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ORIGINAL RESEARCH

Intrathecal Injection of Ropivacaine Reduces Cervical Resistance in Late-Pregnant Rats

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Purpose: Neuraxial (spinal and epidural) anesthesia is the cornerstone of ensuring the satisfaction rate of painless delivery; however, whether it prolongs the first stage of labor remains controversial. Although current clinical research results tend to be negative, the conclusions are not convincing due to the lack of basic research. This study was conducted to provide a theoretical reference for this controversy through basic research.

Materials and Methods: A spinal anesthesia model was established by the intrathecal injection of 0.1% ropivacaine in late-pregnant rats (day 22). The cervical resistance test was used to measure the tension of different groups of isolated cervical tissues. Western blotting and cervical tissue cyclic AMP (cAMP) enzyme-linked immunosorbent assay were performed to clarify the possible related mechanisms.

Results: Cervical resistance experiments showed that the intrathecal injection of ropivacaine decreased the cervical resistance, and norepinephrine injection reversed this effect. Western blotting showed that α2A adrenergic receptor (α_{2A}-AR) levels gradually increased over time in pregnant rats. The cAMP enzyme-linked immunosorbent assay revealed that the intrathecal injection of norepinephrine reversed the increase in cervical tissue cAMP concentration caused by ropivacaine injection.

Conclusion: Ropivacaine relaxes the cervix. Further, α_2-AR may be involved in the process of cervical contraction.

Keywords: neuraxial anesthesia, labor stage, cervical resistance, cAMP

Introduction

Neuraxial anesthesia is widely used for painless delivery because of its excellent analgesic effect,[1,2] and the low concentration of local anesthetics (eg, 0.1% ropivacaine) guarantees safety. However, clinicians are always concerned whether neuraxial anesthesia would prolong the labor stage of the parturient, so that the anesthesiologist might have to wait until the cervix of the parturient opens to 3–4 cm before inducing anesthesia. Existing clinical studies report different conclusions on whether neuraxial anesthesia prolongs the labor stage.[3–7] Therefore, obstetricians and the parturient undergoing painless delivery have the impression that the timing of intraspinal anesthesia intervention is "the later, the better."

In fact, the first stage of labor depends on the tension of the cervix. More importantly, the tension of the cervix at this stage appears to be closely related to the function of the paracervical adrenergic nerves. During pregnancy, the uterus has characteristic but poorly understood innervation remodeling, which involves a deep denervation process.[8–11] The degeneration of adrenergic nerves is most obvious in the uterine body and fundus, whereas the cervical adrenergic innervation remains intact or hardly affected.[12–14]As the adrenergic system plays a vital role in regulating the contractility of the uterus during pregnancy, we speculated that neuraxial anesthesia could relax the cervix by blocking the paracervical adrenergic nerves, thereby shortening the first stage of labor.

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To confirm our speculation, we conducted this study to clarify the impact of neuraxial anesthesia on cervical tension at the two levels of cervical tension changes and molecular biological changes, which would help improve people's correct understanding of painless delivery.

Materials and Methods

All experimental protocols and animal handling procedures were approved by the Animal Care and Use Committee of Xuzhou Medical University (No.202112A149). The protocols are consistent with the National institute of Health Guide for the Care and Use of Laboratory Animals and the International Association for the Study of Pain's guidelines for pain research.

Animal Mating

Sprague–Dawley (SD) rats (Experimental Animal Center, Jinan, China) were maintained at ~21°C ± 0.5°C with alternating lights simulating day and night. Mature female (weighing 180–200 g) and male (weighing 240–260 g) SD rats were mated in a mating cage separated by a metal gate. Within approximately 4–5 h after mating, the presence of mating plugs or vaginal smears was used to confirm mating.

Intrathecal Cannulation

Intrathecal cannulation was performed as previously described.[15] The rats were anesthetized using 5% isoflurane, and a 6-cm long PE-10 catheter (Becton Dickinson, Sparks, MD, USA) was implanted into the subarachnoid space at the L4–L5 level. Isoflurane was administered when necessary during the operation. Tail or hind-limb movements when unconscious were considered as signs of dura penetration. The catheter was then pushed further by 1.5 cm into the subarachnoid space, and the end of the catheter was heat sealed. On the following day, 10 μL of 2% lidocaine was injected to confirm the accuracy of the catheter position. The rats are allowed to recover for 3 days.

