

# Mechanisms of Direct Inhibitory Action of Propofol on Uterine Smooth Muscle Contraction in Pregnant Rats

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**Background:** Although propofol directly inhibits uterine smooth muscle contraction, the mechanisms of this effect are still unknown. The current study aimed to clarify the mechanisms of the inhibitory effect of propofol on oxytocin-induced uterine smooth muscle contraction by measuring (1) the concentration of intracellular free  $\text{Ca}^{2+}$  ( $[\text{Ca}^{2+}]_i$ ) simultaneously with muscle tension, (2) the amount of intracellular inositol 1,4,5-triphosphate ( $[\text{IP}_3]_i$ ), and (3) voltage-dependent  $\text{Ca}^{2+}$  channel (VDCC) activity.

**Methods:** Uterine smooth muscle tissues were obtained from pregnant rats (in late gestation).  $[\text{Ca}^{2+}]_i$  with isometric tension was monitored by the 500-nm light emission ratio of preloaded  $\text{Ca}^{2+}$  indicator fura-2.  $[\text{IP}_3]_i$  and VDCC activity were measured by radioimmunoassay and patch clamp techniques, respectively. The uterine smooth muscle was stimulated by 20 nM oxytocin and exposed to propofol ( $10^{-7}$  ~  $10^{-4}$  M).

**Results:** Propofol had significant inhibitory effects on oxytocin-induced uterine smooth muscle contraction and increased  $[\text{Ca}^{2+}]_i$  in pregnant rats in a dose-dependent manner, without affecting the agonist-receptor binding affinity. Propofol inhibited the increase in  $[\text{IP}_3]_i$  induced by oxytocin. Propofol also inhibited VDCC activity in both activated and inactivated states. The solvent Intralipid® had no effects on these parameters.

**Conclusions:** Propofol inhibits oxytocin-induced uterine smooth muscle contraction, at least in part, by decreasing  $[\text{Ca}^{2+}]_i$  without affecting agonist-receptor binding; the inhibitory effect of propofol on  $[\text{Ca}^{2+}]_i$  might be mediated both by a decrease in  $[\text{IP}_3]_i$  and by inhibition of VDCC activity.

PROPOFOL (2,6-diisopropylphenol) is an alternative to barbiturates for induction of general anesthesia in cesarean section delivery<sup>1</sup> and is also used as a sedative agent for supplementation of regional blockade during cesarean section<sup>2-4</sup> because of its smooth induction, satisfactory maintenance, and rapid recovery with reliable amnestic properties.<sup>2,3</sup> Hypotension is an adverse cardiovascular effect of propofol, mainly caused by a direct vasodilatory effect,<sup>5,6</sup> and direct vasodilation or relaxation of vasoconstriction by propofol has been demonstrated in isolated arteries and veins.<sup>7-9</sup> Propofol has also been reported to prevent bronchoconstriction during anesthesia,<sup>10,11</sup> and a smooth muscle relaxant effect of propofol has been observed in the isolated guinea-pig trachea.<sup>12</sup> Although no record has clinically been found

of definitive studies on the propofol-induced uterine atony followed by hemorrhage during delivery, there is *in vitro* evidence that propofol has a relaxant effect on isolated uterine smooth muscle from pregnant women.<sup>13</sup> However, the mechanism of the direct inhibitory effect of this anesthetic on uterine smooth muscle has not yet been determined.

As in other tissues, intracellular free  $\text{Ca}^{2+}$  is a primary regulator of uterine smooth muscle contraction (fig. 1).<sup>14</sup> An increase in the intracellular concentration of free  $\text{Ca}^{2+}$  ( $[\text{Ca}^{2+}]_i$ ) results in calmodulin activation of myosin light-chain kinase. This kinase phosphorylates the regulatory chains of myosin, resulting in activation of myosin Mg-adenosine triphosphatase and contractile shortening of the uterine myocytes.<sup>14,15</sup> In contrast to other kinds of smooth muscle, uterine smooth muscle becomes hypertrophic, and the number of oxytocin receptors and their binding affinity for oxytocin change during pregnancy.<sup>16,17</sup> Furthermore, uterine smooth muscle can be characterized by efferent nerve-independent phasic contraction.<sup>18</sup> Both  $\text{Ca}^{2+}$  release from intracellular stores by inositol 1,4,5-triphosphate ( $\text{IP}_3$ ) and  $\text{Ca}^{2+}$  influx through membrane-associated voltage-dependent  $\text{Ca}^{2+}$  channels (VDCCs) are important for phasic contraction.<sup>18</sup> We therefore speculated that propofol has some inhibitory effects on the phosphatidylinositol pathway or VDCC activity in pregnant uterine smooth muscles.

The current study was designed to clarify the mechanisms of direct inhibitory effects of propofol on oxytocin-induced pregnant uterine smooth muscle contraction in rats by (1) simultaneously measuring the muscle tension and  $[\text{Ca}^{2+}]_i$  using a fluorescence technique,<sup>19,20</sup> (2) measuring intracellular concentration of  $\text{IP}_3$  ( $[\text{IP}_3]_i$ ) using a radioimmunoassay technique,<sup>21,22</sup> and (3) measuring VDCC activity using patch clamp techniques.<sup>23,24</sup> We also demonstrated in a preliminary study the effect of a high concentration of propofol on the oxytocin-oxytocin receptor affinity using radiolabeled receptor assay techniques.<sup>25,26</sup>

## Materials and Methods

### Tissue Preparation

The experimental protocol used in this study was approved by the Sapporo Medical University Animal Care and Use Committee (Sapporo, Japan). One hundred thirty rats (Sprague Dawley, weighing 200–250 g) in the late stage of gestation (19–21 days after fertilization) were anesthetized with sevoflurane (3–4%). After a sur-

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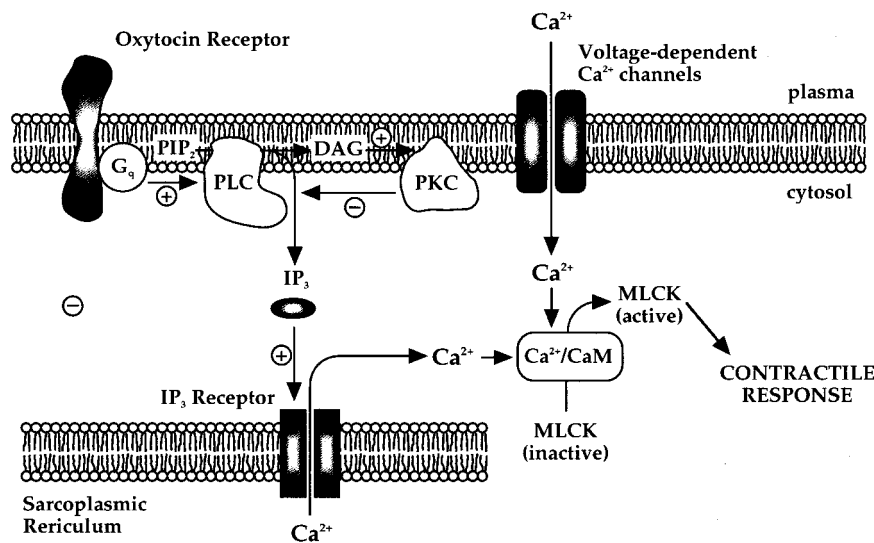


