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Inhibitory effect of alprostadil against sevoflurane-induced myometrial relaxation in rats

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Abstract
Purpose. For anesthetic management of cesarean sections, regardless of the use of regional or general anesthesia, it is crucial to achieve sufficient uterine contraction immediately following the delivery of an infant in order to reduce excessive bleeding. No previous study has investigated the ability of alprostadil, a synthesized prostaglandin, to inhibit myometrial relaxation induced by volatile anesthetics. The aim of the present study was to investigate the inhibitory effects of alprostadil on sevoflurane-induced myometrial relaxation using myometrial strips isolated from pregnant rats.
Methods. Myometrial strips were isolated from Sprague–Dawley rats (300–400 g) in the late stage of gestation (19–21 days). The time course of changes in spontaneous myometrium contraction was studied in the presence and absence of sevoflurane. Additionally, alprostadil was titrated at three different concentrations during continuous introduction of sevoflurane 2%, and myometrium contraction was studied. As an index of contraction, the area under the contraction curve was used, and data were analyzed by repeated measure one-way analysis of variance.
Results. We have shown a significant decrease in myometrium contraction as a result of the use of sevoflurane (2%). Additionally, alprostadil has been shown to inhibit myometrial relaxation induced by sevoflurane in a dose-dependent manner. The areas under the contraction curve were 87%, 87%, 129%, and 172% of the baseline value for the control and at low, medium, and high concentrations of alprostadil, respectively.
Conclusion. The ability of alprostadil to inhibit myometrial relaxation induced by sevoflurane suggests that the use of alprostadil during general anesthesia for cesarean section may be advantageous for the reduction of postpartum bleeding.

Key words Alprostadil · Sevoflurane · Myometrium contraction

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Introduction

For anesthetic management of cesarean section, regardless of the use of regional or general anesthesia, it is crucial to achieve sufficient uterine contraction immediately after the delivery of an infant to reduce excessive bleeding. For this purpose, oxytocin has been routinely used [1,2]; however, it is well known that volatile anesthetics cause myometrial relaxation and reduce the sensitivity to oxytocin. Often, the conventional use of oxytocin does not achieve sufficient uterine contraction under the effect of volatile anesthetics. Therefore, in the case of general anesthesia using volatile anesthetics, two strategies have been recommended. One is reducing the concentration of the volatile anesthetic to the lowest possible level, and the other is shifting the volatile anesthetic to an intravenous anesthetic after the delivery of the infant [1,2]. However, these procedures have been reported to increase the risk of intraoperative awareness in patients [3]. Therefore, an alternative uterotonic agent to inhibit the myometrial relaxation induced by volatile anesthetics is required.

Alprostadil is a synthesized prostaglandin that induces vascular dilation. In Japan, it is widely administered intravenously to treat critical hypertension and to induce deliberate hypotension during operations [4–6]. Alprostadil, like other prostaglandins, possesses a uterotonic effect and is thought by some practitioners to be effective in inducing uterine contractions without increasing blood pressure during cesarean sections [5–8]. Several clinical reports have shown a decrease in blood loss during operations in conjunction with the use of alprostadil [5,6]. Multiple case reports have shown the successful use of alprostadil during cesarean sections [7,8], but none have investigated the ability of alprostadil to inhibit myometrial relaxation induced by volatile anesthetics. The aim of the present study was to investigate the inhibitory effects of alprostadil on

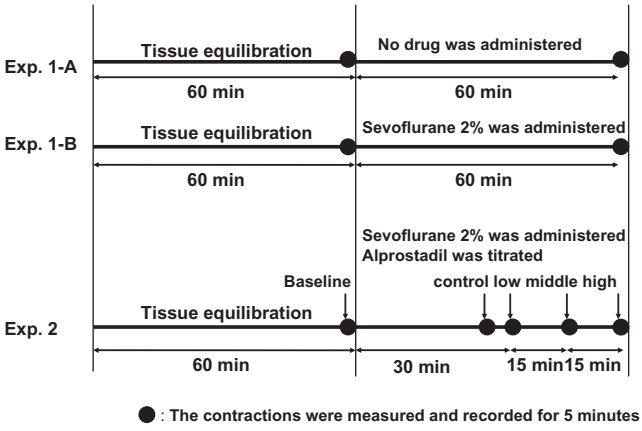

Fig. 1. Schematic protocol for the experiments

sevoflurane-induced myometrial relaxation using myometrial strips isolated from pregnant rats.