Administration of Study Drugs

The catheterized rats were anesthetized using isoflurane and placed into a transparent plexiglass box. Each group of rats (n = 6 per group) was treated with ropivacaine (AstraZeneca AB, Wilmington, US) (0.1%, 10 μL), norepinephrine (NE) (Slleck, Shanghai, China) (1 mM, 10 μL), or normal saline (10 μL). The latter two were used as controls.

Cervical Resistance Test

Cervical tissue was collected at 22 days of pregnancy. The cervix was suspended between the two hooks of the tension transducer (HV-4; Taimeng Software, Chengdu, China). The cervix was bathed in standard Krebs buffer (composition: 130 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1.2 mM NaH_2PO_4, 0.56 mM $MgCl_2$, 25 mM $NaHCO_3$, and 5 mM glucose, pH = 7.4). The temperature of the buffer was maintained at 37°C by perfusing carbon (95% O_2 + 5% CO_2). The cervical tissue was balanced with 1 g of tension in the buffer for 2 h to ensure that the basic value of the data acquisition system (BL-420N; Thai Union Software, Chengdu, China) is zero. The buffer was changed every 15 min. The experimental parameters were set to record the load (g) mi1 for each stretch of 1 mm and the load mi2 after 30 min of standing at 1-mm stretch. The result of (m1i + m2j)/2(i,j = 1, 2 … 8) was taken as the load at that time. The total stretch was 8 mm. It was not necessary to convert into a stress–strain curve after plotting the load–elongation curve. The linear regression was obtained using the least square method, and the slope of the linear regression reflected the Young's modulus.

Western Blotting

Cervical tissues from sham, 18-, 20-, and 22-day-pregnant animals were rapidly removed for evaluation. Briefly, 40 μg of protein was loaded and electrophoresed on a 10% sodium dodecyl sulfate–polyacrylamide gel at 100 V. The proteins were then subjected to wet transfer onto a polyvinylidene fluoride membrane at a constant current of 300 mA. Membranes were blocked with 3% bovine serum albumin at room temperature for 2 h. Rabbit anti- α2A adrenergic receptor (α_{2A}-AR) antibody (1:500 dilution; ab85570, Abcam, UK) and rabbit anti- β-actin antibody (1:5000 dilution; ab8227, Abcam, UK) were used as primary antibodies. The membranes were incubated with primary antibodies

overnight at 4°C. The membranes were then washed three times (5 min per wash) with Tris-buffered saline with Tween-20 (TBST) and subsequently incubated with horseradish peroxidase-labeled goat anti-rabbit Ig (1:5000) for 2 h at room temperature. Membranes were then rinsed six times with TBST (5 min per wash), followed by evaluation using the UVITEC gel camera. Protein band densities were determined and normalized to β-actin band density. The fold change observed in specimens from the control group was set to 1.0 for relative quantification.

Measurement of Cervical cAMP Accumulation

Cervical tissue cAMP concentration was measured using the cAMP ELISA Kit (R&D Systems, Minneapolis, MN, USA). The sample was weighed immediately after it was collected, homogenized in 10 volumes of ice-cold 5% trichloroacetic acid, and centrifuged at 12,000 rpm for 10 min. The supernatant was extracted with three volumes of water-saturated ether. After drying, the extracts were stored at −80°C until the cAMP assay. The tissue cAMP level was expressed in pmol/mg tissue.

Statistical Analysis

All experiments were conducted on at least six animals. All data were expressed as mean ± standard error of the mean. One-way ANOVA was used to evaluate the results; multiple group mean values were compared using the Bonferroni post hoc test. Algorithms in GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA) were used for all analyses.

Results

Changes in Cervical Resistance in Different Groups

The animal study design and timeline are presented in Figure 1A. To confirm the effect of ropivacaine, which is commonly used for painless delivery, on cervical resistance, we first conducted an isolated cervical resistance test on rats in the control group. The obtained results were compared with those of the cervical resistance test on rats in the experimental group injected with intrathecal ropivacaine. The linear regression results of the load–elongation curve showed that the linear slope of the ropivacaine group was significantly lower than that of the control group (Figure 1B and D), indicating that the intrathecal injection of ropivacaine relaxes the cervix. To determine whether the cervical relaxation caused by ropivacaine is related to paracervical sympathetic nerve block, the cervical resistance of the ropivacaine + NE group was also measured. Results showed that the linear slope of the ropivacaine + NE group was significantly higher than that of the ropivacaine group (Figure 1C and D). This indicated that NE intrathecal injection had reversed the effect of ropivacaine in relaxing the cervix. The cervical resistance of each group is presented in Table 1.