Fig. 1. Intracellular signal transduction in a uterine smooth muscle cell in response to oxytocin. Binding of the ligand to the cell membrane oxytocin receptors leads to activation of phospholipase C via G proteins ( $G_q$ ), resulting in the rapid hydrolysis of phosphatidylinositol 4,5-bisphosphate ( $PIP_2$ ) to inositol 1,4,5-trisphosphate ( $IP_3$ ) and 1,2-diacylglycerol (DAG). DAG, along with phosphatidylserine, activates protein kinase C (PKC).  $IP_3$  binds to the  $IP_3$  receptors, leading to the release of stored  $Ca^{2+}$  from the sarcoplasmic reticulum. Stimulation of the oxytocin receptors also activates voltage-dependent  $Ca^{2+}$  channel (VDCC) activity via cell membrane depolarization, leading to the influx of extracellular  $Ca^{2+}$ . The resulting  $Ca^{2+}$  transients activate calmodulin (CaM), leading to the stimulation of myosin light chain kinase (MLCK). Active MLCK phosphorylates the regulatory light chains on myosin, resulting in the smooth-muscle contractile response.

gical level of anesthesia had been attained, the uteri were excised quickly and placed in a modified Krebs solution equilibrated with 95%  $O_2$ -5%  $CO_2$  at room temperature (22–24°C) (composition: 118 mM NaCl, 4.7 mM KCl, 21 mM  $NaHCO_3$ , 1.2 mM  $MgSO_4$ , 1.2 mM  $KH_2PO_4$ , 10 mM glucose, and 2.5 mM  $CaCl_2$ ; pH, approximately 7.4). The longitudinal smooth muscle layer of the uterus was carefully isolated by removing the endometrium and circular smooth muscle layer under a dissecting microscope.

#### Radioligand-binding Receptor Study

In a preliminary study, the effect of a single dose of a high concentration of propofol ( $10^{-4}$  M) on the oxytocin-receptor binding affinity was confirmed according to previously described methods.<sup>25,26</sup> Briefly, smooth muscle tissue from a rat was minced in ice-cold 0.25 M sucrose buffer (10 times volume) with 10 mM Tris-HCl (pH 7.4) and homogenized twice with a homogenizer. The homogenate was centrifuged at 1,500g for 10 min at 4°C, and the supernatant was filtered through 120- $\mu$ m nylon mesh and then centrifuged at 100,000g for 30 min at 4°C. The resting pellet was resuspended in 3 ml of 5 mM HEPES buffer with 1.0 mM  $MgSO_4$  (pH 7.4), yielding a protein concentration of 500–100  $\mu$ g/ml as measured by the method described by Lowry *et al.*<sup>27</sup>

Oxytocin receptor binding was determined using [ $^3H$ ]oxytocin (30–60 Ci/mmol). Nonspecific binding was determined in the presence of oxytocin ( $10^{-4}$  M) and was subtracted from total binding to give specific binding. Labeled and unlabeled drugs were added as 20- $\mu$ l aliquots to give a final assay volume of 540  $\mu$ l. After incubation at room temperature with  $10^{-4}$  M propofol, the plate was placed on ice, and 50- $\mu$ l aliquots of buffer were removed for determination of free (equilibrium) concentration of [ $^3H$ ]oxytocin. The remaining buffer was pipetted out, and the wells were washed twice for 5 min. The wells were finally drained, and punches were

removed by touching with a small square of glass-fiber filter paper. Both were placed in a vial containing 1.8 ml scintillation fluid and counted. The density of receptors ( $B_{max}$ ) and the dissociation constant for the ligand ( $K_d$ ) were determined using linear regression and Scatchard transformation ( $n = 5$  each).

#### Simultaneous Measurement of Muscle Tension and Intracellular Concentration of $Ca^{2+}$

The tissue was cut into small strips (2 mm wide and 15 mm long). The muscle strips were loaded with 5  $\mu$ M acetoxymethyl ester of fura-2, an indicator of  $Ca^{2+}$ , in a physiologic salt solution (PSS) containing 0.02% (vol/vol) cremophor EL for 3–4 h at room temperature. The PSS contained: 136.9 mM NaCl, 5.4 mM KCl, 1.5 mM  $CaCl_2$ , 1.0 mM  $MgCl_2$ , 23.9 mM  $NaHCO_3$ , 5.5 mM glucose, and 0.01 mM EDTA. This solution was saturated with a gas mixture of 95%  $O_2$ -5%  $CO_2$  (pH, approximately 7.4). Each fura-2-loaded muscle strip was held in a temperature-controlled (37°C) organ bath, and one end of the muscle strip was connected to a strain gauge transducer (LVS-20GA; Kyowa, Tokyo, Japan). Experiments were performed using a fluorescence spectrometer (CAF-100; Japan Spectroscopic, Tokyo, Japan). Excitation light was passed through a rotating filter wheel (48 Hz) that contained 340- and 380-nm filters. The light emitted from the muscle strip at 500 nm was measured using a photomultiplier. The ratio of the fluorescence resulting from excitation at 340 nm to that at 380 nm ( $R_{340/380}$ ) was calculated and used as an indicator of  $[Ca^{2+}]_i$ .<sup>20,28</sup>

Physiologic salt solution aerated with 95%  $O_2$ -5%  $CO_2$  was used for the control bath solution, and the uterine smooth muscle strips were allowed to equilibrate for 30 min after being mounted in the bath. To establish an optimal length, the resting tension was adjusted to 0.5 g. As previously reported,<sup>29</sup> this value was selected as the optimal value for maximal active force generation determined in preliminary experiments using repeated oxyto-

cin contractions and various baseline tensions. The tissue was contracted with half-maximal effect ( $ED_{50}$ ) concentration of oxytocin (20 nM), a potent contractile agonist, released reflexively from the pituitary gland. After the force, frequency, and duration of contractions had reached a steady state, a single concentration of propofol ( $10^{-7}$ – $10^{-4}$  M) was introduced into the tissue bath in the presence of oxytocin. To compare the quantitative effect of propofol with those of other intravenous anesthetics, ketamine ( $10^{-7}$ – $10^{-4}$  M) or pentobarbital ( $10^{-7}$ – $10^{-4}$  M) was also introduced into the tissue bath. Similar to this experiment, the tissue strips were exposed to 1  $\mu$ M nifedipine, a dihydropyridine-sensitive VDCC antagonist, or to  $Ca^{2+}$ -free PSS with 5  $\mu$ M EGTA during oxytocin-induced contraction.

To express the quantitative changes in muscle contractility and in  $[Ca^{2+}]_i$ , we measured the areas under the contraction and  $[Ca^{2+}]_i$  curves.<sup>29,30</sup> Briefly, all data were digitized (2,000 samples/s) with an ITC-16 computer interface (Instrutech, Greatneck, NY) and stored on a hard disk. Subsequently, the digitalized data were analyzed using a 8100/100AV Power Macintosh computer (Apple, Cupertino, CA) to determine the areas under the contraction and  $[Ca^{2+}]_i$  curves for 5-min intervals (beginning 3 min after addition of each reagent to the organ bath) using Sigma Plot (Jandel Scientific, Corte Madera, CA).

#### *Measurement of Intracellular Inositol 1,4,5-Triphosphate Concentration*

The longitudinal smooth muscle tissues were cut into large pieces (15 mm wide and 20 mm long) and used for measurement of  $[IP_3]_i$ . Two or three pieces of the muscle tissues were incubated at 37°C in PSS containing 20 nM oxytocin for 20 min, in PSS containing 20 nM oxytocin for 10 min and subsequently in PSS containing 20 nM oxytocin and propofol ( $10^{-7}$ – $10^{-4}$  M) for 10 min, or in PSS alone for 20 min. The muscle tissues were then frozen quickly in a liquid nitrogen and stored at –70°C until used for the assay.

