Sevoflurane inhibits contraction of uterine smooth muscle from pregnant rats similarly to halothane and isoflurane

[Le sévoflurane, pareillement à l'halothane et à l'isoflurane, inhibe la contraction du muscle lisse utérin des rates gravides]

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Purpose: The present study was designed to clarify the direct effects of the volatile anesthetics halothane, isoflurane and sevoflurane on oxytocin-induced uterine smooth muscle contraction from pregnant rats.

Methods: Longitudinal smooth muscle layers were obtained from pregnant rats. Intracellular concentration of free Ca^{++} ($[\text{Ca}^{++}]_i$) was measured, using a fluorescence technique, simultaneously with muscle tension. Inward Ba^{++} current (I_{Ba}) through voltage-dependent Ca^{++} channels (VDCCs) was measured using a whole cell patch clamp technique. After incubation with 20 nM oxytocin, halothane, isoflurane or sevoflurane (1, 2, and 3%) was introduced into the tissue bath.

Results: All volatile anesthetics significantly inhibited muscle contraction concomitant with a decrease in $[\text{Ca}^{++}]_i$. Volatile anesthetics also inhibited the peak I_{Ba} . When the anesthetic concentrations were expressed as multiples of minimum alveolar concentrations, there were no differences in the inhibitory potencies of the three volatile agents tested for muscle tension and VDCC.

Conclusions: Volatile anesthetics halothane, isoflurane and sevoflurane reduce the oxytocin-induced contraction of pregnant uterine smooth muscle. Inhibition of the contraction by the volatile anesthetics is due, at least in part, to the decrease in $[\text{Ca}^{++}]_i$, and the decrease in $[\text{Ca}^{++}]_i$ may be mediated by inhibition of VDCC activity.

Objectif : La présente étude voulait préciser les effets directs d'anesthésiques volatils, halothane, isoflurane et sévoflurane, sur la contraction du muscle lisse utérin induite par l'oxytocine chez des rates gravides.

Méthode : Des couches longitudinales de muscle lisse ont été prélevées chez des rates gravides. La concentration intracellulaire de Ca^{++} ($[\text{Ca}^{++}]_i$) libre a été mesurée par une technique fluorescente, simultanément à la tension musculaire. Le courant entrant de Ba^{++} (I_{Ba}) au travers des canaux calciques potentiel-dépendants Ca^{++} (CCPD) a été mesuré à l'aide d'une technique de "patch clamp" d'une cellule complète. À la suite de l'incubation avec 20 nM d'oxytocine, l'halothane, l'isoflurane ou le sévoflurane (1, 2 et 3 %) a été introduit dans le bain tissulaire.

Résultats : Les trois anesthésiques volatils ont inhibé la contraction musculaire de façon significative et concomitante à la baisse de $[\text{Ca}^{++}]_i$. Ils ont aussi inhibé le I_{Ba} maximal. Les concentrations d'anesthésiques exprimées en multiples des concentrations alvéolaires minimales ne présentaient pas de différence de potentiel inhibiteur pour les trois médicaments testés pour la tension musculaire et les CCPD.

Conclusion : Les anesthésiques volatils halothane, isoflurane et sévoflurane réduisent la contraction induite par l'oxytocine du muscle lisse de l'utérus gravide. Cette inhibition est causée, en partie du moins, par la baisse de $[\text{Ca}^{++}]_i$, cette baisse de diminution de $[\text{Ca}^{++}]_i$ pouvant être le résultat de l'inhibition de l'activité des CCPD.

VOLATILE anesthetics are often used for general anesthesia in obstetric surgery.^{1,2} While these anesthetics have been shown to induce uterine atonicity followed by hemorrhage during abortion or delivery,^{3,4} the direct mechanisms by which these agents produce muscle

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relaxation are not fully known.^{5,6} As in other smooth muscles, intracellular free Ca^{++} is a primary regulator of uterine smooth muscle contraction,⁷ and Ca^{++} influx through membrane-associated voltage-dependent Ca^{++} channels (VDCCs) is important for the regulation of the concentration ($[\text{Ca}^{++}]_i$).⁸ The present study was, therefore, designed to clarify the direct effects of the volatile anesthetics halothane, isoflurane and sevoflurane on oxytocin-induced uterine smooth muscle contraction in the pregnant rat by simultaneously measuring the muscle tension and $[\text{Ca}^{++}]_i$ using a fluorescence technique^{9,10} and by measuring VDCC activity using a patch clamp technique.^{11,12}

Methods

The experimental protocol was approved by the Sapporo Medical University Animal Care and Use Committee. Twenty-four rats (Sprague-Dawley, weighing 200–250 g) in the late stage of gestation (19–21 days) were anesthetized, the uteri were excised quickly, and the longitudinal muscle layer of each uterus was isolated.