Western Blotting and Detection of Cervical cAMP

To investigate the possible mechanism underlying the effect of ropivacaine and NE on cervical tension, we evaluated the cervical α_{2A}-AR levels in the late-pregnant rats. Western blotting showed that cervical α_{2A}-AR levels gradually increased over time in pregnant rats, with the expression peaking on day 22 (Figure 2A and B). Subsequently, we assessed the concentration of cAMP in the cervical tissues of rats from the three groups (Figure 2C). Results showed that the intrathecal injection of ropivacaine increased the cAMP concentration in the cervical tissue, whereas NE could reverse this effect of ropivacaine. The cervical cAMP concentration in each group is presented in Table 2.

Discussion

An innovative finding of this study was that after establishing the neuraxial anesthesia model, the physical experiment of elastic modulus accurately reflected the cervical tension in vitro, and on this basis, the molecular biology experiment further explained the possible causes of cervical tension changes.

The physical experiment method of cervical tissue tension has been introduced in detail in some studies.[16–18] Unlike these previous studies, when plotting the load–elongation curve, we averaged the initial load (m1i) and the load after 30 min of rest (m2j), which can better reflect the resistance of the cervical tissue. In fact, in the static stretching method, which is the classic method for Young's modulus measurement, it is necessary to record the stretched length when the load increases and decreases sequentially.[19] Furthermore, the method of linear regression can intuitively reflect the law of