The technique of Bredt *et al.*<sup>21</sup> was used to measure  $[IP_3]_i$ . The frozen tissue sample was homogenized with 10% (wt/vol) ice-cold  $HClO_4$ . The homogenates were centrifuged at 2,000g for 15 min at 4°C to remove insoluble materials. The pH of the supernatant was adjusted precisely to 7.5 with 1.5 M KOH containing 60 mM HEPES buffer. Insoluble precipitates (primarily  $KClO_4$ ) were removed by centrifugation at 2,000g for 15 min at 4°C. A 200- $\mu$ l aliquot of the resultant supernatant was used to measure concentrations of protein.<sup>27</sup> The extraction of  $IP_3$  from the remaining resultant supernatant was conducted by solid-phase extraction procedures using Amprep SAX minicolumns (Amersham Pharmacia Biotech, Buckinghamshire, United Kingdom).  $[IP_3]_i$  was measured using a Biotrak D-myo- $IP_3$  [ $^3H$ ] assay system (code TRK 1,000; Amersham Pharmacia Biotech). This assay is based on competition between unlabeled  $IP_3$  in the

samples and a fixed quantity of [ $^3H$ ] $IP_3$  for a limited number of high-affinity binding sites on a specific  $IP_3$  binding protein.<sup>22</sup> The determinations were made in duplicate, and the results are expressed as picomoles per milligram of protein.

#### *Measurement of Voltage-dependent $Ca^{2+}$ Channel Activity*

$Ba^{2+}$  was used, instead of  $Ca^{2+}$ , to prevent an indirect inhibitory effect of oxytocin on VDCC activity,<sup>31</sup> and conventional whole cell patch clamp techniques<sup>23,24</sup> were used to observe inward  $Ba^{2+}$  currents ( $I_{BaS}$ ) through VDCCs. Uterine smooth muscle tissues were minced and digested for 20 min at 37°C in  $Ca^{2+}$ -free modified Tyrode solution, to which 0.05% (wt/vol) collagenase (Lot#: 034-10533) was added. Cells were then dispersed by repeated aspiration into a plastic pipette, and debris was removed by filtration through 120- $\mu$ m nylon mesh. The cell suspension was centrifuged at 200g for 3 min, and the pellet was resuspended in modified Kraftbrühe solution<sup>32</sup> and stored at 4°C for up to 5 h before use. The modified Kraftbrühe solution contained: 85 mM KCl, 30 mM  $K_2HPO_4$ , 5.0 mM  $MgSO_4$ , 5.0 mM  $Na_2ATP$ , 5.0 mM pyruvic acid, 5.0 mM creatine, 20 mM taurine, 5.0 mM  $\beta$ -hydroxybutyrate, and 0.1% (wt/vol) fatty acid-free bovine serum albumin, with the pH adjusted to 7.25 with 0.5 M Tris.

The experiments were performed at room temperature (22–24°C). Micropipettes were pulled from soda lime tubing (GC-1.5; Narishige, Tokyo, Japan) using a brown-flaming horizontal puller (model P-97; Sutter Instrument, Novato, CA). These had resistances of 3–5 M $\Omega$  when filled with solution. An aliquot (approximately 0.5 ml) of the cell suspension was placed in a perfusion chamber on the stage of an inverted microscope (IX-70; Olympus, Tokyo, Japan). At  $\times 600$  magnification, a three-dimensional oil-driven micromanipulator (ONM-1; Narishige) was used to position the patch pipettes against the membrane of the uterine smooth muscle cells. After obtaining a high-resistance seal (5–20 G $\Omega$ ) with slight suction (10–20 cm  $H_2O$ ), the patch membrane was disrupted by strong negative pressure, which allowed the voltage of the entire cell membrane to be controlled<sup>23</sup> and permitted the pipette solution to diffuse into the cytoplasm. Membrane currents were monitored using a CEZ-2400 patch clamp amplifier (Nihon Kohden, Tokyo, Japan), and the amplifier output was low-pass filtered at 2,000 Hz. Leak currents, estimated by appropriate scaling of currents during 20-mV hyperpolarizing pulses, were subtracted from each of these records. Membrane capacitance and series resistance were compensated for by using the internal circuitry of the patch clamp amplifier. All data were digitized (10,000 samples per second), stored on a hard disk, and analyzed using a Power Macintosh computer (Apple) with Pulse+PulseFit 8.02 and

Igor Pro 2.04 analysis software programs (Heka, Wiesenstrasse, Lambrecht, Germany).

To measure  $I_{Ba}$  through VDCCs, recording solutions were chosen to inhibit  $K^+$  currents and enhance  $Ba^{2+}$  currents. The pipette solution contained: 20 mM CsCl, 110 mM CsOH, 5.2 mM  $MgCl_2$ , 112 mM L-glutamate, 10 mM EGTA, 5.0 mM  $Na_2ATP$ , and 10 mM HEPES, with the pH adjusted to 7.2 with Tris. The bath solution contained: 130 mM tetraethylammonium chloride, 1.0 mM  $MgCl_2$ , 5.0 mM  $BaCl_2$ , 10 mM glucose, and 10 mM HEPES, with the pH adjusted to 7.35 with Tris. Whole cell  $I_{Ba}$ s were elicited at 5-s intervals by 150-ms depolarizing pulses ( $-50$  to  $+40$  mV in 10-mV increments) from a holding potential of  $-60$  mV. To identify the characteristics of the  $I_{Ba}$  observed in this study, the effect of an L-type VDCC antagonist, nifedipine ( $10^{-6}$  M), on  $I_{Ba}$  was evaluated. Inactivation curves were determined using a double-pulse protocol that consisted of a 3-s prepulse to a potential of  $-60$  to  $+10$  mV, followed by a 150-ms depolarization to  $+10$  mV. The peak change in the current during the test pulse was expressed as a fraction of that obtained with the  $-60$ -mV prepulse, and this quantity was fitted to a Boltzmann expression<sup>33,34</sup> using least-squares analysis to estimate the potential of half-maximal inactivation ( $V_{1/2}$ ) and the slope factor ( $k$ ). Data from the cells that showed unstable current amplitudes, less than 100 pA of peak  $I_{Ba}$ , or a greater than 10% reduction in amplitude during the control recording period were discarded. The G $\Omega$  seal was maintained for a period sufficient to evaluate the reversibility of the effects of propofol in 144 of 192 experiments (75%).

To measure outward  $K^+$  currents ( $I_{Ks}$ ), recording solutions were chosen to enhance the  $K^+$  currents. The pipette solution contained: 70 mM KCl, 60 mM  $K^+$ -glutamate, 5.0 mM  $K_2ATP$ , 1.0 mM  $MgCl_2$ , 2.5 mM EGTA, 1.8 mM  $CaCl_2$ , and 10 mM HEPES, with pH adjusted to 7.2 with Tris. The computer-calculated  $[Ca^{2+}]_i$  was approximately  $10^{-6}$  M. The bath solution contained: 135 mM NaCl, 5.2 mM KCl, 1.8 mM  $CaCl_2$ , 1.0 mM  $MgCl_2$ , 10 mM HEPES, and 10 mM glucose, with pH adjusted to 7.35 with Tris. Whole cell  $I_{Ks}$  were elicited at 5-s intervals by 150-ms depolarizing pulses ( $-40$  to  $+60$  mV) from a holding potential of  $-60$  mV.