Materials and methods

The protocol for this study was approved by the animal care and use committee at St. Marianna University School of Medicine. Two series of experiments were carried out. The first experiment was designed to confirm the induction of myometrial relaxation by sevoflurane and the second investigated the ability of alprostadil to inhibit sevoflurane-induced myometrial relaxation. A schematic of the experimental protocol is shown in Fig. 1.

Tissue preparations

Sprague–Dawley rats (300–400 g) in the late stage of gestation (19–21 days) were anesthetized via intraperitoneal administration of pentobarbital and the uterus was excised. The excised uterus was bathed in a flat container containing Krebs solution with the following composition ($mM \cdot l^{-1}$): NaCl 113, KCl 4.7, $MgSO_4 \cdot 7H_2O$ 0.9, KH_2PO_4 1.2, $CaCl_2 \cdot 2H_2O$ 1.4, Glucose 11.5, $NaHCO_3$ 25. The uterus was immediately dissected in the longitudinal direction from the mesometrium side and the fetuses and placentas were carefully removed. Longitudinal myometrial strips (approximately 5×10 mm) were made from the uterus. From one rat, four strips were taken and the average contraction was calculated. In some cases, however, only three strips were available for various technical reasons.

Measurements of myometrium contraction

The strips were positioned perpendicularly in organ baths, and the baths were filled with Krebs solution bubbled with 95% oxygen and 5% carbon dioxide at 37°C. One end of each strip was connected to the bottom of the organ bath and the other end was connected to a strain gauge transducer (LVS-50GA, Kyowa, Tokyo, Japan). The data from the transducer were recorded on a computer via an interface (PCD-300A, Kyowa, Tokyo, Japan). As an index of myometrium contraction, the amplitude was calculated as the average for the myometrium contractions in a 5-min period, while the frequency was determined by the number of myometrium contractions in a 5-min period. The area under the curve (AUC) of contractions over a 5-min period was calculated using specialized software developed for analyzing the data (PCD-30A, Kyowa, Tokyo, Japan).

The strips were allowed to equilibrate at resting tension (10 mN) for 60 min. During the last 5 min of equilibration, the baseline contraction data were measured and recorded.

Effect of time and sevoflurane on spontaneous myometrium contraction

In the first experiment, designed to study the changes in spontaneous myometrium contraction over time, the strips remained in the organ bath for an additional 65 minutes following equilibration without the addition of any drug. The contractions were measured and recorded during the last 5 min ($n = 8$, experiment 1A).

The effect of sevoflurane on spontaneous myometrium contraction was studied using another eight strips. Following equilibration of the strips, sevoflurane 2% [approximately 1 minimum alveolar concentration (MAC)], was administered to the gas mix (95% oxygen and 5% carbon dioxide) for an additional 65 minutes. Contractions were measured during the last 5 min ($n = 8$, experiment 1B).

Inhibitory effect of alprostadil on sevoflurane-induced myometrial relaxation

To elucidate the inhibitory effect of alprostadil on sevoflurane-induced myometrial relaxation, alprostadil (Sigma-Aldrich, St. Louis, MO, USA) was titrated at three different concentrations. Myometrium contractions were measured while sevoflurane was continuously administered ($n = 8$).

After 60 min of tissue equilibration, baseline contraction values were measured and sevoflurane 2% was administered into the gas mix (95% oxygen and 5% carbon dioxide) and bubbled for 65 min continuously. Twenty five minutes following the addition of sevoflurane, the control contraction data were measured for 5 min. Alprostadil was then titrated at 1×10^{-8} $g \cdot ml^{-1}$ (low concentration), and immediately after the addition of alprostadil the contraction data were measured for

5 min. After a 15-min interval, alprostadil was titrated at 1×10^{-7} g·ml^{-1} (medium concentration), and immediately after the addition of alprostadil the contraction data were measured for 5 min. After an additional 15 min interval, alprostadil was titrated at 1×10^{-6} g·ml^{-1} (high concentration), and immediately after the addition of alprostadil the contraction data were again measured for 5 min. A total of 65 min was taken for all measurements from the onset of sevoflurane administration (experiment 2).