The muscle tissue was cut into small strips (2-mm wide and 15-mm long), and the strips were loaded with 5 μM acetoxymethyl ester of fura-2, an indicator of Ca^{++} . Each fura-2-loaded muscle strip was held in an organ bath (37°C), and one end of the muscle strip was connected to a strain gauge transducer. Experiments were performed using a fluorescence spectrometer (CAF-100; Japan Spectroscopic, Tokyo, Japan). Excitation light was passed through 340- and 380-nm rotating filters. The light emitted from the muscle strip at 500 nm was measured, and the ratio of the fluorescence ($R_{340/380}$) was used as an indicator of $[\text{Ca}^{++}]_i$.^{9,10} The tissue was contracted with 20 nM oxytocin, a potent contractile agonist. Halothane, isoflurane, or sevoflurane (1, 2, and 3%, respectively) were randomly introduced into the tissue bath through bubbling in the presence of oxytocin. Volatile anesthetic concentrations in the gas phase during the bubbling were continuously monitored with a calibrated mass spectrometer (ATOM 303; ATOM, Tokyo, Japan). In order to express the quantitative changes in muscle contractility and in $[\text{Ca}^{++}]_i$, the areas under the contraction and $[\text{Ca}^{++}]_i$ curves were measured for five minutes using Sigma Plot (Jandel Scientific, Corte Madera, CA, USA).¹³

Conventional whole cell patch clamp techniques^{11,12} were used to observe the inward current through VDCCs. Uterine smooth muscle tissues were minced and digested in Ca^{++} -free modified Tyrode's solution to which 0.05% (w/v) collagenase (Lot#: 034–10533) had been added. Cells were then dispersed by tritura-

tion, filtered by nylon mesh, and centrifuged. The pellet was resuspended in a modified Kraftbrühe solution¹⁴ and stored at 4°C for up to five hours before use. Micropipettes had resistances of 3–5 M Ω , and the pipette solution contained (in mM) CsCl 20, CsOH 110, MgCl_2 5.2, L-glutamate 112, EGTA 10, Na_2ATP 5.0, and HEPES 10, with the pH adjusted to 7.2 with Tris. The bath solution contained (in mM) tetraethylammonium chloride 130, MgCl_2 1.0, BaCl_2 5.0, glucose 10, and HEPES 10, with the pH adjusted to 7.35 with Tris. Ba^{++} was used, instead of Ca^{++} , in order to prevent an indirect inhibitory effect of oxytocin on VDCC activity. An aliquot of the cell suspension was placed in a temperature-controlled chamber (37°C), and the patch membrane was disrupted by strong negative pressure. Membrane currents were monitored using a patch clamp amplifier (CEZ–2400; Nihon Kohden, Tokyo, Japan), and the amplifier output was low-pass-filtered at 2,000 Hz. Inward Ba^{++} currents through VDCCs (I_{Ba} s) were elicited by 100-msec depolarizing pulses from a holding potential of -60 mV. Leak currents were subtracted, and membrane capacitance and series resistance were compensated. After obtaining a stable baseline of peak I_{Ba} with 20 nM oxytocin, cells were then exposed to bath solution equilibrated with one of three volatile anesthetics: halothane, isoflurane or sevoflurane (1, 2, or 3%, respectively).

The following drugs and chemicals were used: acetoxymethyl ester of fura-2 (Dojindo, Kumamoto, Japan), oxytocin (Sigma Chemical, St. Louis, MO, USA), collagenase (Wako Pure Chemical, Osaka, Japan), halothane (ICI, Dighton, MA, USA), isoflurane (Ohio Medical Products, Madison, WI, USA), and sevoflurane (Maruishi Pharmaceutical, Osaka, Japan).

Data are expressed as means $\pm$ SD. For the measurement of $[\text{Ca}^{++}]_i$ and muscle tension, oxytocin-induced changes in $[\text{Ca}^{++}]_i$ ($R_{340/380}$) and muscle tension were used as references.^{9,10} Changes in peak I_{Ba} with exposure to anesthetics were compared at each applied potential by the paired, two-tailed t test. Other data were analyzed using one-way ANOVA for repeated measurements with Fisher's test. In all comparisons, $P < 0.05$ was considered significant.