Voltage-pulse protocols were performed in control solutions for more than 5 min to obtain a stable baseline. Cells were exposed to a single concentration of propofol ( $10^{-7}$ – $10^{-4}$  M) with or without 20 nM oxytocin by changing the inflow perfusate of the chamber. The perfusion chamber consisted of a glass coverslip bottom, with needles placed for rapid solution changes. The chamber volume was approximately 1 ml, and complete solution changes in the chamber could be obtained within 1 min using a peristaltic pump (CTP-3; Iuchi, Tokyo, Japan) attached to the input and output ports. After 6-min exposure, the perfusate was switched again to the control solution.

### Materials

The following drugs and chemicals were used: acetoxymethyl ester of fura-2, EDTA (Dojindo, Kumamoto, Japan), fatty acid-free bovine serum albumin, pyruvic acid, creatine,  $Na_2ATP$ , taurine,  $\beta$ -hydroxybutyrate, pentobarbital, ketamine hydrochloride, nifedipine, EGTA, tetraethylammonium chloride, cremophor EL, oxytocin (Sigma Chemical, St. Louis, MO), collagenase (Wako Pure Chemical, Osaka, Japan), sevoflurane (Maruishi Pharmaceutical, Osaka, Japan), [ $^3H$ ]oxytocin (New England Nuclear, Boston, MA) and propofol (Diprivan®; Astrazeneca, London, United Kingdom). Nifedipine and pentobarbital were dissolved in ethanol and in dimethyl sulfoxide, respectively (0.1% final concentration). We used commercially available propofol, which included 10% Intralipid® (Pharmacia & Upjohn, Stockholm, Sweden) as the solvent (10% [vol/vol] soybean oil, 2.5% glycerol, and 1.2% purified egg lecithin). The effects of Intralipid *per se* were therefore tested in each experiment, and the concentration of the solvent used alone corresponded to each concentration of propofol.

### Statistical Analysis

Data are expressed as mean  $\pm$  SD. For the measurement of  $[Ca^{2+}]_i$  and muscle tension, oxytocin-induced changes in  $[Ca^{2+}]_i$  (indicated by  $R_{340/380}$ ) and muscle tension were used as references.<sup>20,25</sup> Changes in peak  $I_{Ba}$  or in the inactivation parameters  $V_{1/2}$  and  $k$  with exposure to propofol were compared at each applied potential by the paired, two-tailed *t* test. Other data were analyzed using one-way analysis of variance for repeated measurements, and the Fisher exact test was used as a *post hoc* test. In all comparisons, a *P* value  $< 0.05$  was considered significant.

## Results

### Influence of Propofol on Oxytocin Binding Receptors

Oxytocin binding experiments using [ $^3H$ ]oxytocin revealed saturable and high-affinity binding. Scatchard transformation of the data yielded linear plots from which  $B_{max}$  and  $K_d$  could be determined. Treatment of uterine cell membranes with a single dose of a high concentration of propofol ( $10^{-4}$  M) had no effect on oxytocin receptor density or agonist affinity ( $n = 5$  each, table 1). This result indicates that propofol does not interfere with the binding characteristics of the oxytocin receptors.

### Effects of Propofol on Tension and Intracellular $Ca^{2+}$ Concentration in Uterine Smooth Muscle Strips

Even in the resting condition with PSS alone, oscillatory phasic contractions of the uterine smooth muscle

**Table 1. Effects of Propofol and Intralipid on Oxytocin Receptor-binding Characteristics in the Uterine Smooth Muscle Membranes**

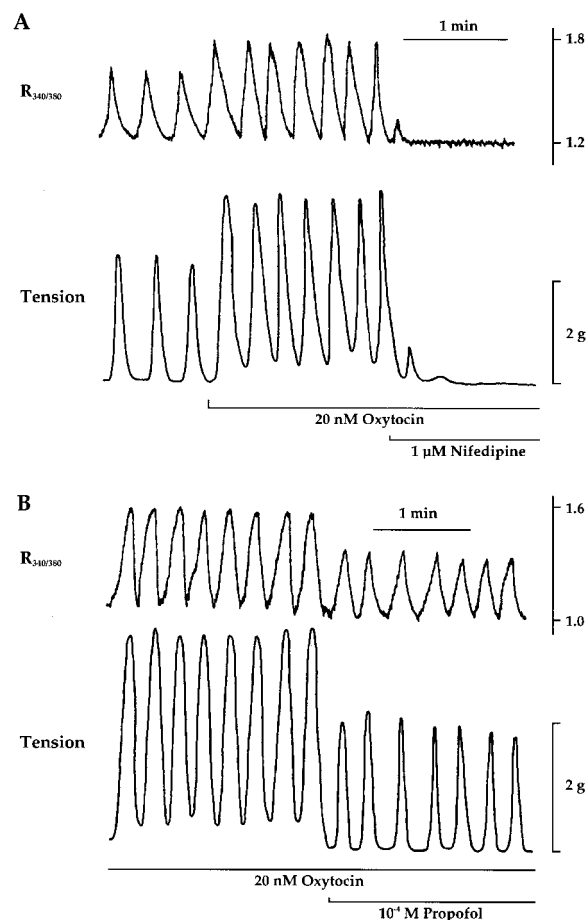
	$B_{\max}$ (fmol/mg protein)	$K_d$ (nM)
Control	$86.2 \pm 12.8$	$1.69 \pm 0.52$
$10^{-4}$ M propofol	$88.5 \pm 10.6$	$1.72 \pm 0.43$
Intralipid	$85.3 \pm 14.2$	$1.66 \pm 0.28$

Data are expressed as mean  $\pm$  SD from five experiments. Statistical significance was not evident using analysis of variance.

$B_{\max}$  = density of receptors;  $K_d$  = dissociation constant for the ligand.

strips were observed with a concomitant oscillatory increase in  $[Ca^{2+}]_i$  (fig. 2A). Oscillatory rates were constant ( $3.2 \pm 0.8$  cycles/min). Treatment with 20 nM oxytocin significantly increased oscillatory rates ( $4.5 \pm 1.2$  cycles/min) by approximately 41% and elevated the peaks of the contraction by approximately 35% and of the  $[Ca^{2+}]_i$  by approximately 21%. When calculating the areas under the contraction and  $[Ca^{2+}]_i$  curves to express the quantitative changes in muscle contractility and in  $[Ca^{2+}]_i$ ,<sup>29,30</sup> introduction of 20 nM oxytocin increased muscle contraction by  $123.5 \pm 20.6\%$  and  $[Ca^{2+}]_i$  by  $85.4 \pm 12.6\%$ . Nifedipine ( $1 \mu M$ ), both in the resting condition or in the oxytocin-activated condition, completely blocked the oscillatory changes both in the muscle contraction and in  $[Ca^{2+}]_i$  (fig. 2A). Deletion of free  $Ca^{2+}$  from the bath solution (with  $5 \mu M$  EGTA) also blocked these oscillations ( $n = 3$ , data not shown). These results indicate that  $Ca^{2+}$  influx through VDCCs is important for phasic contraction and increase in  $[Ca^{2+}]_i$ .

