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Effects of levobupivacaine and bupivacaine on rat myometrium*

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Abstract: Objective: To study the effect of levobupivacaine and bupivacaine on the contractility of isolated uterine muscle strips from pregnant and non-pregnant female rats. Methods: Full-thick myometrial strips were prepared from 18- to 21-day pregnant (n=8) and non-pregnant rats (n=7). After contractions became regular, strips were exposed to cumulative concentrations of the two drugs from 10^{-8} to 10^{-4} mol/L, amplitude and frequency of the uterine contraction was recorded. Results: Two local anesthetics caused a concentration dependent inhibition on contractility of myometrial strips from pregnant and non-pregnant rats. In the myometrium from non-pregnant rats, $-\log IC_{50}$ of levobupivacaine and bupivacaine were 4.85 and 4.25 respectively. In the myometrium from pregnant rats, similar concentrations of levobupivacaine and bupivacaine were observed, $-\log IC_{50}$ were 2.7 and 2.9 respectively. Levobupivacaine produced an increase in amplitude of contractions, while bupivacaine showed an increased trend in frequency. Conclusion: These results demonstrate that levobupivacaine and bupivacaine may inhibit myometrium contractility. The inhibitory effect of levobupivacaine or bupivacaine is not enhanced by gestation in rat. Levobupivacaine may have more positive influence than bupivacaine in pregnant myometrium.

Key words: Levobupivacaine, Bupivacaine, Myometrium, Rat
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INTRODUCTION

Bupivacaine as a racemic mixture of its enantiomers, dextrobupivacaine and levobupivacaine, has been the most widely used local anaesthetic for years. Both in vitro and in vivo studies showed that dextrobupivacaine has more inherent central nervous system (CNS) and cardiovascular toxicity than levobupivacaine. So, levobupivacaine as a new long-acting amide local anaesthetic, the pure S-enantiomer of racemic bupivacaine, has been developed. Pharmacological studies demonstrated that levobupivacaine has equal local anaesthetic potency with reduced potential for cardiac and CNS toxicity compared to bupivacaine (Bremerich and Zwissler, 2004).

Regional anesthesia and analgesia have come into widespread use for women in gynecological procedures. Local anesthetic agents are often used for obstetric analgesia and anesthesia. Previous studies reported that bupivacaine inhibited spontaneous contractions of isolated gravid rat myometrium (Arici *et al*., 2004; Karsli *et al*., 2003; Santos *et al*., 1995). But there is no report on the effect of levobupivacaine in pregnant or non-pregnant myometrium in vitro. In this study, we compared the effects of bupivacaine and levobupivacaine on contractions of myometrium isolated from pregnant or non-pregnant rats, then discuss its role in obstetric analgesia and anaesthesia.

MATERIALS AND METHODS

Animals

Pregnant female Sprague-Dawley (SD) rats at 18 ~21 d and non-pregnant female SD rats were used. Non-pregnant female SD rats weighing (200±20) g

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were pretreated with 40 μg diethylstilbestrol subcutaneously for 2 d to induce oestrus and improve the response to drug. The study procedures were approved by the Medical Faculty Ethic Committee of Zhejiang University.

Experiments procedures

Pregnant and non-pregnant rats were anesthetized with urethane (25%, 4 ml/kg) intravenously, then killed by cervical subluxation for the study. The preparation of myometrial strip followed the method of Karsli *et al*.(2003). The uterine horns were rapidly excised and carefully cleaned of surrounding connective tissue and opened longitudinally along the mesenteric border. Fetuses of the late-stage pregnant rats were removed and non-uterine tissues were dissected and discarded. We obtained longitudinal full-thickness myometrial muscle strips (measuring 4 mm×10 mm) from each animal. The uterine tissues of non-pregnant SD rats were rapidly isolated and carefully cleaned of surrounding connective tissue, then two uterine strips from one uterus were prepared.