Statistics

In the first experiment, the maximum amplitude, the frequency of contraction, and the AUC during a 5-min period were adopted as the contraction parameters and were compared via a paired student *t* test.

In the second experiment, only the AUC was used as the index of myometrium contraction, following the results of the first experiment, and data were analyzed between the control and the three different alprostadil concentrations by repeated measure one-way analysis of variance.

Results

Representative myometrium contraction curves are showed in Fig. 2.

In the first experiment, the spontaneous myometrium contractions showed no significant changes with regard to the AUC, maximum amplitude, or frequency over a 1-h period (Fig. 3A). On induction of sevoflurane (2%),

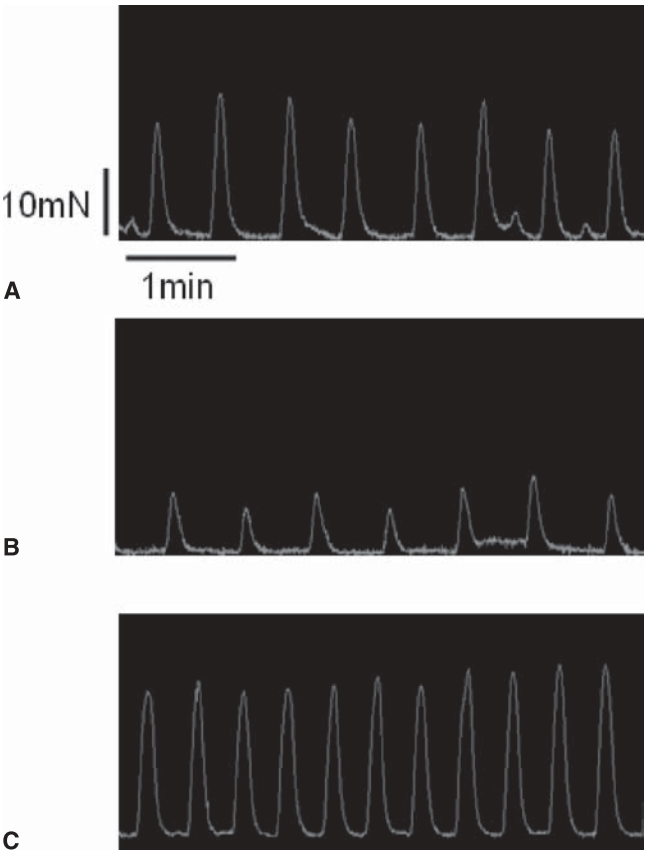

Fig. 2A–C. Representative recordings of myometrium contractions. **A** Spontaneous myometrium contractions, **B** myometrial relaxation induced by introduction of sevoflurane (2%), and **C** inhibition of myometrial relaxation by alprostadil (1×10^{-6} g·ml^{-1})

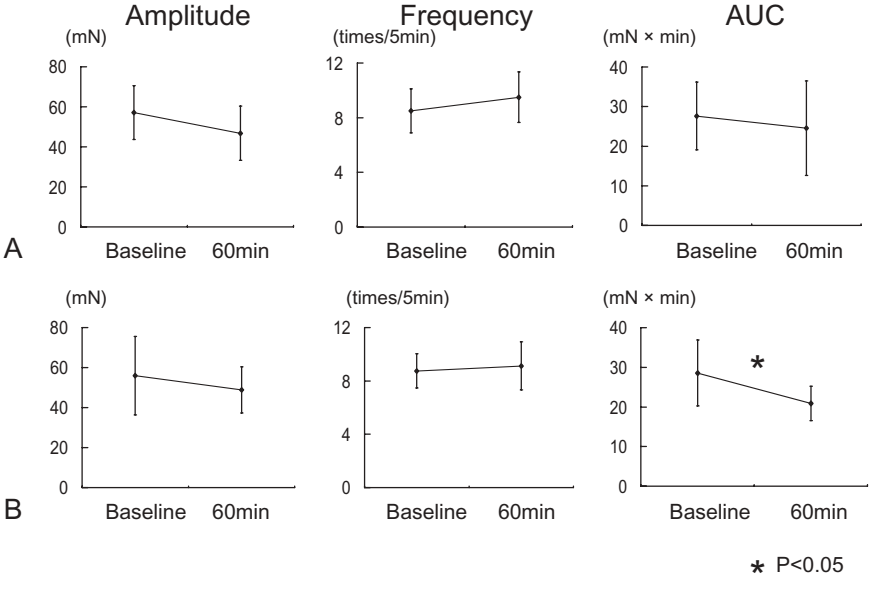

Fig. 3A,B. Effect of sevoflurane on spontaneous myometrium contractions. **A** Without sevoflurane, neither the area under the curve (*AUC*), the maximum amplitude, or the frequency showed significant differences in spontaneous myometrium contraction over time. **B** Sevoflurane (2%) induced a significant decrease in myometrium contractions when the AUC was used as an index, but not when the maximum amplitude or the frequency were used