As shown in figure 2B,  $10^{-4}$  M propofol inhibited both the muscle contraction and increase in  $[Ca^{2+}]_i$  induced by 20 nM oxytocin. Propofol significantly decreased oscillatory rates by approximately 21% and suppressed the oxytocin-induced peaks of muscle contraction by approximately 34% and of  $[Ca^{2+}]_i$  by approximately 41%. We determined the dose dependence of the inhibition of oxytocin-induced muscle contraction and increase in  $[Ca^{2+}]_i$  by propofol, pentobarbital, and ketamine. Figure 3 shows the relations of percent responses of oxytocin (20 nM)-induced contraction (fig. 3A) and increase in  $[Ca^{2+}]_i$  (fig. 3B) with concentrations of the anesthetics. Each intravenous anesthetic significantly decreased muscle contraction and  $[Ca^{2+}]_i$  in a dose-dependent manner ( $n = 6$  each). Based on total concentration in the solution, propofol and pentobarbital had similar potencies, whereas ketamine required somewhat greater concentrations to achieve the same inhibitory effect. These results indicate that propofol as well as pentobarbital and ketamine can inhibit oxytocin-induced uterine muscle contraction, at least in part, by decreasing  $[Ca^{2+}]_i$ , a main regulator of the contraction.<sup>14,18</sup>



**Fig. 2.** Changes in muscle tension and intracellular  $Ca^{2+}$  concentration ( $[Ca^{2+}]_i$ ) (indicated by  $R_{340/380}$ ) during contraction by 20 nM oxytocin. Nifedipine ( $1 \mu M$ ) (A) or propofol ( $10^{-4}$  M) (B) was introduced in the presence of oxytocin. Nifedipine completely blocked the oscillatory changes in the muscle contraction and  $[Ca^{2+}]_i$ . Propofol significantly decreased oscillatory rates and suppressed the oxytocin-induced peaks of muscle contraction and  $[Ca^{2+}]_i$ .

#### Effects of Propofol on Intracellular Concentrations of Inositol 1,4,5-Triphosphate

The  $[IP_3]_i$  in the resting condition was  $4.2 \pm 0.6$  nmol/mg protein ( $n = 6$ ). Because  $[IP_3]_i$  is believed to oscillate phasically, as do muscle tension and  $[Ca^{2+}]_i$ ,<sup>18</sup> we did not measure the time course of  $[IP_3]_i$  activated by oxytocin. Instead,  $[IP_3]_i$  at 20 min after the oxytocin activation was measured and found to be significantly elevated to  $8.4 \pm 1.2$  nmol/mg protein. Figure 4 summarizes the effects of various concentrations of propofol on  $[IP_3]_i$  at 10 min after the propofol introduction ( $10^{-7}$ – $10^{-4}$  M) during the condition of 20-min oxytocin incubation. Propofol significantly inhibited the increase in  $[IP_3]_i$  induced by oxytocin in a dose-dependent manner.

#### Effects of Propofol on $Ba^{2+}$ and $K^+$ Channel Currents

The inward  $Ba^{2+}$  current ( $I_{Ba}$ ) observed in enzymatically dispersed rat uterine smooth muscle cells during

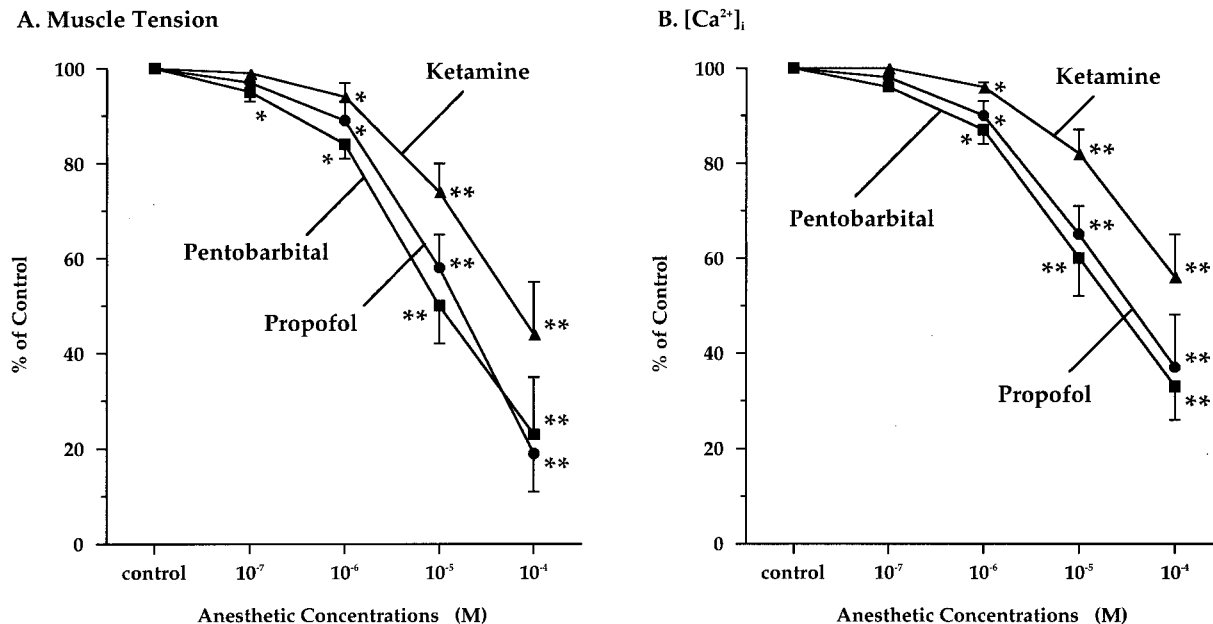


Fig. 3. Relations of percent responses of oxytocin (20 nM)-induced contraction (A) and increases in intracellular  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_i$ ) (B) with concentrations of the intravenous anesthetics tested in this study: propofol (circles), pentobarbital (squares), and ketamine (triangles). Each intravenous anesthetic significantly decreased muscle contraction and  $[\text{Ca}^{2+}]_i$  in a dose-dependent manner. The potency order of these anesthetics is propofol = pentobarbital > ketamine. Symbols represent mean  $\pm$  SD ( $n = 6$  at each point). \* $P < 0.05$ , \*\* $P < 0.01$  versus control without the anesthetics.

depolarizations from  $-60$  mV peaked at approximately 10–15 ms and was inactivated slowly with a time constant of approximately 300–500 ms (fig. 5A: control). In baseline conditions, threshold activation of  $I_{\text{Ba}}$  occurred at approximately  $-40$  mV, and maximum peak current amplitude was obtained at approximately  $+10$  mV (fig. 5B). In 72 cells, the maximum peak  $I_{\text{Ba}}$  was  $-421 \pm 46$  pA

(range,  $-259$  to  $-514$  pA). The inactivation parameters obtained in 24 cells in control conditions were  $V_{1/2} = -28.6 \pm 3.3$  mV and  $k = 5.1 \pm 0.9$  mV. The addition of  $10^{-6}$  M nifedipine virtually eliminated the  $I_{\text{Ba}}$  of rat uterine smooth muscle cells by  $91 \pm 3\%$  (data not shown,  $n = 4$ ).

