be given intravenously and the anesthesiologists are familiar with it. By intravenous route, it acts within a few seconds, and has a half-life of only a few minutes. It also dose not produce toxic metabolites clinically. Smooth muscle exists not only in the blood vessels but also in other organs such as the bronchus, the uterus, etc. Since it was thought that vasodilating effect of nitroglycerin may also act on the uterine smooth muscle, this study was performed to examine its effects on the uterine smooth muscle.

In our study, when the concentration of nitroglycerin was increased, the amplitude and frequency of spontaneous uterine contraction were decreased. Complete muscular relaxation was obtained at a concentration of 50μg/ml.

In such manners, it was found that nitroglycerin inhibited the contraction of uterine muscle. Since nitroglycerin forms reactive free radical nitric oxide (NO), when NO is released, it stimulates guanylate cyclase located in smooth muscles, increases cyclic 3’5’ guanosine monophosphate (cGMP), suppresses the influx of calcium to smooth muscles, decreases the concentration of intracellular calcium, and induces the relaxation of smooth muscle.

In addition, when the bath solution was washed again with fresh Krebs solution, myometrial activity was immediately restored, which showed that uterine muscles have spontaneous rhythmic contractions. Furthermore, when the con-
centration of nitroglycerin was increased, the amplitude and frequency of oxytocin induced uterine contraction were decreased. Complete muscular relaxation on the oxytocin-induced uterine activity was obtained at a concentration of 75μg/ml. Our results showed that relaxation for oxytocin induced uterine contraction is required higher concentration of nitroglycerin than spontaneous muscle activity. Diamond and Marshall\(^5\) have reported that “nitroglycerin reduced the force of contractions, decreased the frequency of spontaneous action potential generation on isolated rat myometrium,” and “nitroglycerin also reduced the contractions elicited by supramaximal electrical field stimulation, and the contractures caused by isotonic potassium solutions,” which are in agreement with our results. Already in 1881, Barnes reported a patient with a retained placenta due to an ergotamine-induced contraction ring.\(^10\) This patient inhaled amyl nitrite resulting in uterine relaxation and delivery of placenta. Bayhi et al\(^6\) used intravenous nitroglycerin to treat uterine inversion. Some case reports have shown that the removal of the placenta was carried out successfully by relaxing uterine muscles by intravenous nitroglycerin.\(^5,6\) Mayer and Weeks\(^7\) used it to facilitate cesarean delivery under spinal anesthesia at twin extraction, and Belfort\(^8\) has reported the intravenous injection of nitroglycerin for the uterine relaxation in the external cephalic version during intrapartum. Although nitroglycerin passes through the placenta, it has no side effect on neonate.\(^7,8\) In the mothers an occasional mild hypotension has been noticed, but there was neither increased heart rate nor prolonged uterine relaxation.\(^4,10\) Nitroglycerin was used to suppress the uterine contraction in mothers who have the tendency of premature labor,\(^10\) and to treat severe pregnancy-induced hypertension.\(^8,17\) Yallampalli and Garfield\(^3\) have reported that chronic inhibition of nitric oxide synthesis in rats during pregnancy produced signs similar to those of preeclampsia.

Segal et al\(^9\) reported that sensitivity to nitroglycerin increased 3 times by adding placental tissue. In conclusion, nitroglycerin inhibited the uterine contraction in rats and induced relaxation of uterine smooth muscle in dose-dependent manner, and it induced the inhibition of the uterine contractile response to spontaneous as well as oxytocin-induced uterine activity. So, nitroglycerin can be used for rapid uterine relaxation under urgent obstetric condition that requires fast uterine relaxation and can be used as an alternative to general anesthesia. However, since this study used non-pregnant white rats for experiment, further study using pregnant white rats will be necessary.

### REFERENCES