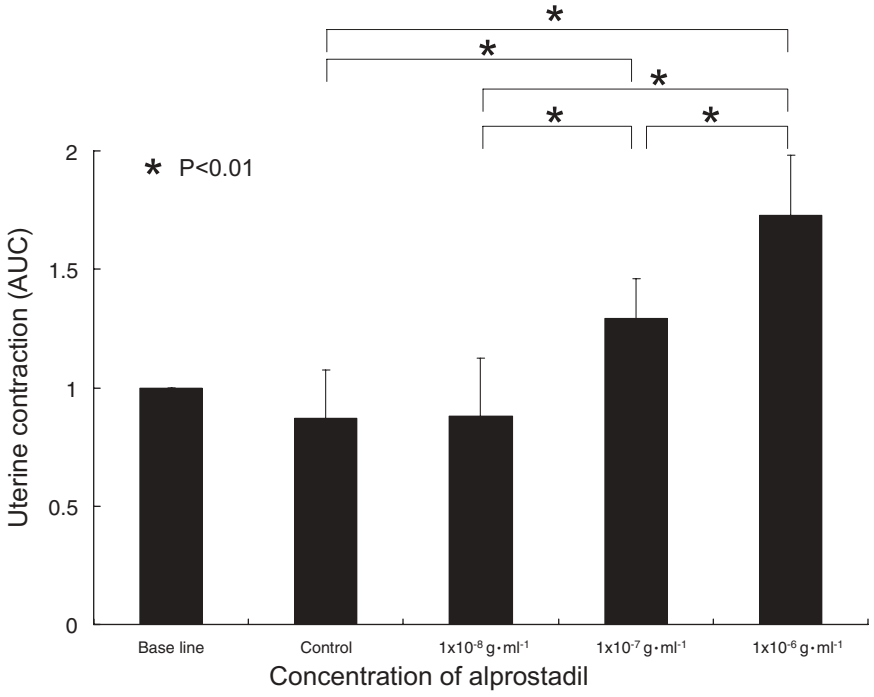

Fig. 4. Sevoflurane (2%) decreased the AUC by 13%, but alprostadil counteracted the myometrial relaxation induced by sevoflurane in a dose-dependent manner. The AUC was 87%, 87%, 129%, and 172% of the baseline value for the control and at low, medium, and high concentrations of alprostadil, respectively

there was a significant decrease of myometrium contraction when the AUC was used as the measurement index, but not when the maximum amplitude or the frequency were used (Fig. 3B).

In the second experiment, sevoflurane (2%) decreased the AUC by 13%. The addition of alprostadil increased myometrium contraction in a dose-dependent manner, reversing sevoflurane-induced myometrial relaxation. The AUC values were 87%, 87%, 129%, and 172% of the baseline value for the control and at low, medium, and high concentrations of alprostadil, respectively (Fig. 4).

Discussion

In the first experiment, we showed that spontaneous myometrium contractions are constant over a period of 60 min. The AUC revealed sevoflurane-induced myometrial relaxation; however, neither the amplitude nor the frequency indicated sevoflurane-induced myometrial relaxation.

The effect of drugs on myometrium contraction has been studied using isolated myometrial strips from humans [9,10] and rats [11,12]. The amplitude and frequency have traditionally been used as an index of myometrium contraction because they can be easily measured from analog data. Recently, new computer software has made it possible to calculate the AUC [13–15]. Previous studies have found that sevoflurane reduces both the amplitude and frequency of myometrium contractions in myometrium isolated from humans [9,10] and rats [11,12]. Sevoflurane-induced myometrial relaxation has also been established using the AUC [13–15]. The failure to detect myometrial relaxation induced by sevoflurane via the amplitude or frequency in our study may be attributable to the small number of strips used. However, the AUC, which may decrease synergistically with decreased amplitude and frequency, revealed myometrial relaxation in the current small sample size. The superiority of the AUC as an index of myometrium contraction is supported by the clinical use of the Montevideo unit by obstetricians; this unit is defined as the average intensity times frequency in a 10-min period [1].