As shown in a representative trace for depolarization from  $-60$  to  $+10$  mV (fig. 5A: propofol), propofol ( $10^{-4}$  M) inhibited the magnitude of  $I_{\text{Ba}}$  but did not obviously alter the time course of the current. Figure 5B shows the relation between peak  $I_{\text{Ba}}$  against applied potential before and after exposure to  $10^{-4}$  M propofol. Propofol significantly inhibited  $I_{\text{Ba}}$  at step potentials ranging from  $-20$  to  $+40$  mV and decreased peak  $I_{\text{Ba}}$  at  $+10$  mV by approximately 68%. There was no apparent shift in the voltage dependence of  $I_{\text{Ba}}$  with propofol. We determined the dose dependence of the inhibition of peak  $I_{\text{Ba}}$  by propofol. Figure 5C shows the relation between the percentage of control peak  $I_{\text{Ba}}$  at  $+10$  mV and the molar concentration of propofol in the bath solution. Propofol significantly inhibited peak  $I_{\text{Ba}}$  in a dose-dependent manner ( $\text{IC}_{50} = \text{approximately } 3 \times 10^{-6}$  M). Introduction of 20 nM oxytocin had no effect either on the magnitude of  $I_{\text{Ba}}$  or on the inhibitory effect of propofol ( $n = 4$ , data not shown). Figure 6 shows the effect of  $10^{-4}$  M propofol on the inactivation curve of  $I_{\text{Ba}}$ . Propofol significantly shifted the inactivation curve to a more negative potential ( $V_{1/2} = -32.2 \pm 2.3$  mV) without changing the shape of the curve ( $k = 5.2 \pm 1.1$  mV).

Figure 7A shows a macroscopic outward  $\text{K}^+$  current ( $I_{\text{K}}$ ) obtained from a freshly dispersed rat uterine smooth muscle cell dialyzed with a pipette solution containing a

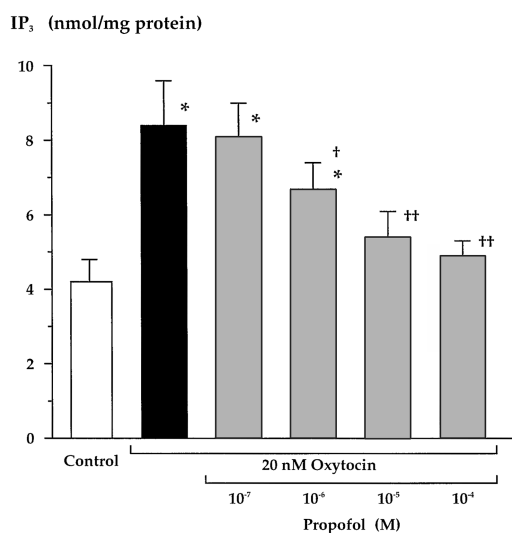


Fig. 4. Effects of various concentrations of propofol on intracellular concentrations of  $\text{IP}_3$  ( $[\text{IP}_3]_i$ ) after the introduction of propofol ( $10^{-7}$ – $10^{-4}$  M) during the condition of 20-min oxytocin incubation. Propofol significantly inhibited the increase in  $[\text{IP}_3]_i$  induced by 20 nM oxytocin in a dose-dependent manner. Symbols represent mean  $\pm$  SD ( $n = 6$  at each point). \* $P < 0.05$  versus control; † $P < 0.05$ , †† $P < 0.01$  versus control with oxytocin alone.

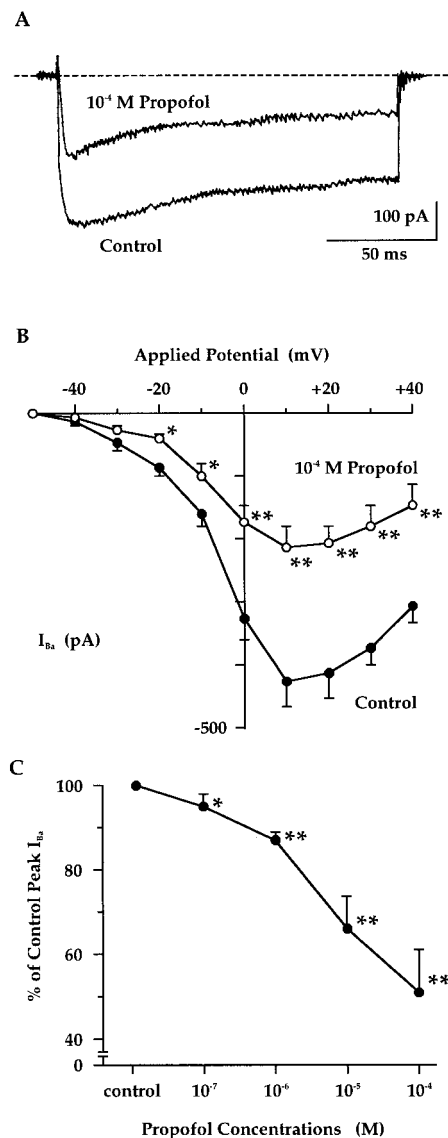


Fig. 5. Effects of propofol on depolarization-induced whole cell inward  $\text{Ba}^{2+}$  currents ( $I_{\text{Ba}}$ ). (A) Typical recordings of  $I_{\text{Ba}}$  induced by pulses to +10 mV without or with propofol ( $10^{-4}$  M). The dashed line denotes zero current. (B) The relation between peak whole cell  $I_{\text{Ba}}$  and applied potential before (filled circles) and after (open circles) exposure to propofol. (C) The relation between peak  $I_{\text{Ba}}$  at +10 mV, expressed as a percentage of control, and the propofol concentrations in the bath. Propofol significantly inhibited peak  $I_{\text{Ba}}$  at +10 mV in a dose-dependent manner, and there was no apparent shift in the voltage dependence of  $I_{\text{Ba}}$  with propofol. Symbols represent mean  $\pm$  SD ( $n = 6$  at each point). \* $P < 0.05$ , \*\* $P < 0.01$  versus control without propofol.

$[\text{Ca}^{2+}]_i$  of approximately  $10^{-6}$  M to enhance  $I_{\text{K}}$  through  $\text{Ca}^{2+}$ -activated  $\text{K}^{+}$  channels. The  $I_{\text{K}}$  was activated progressively by 150-ms depolarizing pulses from a holding potential of -60 mV to consecutively more positive membrane potentials. Stepwise depolarization from a holding potential of -60 mV to more than -30 mV elicited an outward  $I_{\text{K}}$  with a mean peak amplitude of  $2.11 \pm 0.18$  nA at +60 mV (fig. 7B,  $n = 6$ ). The effect of propofol on  $I_{\text{K}}$  was examined in 24 cells. Only the

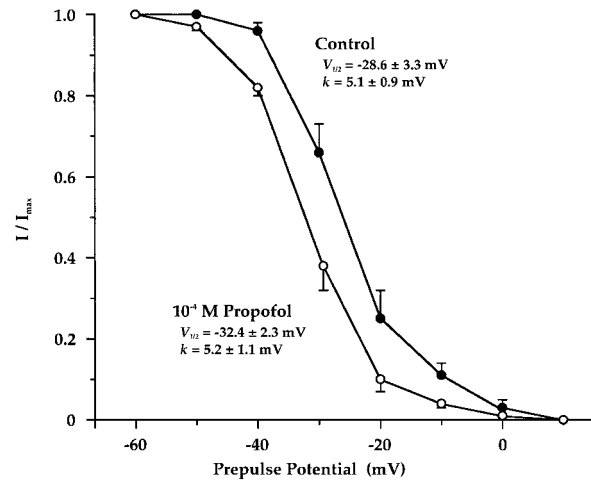


Fig. 6. Effect of propofol on voltage-dependent steady state inactivation of the whole cell inward  $\text{Ba}^{2+}$  current. The inactivation curve was generated during control conditions (filled circles;  $V_{1/2} = -28.6 \pm 3.3$  mV,  $k = 5.1 \pm 0.9$  mV), and propofol significantly shifted the inactivation curve to a more negative potential ( $V_{1/2} = -32.4 \pm 2.3$  mV) without changing the shape of the curve ( $k = 5.2 \pm 1.1$  mV) (open circles). Symbols represent mean  $\pm$  SD ( $n = 6$  at each point).

highest concentration of propofol ( $10^{-4}$  M) had a slight inhibitory effect on  $I_{\text{K}}$  (by approximately 8%) without any apparent effect on the time course of the current (fig. 7C).