Results

In resting conditions, oscillatory phasic contractions of the uterine smooth muscle strips with a concomitant oscillatory increase in $[\text{Ca}^{++}]_i$ were observed (Figure 1A). Treatment with 20 nM oxytocin significantly increased oscillatory rates (3.3 ± 1.0 to 4.3 ± 1.2 cycles $\cdot\text{min}^{-1}$) by approximately 30% and elevated the peaks of the contraction by approximately 35% and of the $[\text{Ca}^{++}]_i$ by approximately 21%. Nifedipine

FIGURE 1 Effects of volatile anesthetics on pregnant uterine smooth muscle tension and $[Ca^{2+}]_i$ (indicated by $R_{340/380}$) during contraction caused by 20 nM oxytocin. (A) Halothane (3%) was introduced in the presence of oxytocin. (B) Relationships of percent responses of contraction and $[Ca^{2+}]_i$ with the concentrations of the volatile anesthetics tested in this study: halothane ($\bullet$), isoflurane ($\square$), and sevoflurane ($\circ$). Symbols represent means $\pm$ SD ($n=6$ at each point). * $P < 0.05$, ** $P < 0.01$ vs control without the anesthetic.

(1 μ M), a potent L-type VDCC antagonist, completely blocked the oscillatory changes both in the muscle contraction and in $[Ca^{2+}]_i$ (data not shown, $n=3$). As shown in Figure 1A, 3% halothane inhibited both the muscle contraction and increase in $[Ca^{2+}]_i$ induced by 20 nM oxytocin. Halothane significantly decreased oscillatory rates by 15% and suppressed the oxytocin-induced peaks of muscle contraction by approximately 67% and of $[Ca^{2+}]_i$ by 57%. The other two volatile anesthetics, isoflurane and sevoflurane, showed similar inhibitory effects both on the muscle contraction and on $[Ca^{2+}]_i$ (data not shown). Figure 1B shows the relationships of percent responses of contraction (a) and $[Ca^{2+}]_i$ (b) with anesthetic concentrations (%). Each volatile anesthetic significantly decreased muscle contraction and $[Ca^{2+}]_i$ in a dose-dependent manner ($n=6$ each). Based on the percent anesthetic concentrations in the gas phase, the order of inhibitory

FIGURE 2 Effects of volatile anesthetics on depolarization-induced whole-cell Ba^{2+} currents (I_{Ba}). (A) Typical recordings of I_{Ba} induced by pulses as long as +10 mV with or without 3% sevoflurane. (B) Relationships between peak I_{Ba} and applied potential before ($\bullet$) and after exposure to sevoflurane ($\circ$). (C) Relationships between peak I_{Ba} at +10 mV, expressed as percentage of control, and the concentrations of volatile anesthetics in the gas phase. Symbols represent means $\pm$ SD ($n=6$ at each point). * $P < 0.05$, ** $P < 0.01$ vs control without the anesthetic.

potencies for both the muscle tension and $[Ca^{++}]_i$ was halothane > isoflurane > sevoflurane.

The I_{Ba} seen in dispersed rat uterine smooth muscle cells peaked at approximately 10–15 msec and was inactivated slowly with a time constant of approximately 300–500 msec (Figure 2A: control). In baseline conditions, threshold activation of I_{Ba} occurred at approximately -40 mV, and maximum peak current amplitude was obtained at approximately +10 mV (Figure 2B). In 12 cells, the maximum peak I_{Ba} was -389 ± 89 pA. Nifedipine (1 μ M) completely inhibited the currents (data not shown, $n=3$). As shown in a representative trace for depolarization from -60 to +10 mV (Figure 2A: sevoflurane), 3% sevoflurane inhibited the magnitude of I_{Ba} but did not obviously alter the time course of the current. Sevoflurane significantly inhibited I_{Ba} at step potentials ranging from -20 mV to +40 mV and decreased peak I_{Ba} by approximately 33% (Figure 2B). There was no apparent shift in the voltage dependence of I_{Ba} with sevoflurane. The other two volatile anesthetics showed similar inhibitory effects on I_{Ba} (data not shown). Figure 2C shows the relationships between the percentage of control peak I_{Ba} at +10 mV and the % anesthetic concentrations of volatile anesthetics in the gas phase. All three volatile anesthetics significantly inhibited the peak I_{Ba} in a dose-dependent manner, and the order of inhibitory potencies for I_{Ba} was halothane > isoflurane > sevoflurane.