In the second experiment, the AUC was used as an index of myometrium contraction, following the results of the first experiment. Alprostadil successfully counteracted the myometrial relaxation induced by sevoflurane in a dose-dependent manner, restoring myometrium contraction. In this experiment, we measured myometrium contractions for 5 min immediately after the addition of alprostadil. Although it is not clear how long it takes alprostadil to induce myometrium contractions and to disappear after administration, our results clearly showed that alprostadil induced myometrium contraction during this 5-min period.

Alprostadil, a synthesized prostaglandin, serves as an important messenger for a wide variety of functions [16]. The effects of alprostadil on myometrial contractility have been used for the termination of pregnancies and the induction of labor [17,18]. It also possesses a

vasodilatory effect on vascular smooth muscle, and it has been used to treat peripheral vascular disease and erectile dysfunction [19,20]. Furthermore, alprostadil has been administered intravenously to treat critical hypertension and to induce deliberate hypotension during anesthesia in Japan [4–6], where it is available for intravenous administration.

Several clinical reports have indicated a decrease in blood loss as a result of deliberate hypotension when alprostadil is used during operations [5,6]. Considering the effects of alprostadil on myometrium contraction and systemic vascular dilation, it is hypothesized that alprostadil may reduce postpartum bleeding during a cesarean section. While successful use of alprostadil for treatment of cesarean sections for patients with hemolysis, elevated liver enzymes, and low platelet count syndrome (HELLP) has been reported [7,8], clinical use of prostaglandins, including alprostadil, during cesarean section has not been well studied or commonly practiced.

It is well recognized that volatile anesthetics induce myometrial relaxation, and it is recommended that volatile anesthetics be avoided after the delivery of an infant during a cesarean section. However, an increased risk of intraoperative awareness has been reported after discontinuation of volatile anesthetics [3], therefore, a uterotonic agent inhibiting the myometrial relaxation induced by volatile anesthetics would be useful for cesarean sections. To support the practical use of a uterotonic agent in combination with volatile anesthetics, the confirmation that uterotonic agents effectively inhibit myometrial relaxation induced by volatile anesthetics is necessary.

Decreased intracellular calcium concentration has been proposed as a mechanism of myometrial relaxation [21–23]. Recently, it has been suggested that sevoflurane decreases intracellular Ca^{++} by inhibiting activation of voltage-dependent Ca^{++} channels (VDCCs) [13]. It has also been reported that oxytocin and prostaglandins induce myometrium contraction by increasing the intracellular calcium concentration [24,25]. Two different mechanisms have been proposed, an extracellular mechanism and an intracellular mechanism, both leading to increased Ca^{++}. Increased Ca^{++} influx through VDCCs has been proposed for the extracellular mechanism, while increased Ca^{++} release from intracellular stores has been suggested for the intracellular mechanism [26–28]. The dominant mechanism by which prostaglandins induce myometrium contraction is still under considerable debate. Because volatile anesthetics inhibit VDCCs, it is speculated that prostaglandins may be unable to inhibit myometrial relaxation induced by volatile anesthetics via the extracellular mechanism. Therefore the present results seem to support the idea that alprostadil inhibits myometrial relaxation by increasing intracellular Ca^{++} via the intracellular mechanism.

The present results suggest possible advantages for the use of alprostadil during cesarean sections. Furthermore, there may be specific clinical conditions for which alprostadil is more advantageous. For patients with pregnancy-induced hypertension, alprostadil can be used to induce myometrium contraction while simultaneously reducing blood pressure. Further studies would be needed to determine to what degree alprostadil reduces blood pressure at the concentration required for inducing myometrium contraction. However, it is certain that alprostadil does not induce the hypertension commonly seen with methylergometrine. For preterm patients, alprostadil may be a superior alternative to oxytocin because sensitivity to oxytocin is known to be immatured until the late stage of pregnancy.

In conclusion, we have shown that alprostadil is effective against myometrial relaxation induced by sevoflurane. Therefore, alprostadil may be an advantageous uterotonic agent during cesarean sections in which volatile anesthetics are used.

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