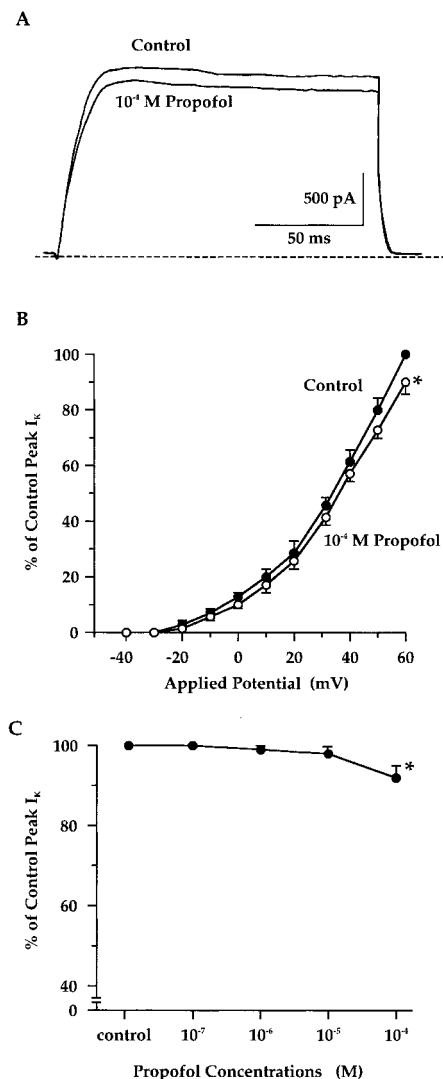
#### *Influence of Intralipid and Vehicles on the Various Parameters Tested*

We used Intralipid as the vehicle for propofol. Intralipid, the concentration of which corresponded to that used as the vehicle for propofol ( $10^{-4}$ – $10^{-7}$  M), did not affect any parameters tested in this study ( $n = 4$  each, data not shown). Neither ethanol nor dimethyl sulfoxide, the final concentration of which was less than 0.1%, affected any parameters tested in this study ( $n = 4$  each, data not shown).

## Discussion

### *Effects of Propofol on Oxytocin Binding Affinity, Uterine Smooth Muscle Contractility, and Intracellular $\text{Ca}^{2+}$ Concentration*

One of the major findings of our study is that propofol had a significant inhibitory effect on oxytocin-induced uterine smooth muscle contraction and increased  $[\text{Ca}^{2+}]_i$  in pregnant rats without affecting the agonist-receptor binding affinity (table 1 and figs. 2 and 3). For the agonist-receptor binding interactions, this result is in agreement with the general observations that volatile anesthetics<sup>35–37</sup> or propofol<sup>38,39</sup> have little effect on the binding of some other agonists. The result for muscle contraction and  $[\text{Ca}^{2+}]_i$  is consistent with that obtained by Shin *et al.*<sup>13</sup> using human pregnant uteri. Similar inhibitory effects were observed in this study with other



**Fig. 7.** Effects of propofol on depolarization-induced whole cell outward  $K^+$  currents ( $I_K$ ) with a pipette solution including 1.8 mM  $CaCl_2$  and 2.5 EGTA. (A) Typical recordings of  $I_K$  induced by pulses up to +60 mV in the absence and presence of propofol ( $10^{-4}$  M). The dashed line denotes zero current. (B) Relative peak current-voltage relations obtained before and after exposure to propofol ( $10^{-4}$  M). (C) The relation between peak  $I_K$  at +60 mV, expressed as a percentage of control, and the bath concentrations of propofol. Only the highest concentration of propofol ( $10^{-4}$  M) had a slight inhibitory effect on  $I_K$  without any apparent effect on the time course of the current. Symbols represent mean  $\pm$  SD ( $n = 6$  at each point). \* $P < 0.05$  versus control without propofol.

intravenous anesthetics, ketamine and pentobarbital. The inhibitory effect of propofol on muscle tension was parallel to its inhibitory effect on  $[Ca^{2+}]_i$ . Because  $[Ca^{2+}]_i$  plays a central role in the regulation of uterine smooth muscle tone,<sup>14,15</sup> it is thought that propofol inhibits uterine smooth muscle contraction, at least in part, by decreasing  $[Ca^{2+}]_i$ .

Oxytocin receptor occupancy, coupled through G proteins, results in stimulation of the phosphatidylinositol-signaling pathway.<sup>40</sup> Phillippe *et al.*<sup>18,29,41</sup> demonstrated that activation of the phosphatidylinositol-signaling path-

way results in the development of intracellular  $Ca^{2+}$  oscillation-like phenomena. Similar intracellular  $Ca^{2+}$  transients have been reported by other investigators in myometrium tissue and cultured uterine myocytes.<sup>42-44</sup> When these phenomena occur, increased  $[Ca^{2+}]_i$  is produced by both the release of stored intracellular  $Ca^{2+}$  and the influx of extracellular  $Ca^{2+}$ . Both events appear to be essential for the maintenance of repetitive myometrial contractions,<sup>14,29,30</sup> because the introduction of  $Ca^{2+}$ -free solution or the blockade of  $Ca^{2+}$  influx through VDCCs by nifedipine completely blocked the oscillatory contractions in this study. Therefore, the mechanisms by which propofol decreased  $[Ca^{2+}]_i$  are thought to be inhibition of the phosphatidylinositol-signaling pathway or inhibition of VDCC activity. To investigate the mechanisms,  $[IP_3]_i$  and the changes in inward  $Ba^{2+}$  currents ( $I_{Ba}$ ) through VDCCs in uterine smooth muscles obtained from pregnant rats were examined using radioimmunoassay and whole cell patch clamp techniques, respectively.