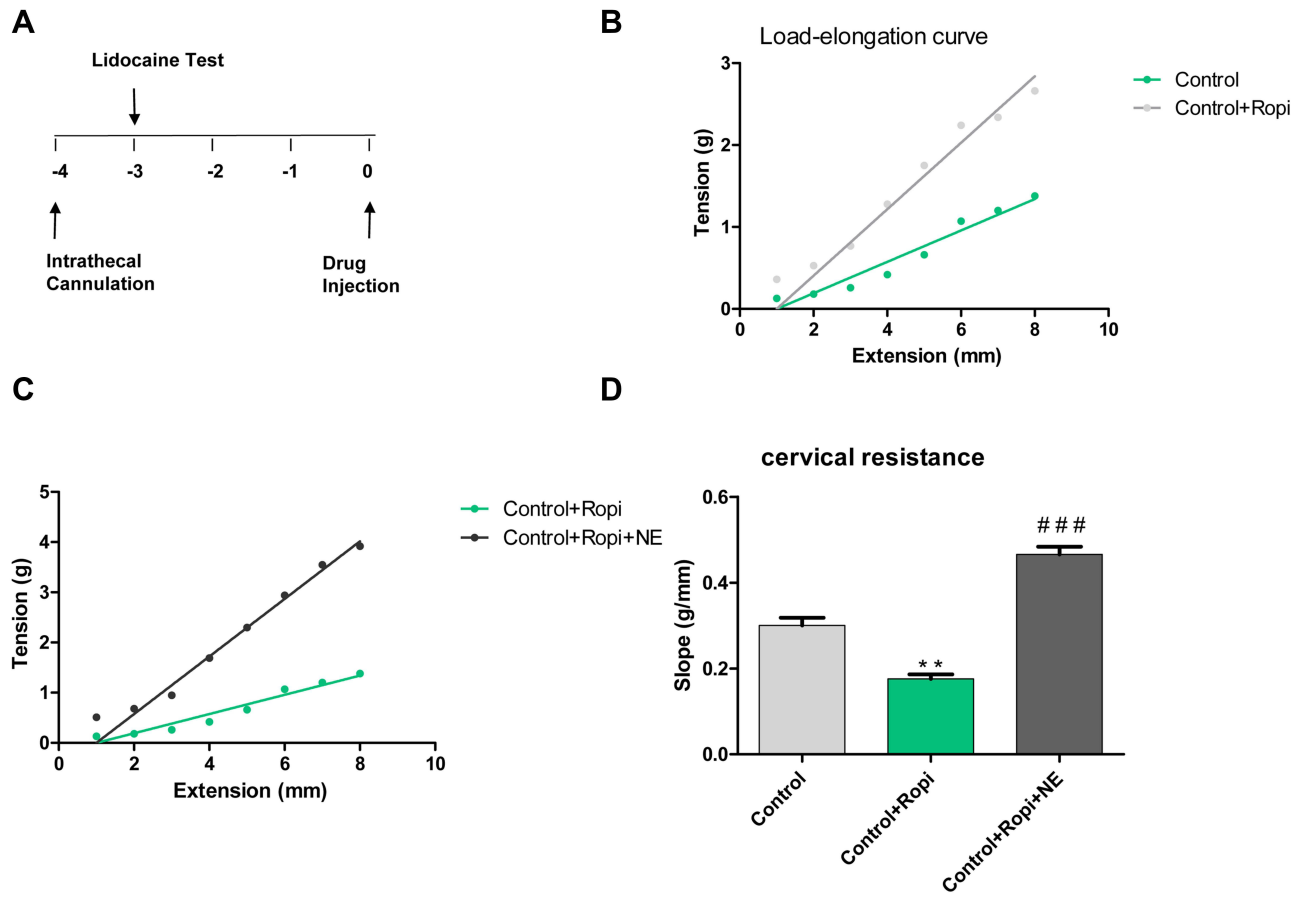

Figure 1 Norepinephrine (NE) reverses the decrease in cervical tone caused by ropivacaine (Ropi). (**A**) Schematic diagram of the study timeline. (**B** and **C**) One of the load–elongation linear regression results of each group, gray represents the test value of the control group; green represents the control + ropivacaine group; black represents the control + ropivacaine + NE group. The slope of the regression line reflects cervical tension. (**D**) Compared with the control group, the cervical resistance in the control + ropivacaine group was significantly reduced (**P < 0.01); compared with the control + ropivacaine group, the cervical resistance in the control + ropivacaine + NE group was significantly enhanced (###P < 0.001) (n = 6 for each group).

data distribution, and it is also one of the methods that can accurately calculate the elastic modulus of an object in addition to the method of successful difference.[20–22] In this study, we found that the intrathecal injection of 0.1% ropivacaine significantly reduced the cervical tension in rats at 22 days of pregnancy. This result can indicate that ropivacaine, a commonly used drug for painless delivery, can promote the progression of the first stage of labor at a common concentration of 0.1%. This result is consistent with several clinical observations.[1,2]

In molecular biology experiments, we evaluated the cervical levels of α_{2A}-AR, the main subtype of α2 adrenergic receptor. Western blotting showed that cervical α_{2A}-AR levels gradually increased over time, with the expression peaking on day 22. We also measured cAMP concentration in the cervical tissue. The enzyme-linked immunosorbent assay results

Table 1 Cervical Resistance in Each Group (g/mm)

Groups	N	Mean	Std. Error	95% Confidence Interval for Mean	
				Lower Bound	Upper Bound
Control	6	0.3002	0.0178	0.2547	0.3459
Control + Ropi	6	0.1760	0.0105	0.1490	0.2030
Control + Ropi + NE	6	0.4659	0.0178	0.4200	0.5117
Total	18	0.3140	0.0300	0.2507	0.3774

Abbreviations: Ropi, ropivacaine; NE, norepinephrine.