The strips were mounted vertically, one end of the strip was connected to the lower hook of the bath and the other end of the strip was connected to a force rod. The strips were incubated in the 10 ml tissue bath containing De-Jalon's solution (composition in mmol/L: NaCl 153.9, KCl 5.6, glucose 2.7, $NaHCO_3$ 5.9, and $CaCl_2$ 0.27), which were aerated continuously with 95% oxygen and 5% carbon dioxide. The pH was kept at 7.4 and the temperature was maintained at (32±0.5) °C. The solution was constituted daily for each experiment. Myometrial strips of pregnant rats were allowed to equilibrate at 1 g tension for 20 min before the contractions became regular. The characteristics of the contraction frequency and amplitude were recorded by a force displacement transducer (JZ100, Xinhang Machine and Equipment, Gaobeidian, China) coupled to MedLab Biological Signal Collection System (Medease Science and Technology, Nanjing, China). When the contractions of the strips became regular, the contraction frequency and amplitude were recorded as baseline activity, then levobupivacaine (Suka, Jiangsu Hengrui Medicine Co. Ltd., Shanghai, China) or bupivacaine (Shanghai Harvest Pharmaceutical Co. Ltd., Shanghai, China) was added at cumulative concentrations, duration of each concentration was 20 min. At the end of the drug exposure, the muscle strips were washed out 3 times. Drug-containing solutions were prepared immediately before the experiment.

The non-pregnant myometrial strips were allowed to equilibrate at 2 g tension for 40 min after the preparation was induced by appropriate concentration of oxytocin (0.05 U/10 ml). When the contractions became regular, the contractions frequency and amplitude were recorded as baseline activity, then the experimental drugs were added and recorded as above described. Each concentration duration was 15 min, washed out at the end of the experiment, wash interval was 5 min for 3 times. Each strip was exposed to only one anesthetic agent.

Data analysis

All data were expressed as mean±*SD* and intergroup and intragroup differences analyzed by unpaired *t*-test and one-way ANOVA using SPSS software (version 11.0 for Windows, SPSS Inc.). $P<0.05$ was considered to be statistically significant.

RESULTS

Effects of bupivacaine and levobupivacaine on non-pregnant rat myometrium

The exposure to bupivacaine and levobupivacaine with cumulative concentrations from 10^{-8} mol/L to 10^{-4} mol/L significantly decreased the contractile activity, both on the amplitude and on frequency in dose-dependent manner (Figs.1 and 2). Meanwhile levobupivacaine was stronger than bupivacaine ($P<0.05$). The $-\log IC_{50}$ of bupivacaine and levobupivacaine on contraction amplitude were 4.25 and 4.85, respectively. In two groups, the baseline values of the contractile forces were (3.11±0.59) g (bupivacaine), (3.31±0.48) g (levobupivacaine), respectively, and the baseline values of the frequency were (8.14±2.27)/(15 min) (bupivacaine) or (8.08±2.20)/(15 min) (levobupivacaine), respectively. There was no significant difference between the two groups.

Effects of bupivacaine and levobupivacaine on pregnant rat myometrium

Bupivacaine and levobupivacaine at cumulative concentrations did not show significant decrease in contractile amplitude and frequency of myometrial strips isolated from pregnant rat. Furthermore

levobupivacaine produced significant increase in the amplitude of contractions from 3×10^{-7} mol/L to 3×10^{-6} mol/L. However, bupivacaine did not. Higher concentration of bupivacaine and levobupivacaine both produced significant reduction in amplitude (Figs.3 and 4). $-\log IC_{50}$ of bupivacaine and levobupivacaine on contraction amplitude were very close, 2.9 and 2.7, respectively.

Bupivacaine produced an increase in the frequency in concentration dependent manner (Fig.3b), and reaching statistical significance at a concentration of 3×10^{-5} mol/L; however, levobupivacaine had no similar action. But at higher concentration, 10^{-4} mol/L of the two drugs showed slight inhibitory effects without statistical significance. The baseline values of the contractile amplitude were (3.01±0.31) g (bupivacaine) and (2.94±0.45) g (levobupivacaine), respectively. The baseline values of contraction frequency were (8.33±0.58)/(20 min) (bupivacaine) and (7.94±0.52)/(20 min) (levobupivacaine), respectively.