#### *Effect of Propofol on Intracellular Inositol 1,4,5-Triphosphate Concentration*

The results of the current experiment showed that propofol inhibited the increase in  $[IP_3]_i$  in a dose-dependent manner (fig. 4). The decrease in  $[IP_3]_i$  caused by propofol lead to a decrease in  $[Ca^{2+}]_i$ , resulting in inhibition of the uterine contraction. This result is in agreement with that in a study on canine smooth muscle<sup>39</sup> showing that treatment with propofol is associated with inhibition of the increase in  $[Ca^{2+}]_i$  mediated by  $IP_3$ . Hirakata *et al.*,<sup>45</sup> however, showed that high concentrations of propofol ( $3 \times 10^{-5}$ – $10^{-4}$  M) induced formation of  $IP_3$  in human platelets. This discrepancy may result from the differences in cell types and species or in the selective effects of propofol on certain receptors, G proteins, or phospholipase C isozymes.<sup>46</sup>

As shown in figure 1, stimulation of the oxytocin receptor activates the G protein-linked phospholipase C, resulting in hydrolysis of phosphatidylinositol 4,5-bisphosphate to the two potent stimulatory second messengers  $IP_3$  and 1,2-diacylglycerol.<sup>47,48</sup>  $IP_3$  is rapidly metabolized to inositol 1,4-bisphosphate and inositol 1,3,4,5-tetrakisphosphate by  $IP_3$  phosphatase and  $IP_3$  kinase, respectively. Therefore, it is possible that propofol affects any point of the phosphatidylinositol-signaling pathway (e.g., inhibition of G protein-phospholipase C or activation of  $IP_3$  phosphatase-kinase) to decrease  $[IP_3]_i$ . Nagase *et al.*<sup>49</sup> and Minami *et al.*<sup>50</sup> reported that propofol had an inhibitory effect on receptor-G protein coupling of muscarinic and 5-hydroxytryptamine receptors, respectively; however, further studies are needed to clarify this point in uterine smooth muscle. There is the evidence that  $IP_3$  also facilitates the influx of extracellular  $Ca^{2+}$  through specific channels in the cell membrane.<sup>47,48,51</sup> Therefore, inhibition of the increase in

$[IP_3]_i$  by propofol might in itself account for the observed effects on both  $[Ca^{2+}]_i$  and muscle contractility.

#### *Effect of Propofol on Voltage-dependent $Ca^{2+}$ Channel Activity*

Using whole cell patch clamp techniques, we measured depolarization-induced inward  $Ba^{2+}$  currents ( $I_{Ba}$ ) in rat uterine smooth muscle cells. Based on their time and voltage dependences and their sensitivity to blockade by nifedipine, these currents are presumed to reflect the activity of L-type VDCCs.<sup>52,53</sup> Young *et al.*,<sup>52</sup> however, reported that freshly isolated human uterine smooth muscle cells showed two types of  $Ca^{2+}$  currents (T and L types) that were analogous to those found in cardiac myocytes. Because it has been reported that the resting membrane potential is approximately  $-60$  mV in the late stage of gestation,<sup>52</sup> the L-type VDCCs might be primarily involved in increasing  $[Ca^{2+}]_i$  by bulk  $Ca^{2+}$  transport.<sup>54</sup>

Propofol significantly inhibited  $I_{Ba}$  through L-type VDCCs of the uterine smooth muscle cells without an apparent change in the voltage dependence of  $I_{Ba}$  (fig. 5), suggesting that the anesthetic has no effect on membrane surface charge or on the voltage sensor of the channel. These data indicate one of the cellular effects of propofol that can account for the uterine smooth muscle relaxant effects of the anesthetic.<sup>13</sup> To further evaluate the inhibitory action of propofol on VDCCs of uterine smooth muscle cells, we studied the effects of propofol on steady state, voltage-dependent inactivation of  $I_{Ba}$ . During prolonged depolarization (prepulse), a fraction of the VDCCs enters an unavailable or "inactivated" state. Propofol significantly shifted the inactivation curve to a more negative potential without changing the sigmoid shape of the curve (fig. 6). A qualitatively similar shift induced by a dihydropyridines-sensitive  $Ca^{2+}$  antagonist, such as nifedipine, has been interpreted as evidence of a drug-induced stabilization of the inactivated state.<sup>55</sup> We therefore speculate that propofol has a dihydropyridines-sensitive  $Ca^{2+}$  antagonist-like inhibitory effect on VDCCs in uterine smooth muscle cells.

#### *Other Possibilities for the Inhibitory Mechanisms of Propofol on Uterine Smooth Muscle Contraction*

Two other possibilities for the inhibitory mechanisms of propofol on uterine smooth muscle contraction should be considered. Franks and Lieb<sup>56</sup> reported that volatile general anesthetics activated a novel neuronal  $K^+$  current. The resulting enhanced  $K^+$  efflux can induce membrane repolarization or hyperpolarization, reduce the open-state probability of VDCCs, and in turn cause relaxation of uterine smooth muscle. In this study, the whole cell outward  $I_K$  was inhibited significantly by only a high concentration ( $10^{-4}$  M) of propofol (fig. 7).

This result suggests that mechanisms other than  $K^+$  channel opening are likely to mediate propofol-induced relaxation.

Another possibility is protein kinase C. As previously described, stimulation of the oxytocin receptor can produce 1,2-diacylglycerol as well as  $IP_3$ . Increased 1,2-diacylglycerol can activate protein kinase C. Although it has been known that protein kinase C has an inhibitory effect on  $IP_3$  production and plays a role in generation of intracellular  $Ca^{2+}$  oscillations in some cell types,<sup>57,58</sup> there is no clear evidence of protein kinase C playing a role in uterine smooth muscle contractility. In airway smooth muscle, it is conceivable that protein kinase C plays a role in  $Ca^{2+}$ -independent smooth muscle contraction.<sup>20,28</sup> Further studies are needed to clarify the role of protein kinase C in uterine smooth muscle and the effect of propofol on the protein kinase C activity.

#### *Concentration Dependence and Clinical Relevance*

Propofol, ketamine, and pentobarbital each showed concentration-dependent inhibition of uterine smooth muscle contraction and  $[Ca^{2+}]_i$  (fig. 3). Based on the total solution concentration, ketamine was less potent than the other agents by a factor of approximate 3. Because propofol was added as an emulsion, it is likely that its free concentration was substantially less than the total concentration and that this drug is, in fact, more potent than pentobarbital for inhibition of muscle contraction. We suggest that the potency order of these agents is propofol > pentobarbital > ketamine. For the measurement of  $IP_3$  concentrations and VDCC activity, a significant decrease and inhibition of these parameters were observed using the same range of concentrations as that used for investigation of contractions and  $[Ca^{2+}]_i$  ( $10^{-7}$ – $10^{-4}$  M). Extrapolation of our data to the clinical situation must be viewed with caution because of possible species differences, *in vivo-in vitro* differences, and the fact that our patch clamp experiments were conducted during nonphysiologic conditions of low (ambient) temperature and high (5 mM) extracellular  $Ba^{2+}$  concentration.

Given that more than 95% of propofol ( $2.5 \times 10^{-5}$  M) in blood bound to plasma protein,<sup>59</sup> the free propofol concentration of  $4\text{--}10 \times 10^{-7}$  M *in vitro*, which has little effect on muscle tension, would be comparable to 9–10  $\mu\text{g/ml}$  propofol *in vivo*.<sup>7</sup> Because the therapeutic ranges of plasma propofol concentrations were 2–9  $\mu\text{g/ml}$ ,<sup>59</sup> the effective propofol concentrations tested in this study were rather higher than the free concentrations observed clinically in serum. Whether the direct decrease in muscle tension produced by propofol would affect uterine atony-bleeding during cesarean section needs further study.

In conclusion, propofol at concentrations ranging from  $10^{-7}$  to  $10^{-4}$  M significantly reduces the oxytocin-induced contraction of uterine smooth muscle obtained from pregnant rats in isolated preparations. The inhibi-

tion of the contraction by propofol is, at least in part, caused by the decrease in  $[Ca^{2+}]_i$  without inhibition of agonist-receptor binding. The decrease in  $[Ca^{2+}]_i$  may be mediated by a decrease in  $[IP_3]_i$  and by an inhibition of VDCC activity.

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