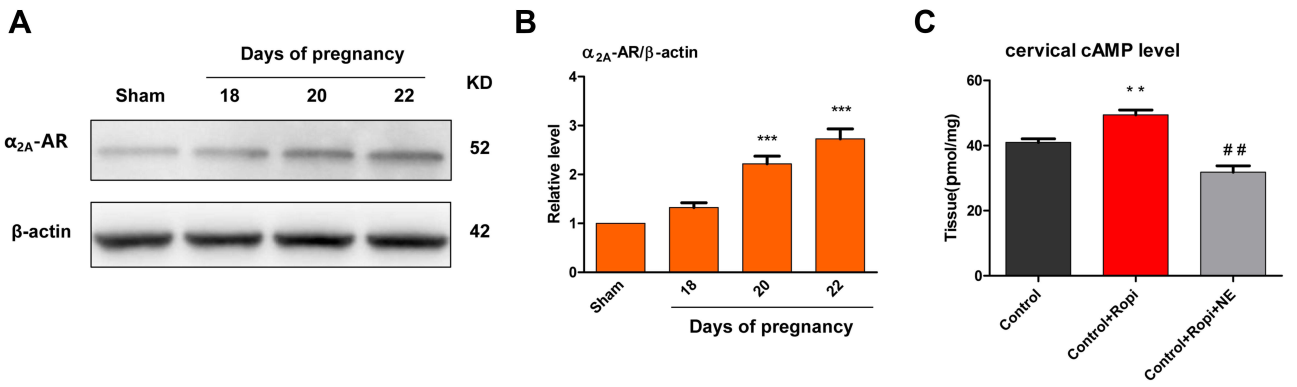

Figure 2 α2A adrenergic receptor (α_{2A}-AR) levels and cAMP concentration in the cervical tissue. (**A** and **B**) Cervical α_{2A}-AR levels gradually increased over time in pregnant rats, with the expression peaking on day 22 (***P < 0.001) (n = 4 for each group). (**C**) Compared with the control group, the cervical tissue cAMP concentration in the control + ropivacaine group was significantly increased (**P < 0.01); compared with the control + ropivacaine group, the cervical tissue cAMP concentration in the control + ropivacaine + NE group was significantly reduced (##P < 0.01) (n = 6 for each group).

showed that the intrathecal injection of ropivacaine increased the cAMP concentration in the cervical tissue of rats at 22 days of pregnancy. It is well known that cAMP can relax the uterine smooth muscle by inhibiting the activity of myosin light-chain kinase (MLCK).[23] In this study, NE reversed the effect of ropivacaine in increasing the cAMP concentration of the cervical tissue. In the cervical resistance test, NE also reversed the effect of ropivacaine in reducing cervical tension in late-pregnant rats. These results suggested that α2 adrenergic receptors are involved in the process of cervical contraction. The known adrenergic receptors are all G protein-coupled receptors. The activation of adrenergic receptors (which are approximately divided into α1, α2, and β receptors) requires the mediation of G protein to couple with the second messenger to produce a series of signal transduction and physiological effects.[24,25] In contrast to the effect of β receptors in increasing the concentration of cAMP in smooth muscle cells by coupling to Gs, α2 receptors couple with Gi, which can inhibit the activity of adenylate cyclase and reduce the synthesis of cAMP, thereby reducing MLCK activity inhibition with the result of smooth muscle cell contraction.[26–28] Some studies concerning the relationship between cervical tone and adrenergic receptors also support the abovementioned discussion.[29–32]

To summarize, ropivacaine, a commonly used drug for painless delivery, can significantly relax the cervical tension of late-pregnant rats by intrathecal injection at a common concentration of 0.1%. This finding provides meaningful theoretical support for the concept that neuraxial anesthesia shortens the first stage of labor. In other words, the intervention of neuraxial anesthesia for painless delivery need not wait until the cervical opening reaches 3–4 cm, but it should be provided as soon as possible when the parturient feels pain due to regular uterine contractions. Furthermore, α2 adrenergic receptors may play an important role in cervical contraction. Perhaps intrathecal injection of long-acting α2 receptor agonists could play a positive role in the treatment of preterm labor.

Of course, this study has several shortcomings. For instance, we did not analyze the effect of NE on the tension of isolated cervical tissue at different concentrations. Moreover, NE receptors were not investigated in detail, such as the contribution of α1 receptor-coupled Gq to smooth muscle cell contraction. Nonetheless, our study results provide

Table 2 Cervical cAMP Concentration in Each Group (pmol/mg Tissue)

Groups	N	Mean	Std. Error	95% Confidence Interval for Mean	
				Lower Bound	Upper Bound
Control	6	40.9817	1.11768	38.1086	43.8548
Control + Ropi	6	49.4117	1.51390	45.5201	53.3033
Control + Ropi + NE	6	31.8467	1.91680	26.9194	36.7740
Total	18	40.7467	1.93230	36.6699	44.8235

Abbreviations: Ropi, ropivacaine; NE, norepinephrine.

theoretical support for the necessity of early intervention by anesthesiologists for labor analgesia, which has positive social significance in improving the understanding of labor.

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Disclosure

The authors declare that they have no competing interests in this work.

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