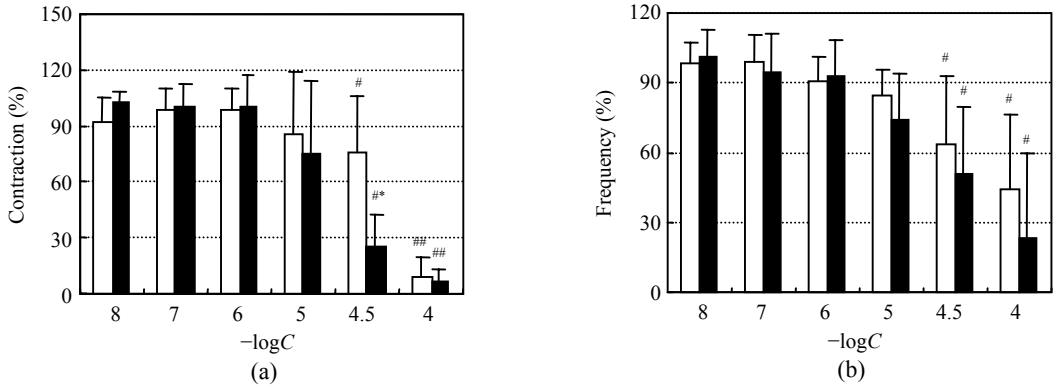

Fig.1 The effects of levobupivacaine (■) and bupivacaine (□) on the amplitude (a) and frequency (b) of contractions of myometrial strips isolated from non-pregnant rats

Data (mean±*SD*) expressed relative to baseline; n=7; $^{*}P$<0.05 levobupivacaine vs bupivacaine; $^{\#}P$<0.05, $^{\#\#}P$<0.01 vs baseline; *C*: Concentration (mol/L)

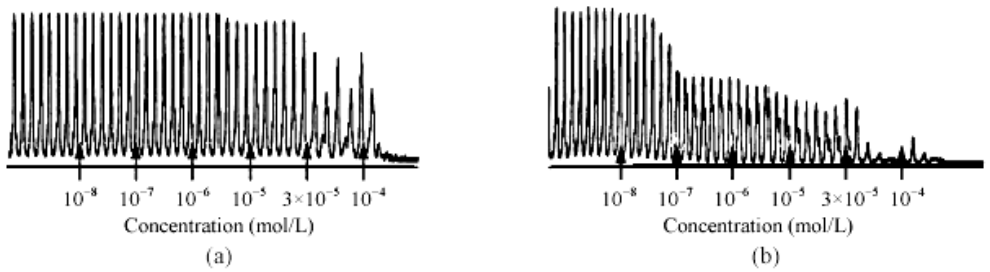

Fig.2 Respresentative trace showing the effect of bupivacaine (a) and levobupivacaine (b) on contraction of isolated non-pregnant rat myomerium at cumulative concentrations (10^{-8}~10^{-4} mol/L)

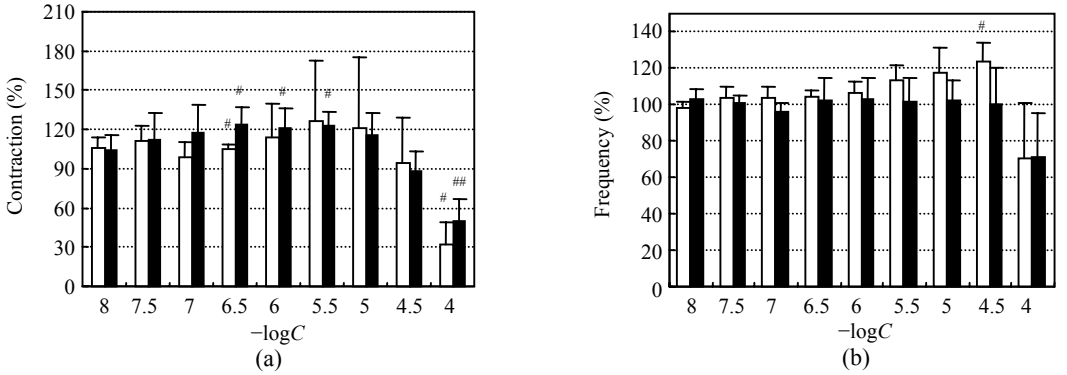

Fig.3 The effects of levobupivacaine (■) and bupivacaine (□) on the amplitude (a) and frequency (b) of contractions of myometrial strips isolated from pregnant rats