Discussion

The present study showed that the volatile anesthetics tested significantly inhibited agonist-induced pregnant uterine smooth muscle contraction, at least in part, by decreasing $[Ca^{++}]_i$. This result for muscle tension is consistent with those obtained by Naftalin *et al.*¹⁵ and by Munson *et al.*¹⁶ using rat and human uterine smooth muscles, respectively. This result is also in agreement with the general observations in airway^{10,17} and in vascular¹⁸ smooth muscles. Inositol 1,4,5-triphosphate (IP_3)-induced Ca^{++} release from intracellular Ca^{++} stores, especially the sarcoplasmic reticulum, and Ca^{++} influx through VDCCs are both important for the oxytocin-induced uterine smooth muscle contraction during delivery.^{8,13} Phillippe *et al.*^{8,13,19} demonstrated that activation of the IP_3 -signaling pathway results in the development of intracellular Ca^{++} oscillation phenomena. Ca^{++} influx through VDCCs is also essential for the maintenance of repetitive contractions,^{7,13} because the blockade of Ca^{++} influx through VDCCs by nifedipine completely blocked the oscillatory contractions in this study. Therefore, the mechanisms by which volatile anesthetics decreased $[Ca^{++}]_i$ are thought to be, at least in part,

due to an inhibition of VDCC activity.

Using a whole-cell patch clamp technique, we measured depolarization-induced I_{Ba} in rat uterine smooth muscle cells. Based on their time and voltage dependencies and their sensitivity to blockade by nifedipine, these currents are presumed to reflect the activity of L-type VDCCs.^{20,21} Volatile anesthetics significantly inhibited I_{Ba} through L-type VDCCs of the uterine smooth muscle cells without an apparent change in the kinetics of activation and inactivation (Figure 2). Volatile anesthetics did not alter the voltage dependence of I_{Ba} , suggesting that these anesthetics have no effect on membrane surface charge or on the voltage sensor of the channel. These data indicate one of the cellular effects of volatile anesthetics that can account for the uterine smooth muscle relaxant effects of the anesthetics.^{15,16}

We did not investigate other second messengers such as IP_3 and cyclic AMP. IP_3 can release Ca^{++} from the sarcoplasmic reticulum and also activates VDCCs directly.¹³ Since it was reported that halothane inhibits the agonist-induced increase in IP_3 concentration in canine tracheal smooth muscle,²² it is possible that the anesthetic also decreases IP_3 concentration in uterine smooth muscle. Furthermore, Yang *et al.*⁶ reported that halothane increases intracellular concentration of cyclic AMP in rat uterine smooth muscle. An increase in cyclic AMP concentration can decrease $[Ca^{++}]_i$, leading to uterine smooth muscle relaxation. Further studies are, therefore, needed to clarify the relative importance of the inhibitory effect of the volatile anesthetics on VDCC activity.

We did not measure the exact concentrations of the volatile anesthetics in the bathing chamber and showed our data using percent anesthetic concentrations in the gas phase, because the systems (both fluorescence and patch-clamp techniques) used in this study were the same as those we had used in our previous studies.^{10,17,23} Minimum alveolar concentrations (MACs) of the volatile anesthetics halothane, isoflurane and sevoflurane in adult rats are 1.03%,²⁴ 1.46%²⁴ and 2.68%,²⁵ respectively. Although the effect of pregnancy on the MAC of sevoflurane has not been reported in rats, it has been shown that the MACs of halothane and isoflurane are not affected by either sex or pregnancy.²⁴ When the anesthetic concentrations were expressed as MACs, differences in inhibitory potencies of the volatile anesthetics tested for muscle tension, $[Ca^{++}]_i$ or VDCC activity became indistinguishable. Therefore, the three volatile anesthetics tested in this *in vitro* study appear to be equivalent for general anesthesia in obstetric surgery, as far as uterine contraction is concerned and in as much as our *in vitro* results apply to the clinical situation.

In conclusion, the volatile anesthetics halothane, isoflurane and sevoflurane at clinically relevant concentrations (1–3%) significantly reduce the oxytocin-induced contraction of isolated preparations of uterine smooth muscle obtained from pregnant rats. Inhibition of the contraction by the volatile anesthetics is due, at least in part, to the decrease in $[Ca^{2+}]_i$, and the decrease in $[Ca^{2+}]_i$ may be mediated by inhibition of VDCC activity.

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