Data (mean±*SD*) expressed relative to baseline; n=8; $^{\#}P$<0.05, $^{\#\#}P$<0.01 vs baseline; *C*: Concentration (mol/L)

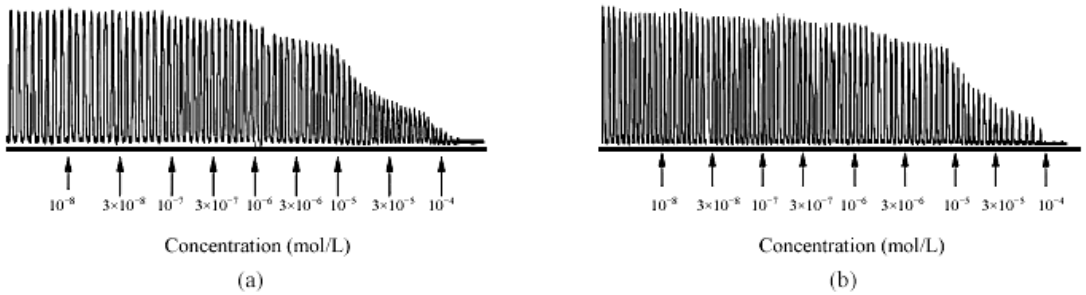

Fig.4 Respresentative trace showing the effect of bupivacaine (a) and levobupivacaine (b) on the contraction of isolated pregnant rat myomerium at cumulative concentrations (10^{-8}~10^{-4} mol/L)

DISCUSSION

The goal of obstetric analgesia is to provide optimal pain relief for the parturient with minimal risks to herself and fetus. Local anesthetics are the most effective agents for reducing pain during labor and postoperative period. Bupivacaine is the most commonly used local anesthetic agent in obstetric patients. In vivo clinical studies suggested that bupivacaine had no effect on uterine contractility (Nielsen *et al*., 1996; Scull *et al*., 1998). Other clinical trials showed that minimum local analgesic concentration of extradural bupivacaine accelerated the progression of labour (Capogna *et al*., 1998). These conflicting results may be involved under different condition of clinical trials and different administration concentration. Strümper *et al*.(2005) reported that continuous infusion of 0.175% bupivacaine via epidural catheter at a rate of 5 ml/h might improve placental blood flow and have beneficial outcome in intrauterine growth restriction independent of the underlying cause. Furthermore, many studies demonstrated that bupivacaine decreased contractile activity of muscles, such as myocardial papillary muscles, bladder smooth muscles, and tracheal smooth muscles (Oh *et al*., 2005; Shibuya *et al*., 1993; Wali, 1987).

Levobupivacaine, a relatively new agent, is being used increasingly for obstetric patients recently. The reduced toxicity of levobupivacaine gives wider safety margin in the daily clinical practice for single shot or continuous infusion during various surgical procedures, postoperative pain control and analgesia in labour (Ivani *et al*., 2001). Recent experimental study suggested that bupivacaine and its R(+)- and S(−)-enantiomers are similar for somatic antinociception and neurotoxicity but slightly different in visceral antinociception and motor paralysis, in which levobupivacaine is less potent than the others (Muguruma *et al*., 2006). Some clinical studies suggested that local anaesthetic effect of levobupivacaine did not differ from that of racemic bupivacaine under ulnar nerve blockade (Bardsley *et al*., 1997), paracervical block (Palomaki *et al*., 2005), spinal anesthesia (Glaser *et al*., 2002; Alley *et al*., 2002), and inferior alveolar nerve block (Branco *et al*., 2006). For caesarean section, levobupivacaine had the efficacy and safety profile equivalent to bupivacaine in epidural anesthesia (Cheng *et al*., 2002; Faccenda *et al*., 2003), but contrary evidence suggested that intrathecal levobupivacaine has similar clinical profile as racemic bupivacaine, but at equal doses it produced less motor block (Vercauteren *et al*., 2001), and supported by the study of Gautier *et al*.(2003). However, few studies investigated the effect of levobupivacaine on smooth muscle contractions. Moreover, in vitro effects of bupivacaine and levobupivacaine on uterine contractions are not well documented.

On pregnant uterine contractions, previous studies suggested that bupivacaine caused a concentration dependent inhibition of uterine contraction amplitude, but on frequency, there exists controversy. One study reported that bupivacaine had no effect on frequency of uterine contractions of pregnant 18- to 21-day Wistar rat (Arici *et al*., 2004). Another study indicated bupivacaine elevated the frequency of uterine contractions in albino rats pregnant for 18~21 d at 3×10^{-4} mol/L concentration (Karsli *et al*., 2003). In this study, we found that bupivacaine inhibited uterine contraction amplitude in a concentration dependent manner similar to the previous studies. On frequency, results showed that bupivacaine produced

an increase in the frequency in concentration dependent manner, but at higher concentration 10^{-4} mol/L showed slight inhibitory effects without statistical significance. These results being different from those of latter studies, may suggest that different strain of rat have different response for bupivacaine. In this study, levobupivacaine also inhibited contractile activity in pregnant myometrial strips in a concentration dependent manner. Meanwhile, levobupivacaine enhanced contraction of amplitude at lower concentration. But in highest concentration, they all showed significant inhibitory effects. Pharmacokinetics of the enantiomers of bupivacaine suggested that peak plasma concentrations of bupivacaine or levobupivacaine during epidural administration were (389±93) ng/ml and (449±109) ng/ml, respectively, approximately 10^{-6} mol/L (Groen *et al*., 1998). At this concentration, the influence is slight on the contraction amplitude or frequency of the uterine muscles from pregnant rats. Levobupivacaine even enhanced uterine contraction amplitude and may provide positive influence during labor and postoperative period.

On non-pregnant uterine contraction, our results suggested that bupivacaine and levobupivacaine inhibited contractile activity, in both amplitude and frequency, in a concentration dependent manner. Levobupivacaine was stronger than bupivacaine at 3×10^{-5} mol/L, these results may suggest that levobupivacaine has advantages for use in operations during pregnancy. Moreover bupivacaine and levobupivacaine had stronger inhibition on myometrial strips from non-pregnant rats than from pregnant rats. These suggested that the responses of pregnant and non-pregnant myometrial strips were different, but the reason is unclear so that further studies are required. Santos *et al*.(1995) compared the systemic toxicity of ropivacaine and bupivacaine in non-pregnant and pregnant ewes, which showed that systemic toxicity of ropivacaine or bupivacaine is not enhanced by gestation in sheep. This is in contrast to an earlier study in which the cardiotoxicity of bupivacaine was enhanced during ovine pregnancy (Santos *et al*., 1991). Similarly to those results, our study demonstrated that exposure to higher concentration of bupivacaine decreased contractile amplitude in pregnant myometrial strips. But greater doses of levobupivacaine are needed to produce the same manifestations as compared with bupivacaine.

In conclusion, two local anesthetic agents, bupivacaine and levobupivacaine were tested with regard to their influence on the contraction amplitude and frequency of contractions of pregnant and non-pregnant rat myometrium in vitro. The two drugs had inhibitory effect on contractions. Minimal differences were observed between the two drugs, in non-pregnant rats. The potency was levobupivacaine>bupivacaine, but in pregnant rats was bupivacaine≈levobupivacaine. However, the reduced toxic potential of levobupivacaine supports the clinical use to decrease the risk of systemic toxicity related to either overdosing or unwanted intravascular injection, such as during epidural or peripheral nerve blocks. Meanwhile, the slightly different action of levobupivacaine on contraction amplitude of pregnant uterus and frequency of non-pregnant uterine muscles may provide positive influence in operations during pregnancy. This result may provide evidence for clinical implication, but further studies are required to verify its efficacy in pregnant women.

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