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Y-27632, a Rho-Kinase Inhibitor, Inhibits Oxytocin-Stimulated Actin Reorganization in Human Myometrial Cells
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The mechanisms by which contractile agonists such as oxytocin regulate myometrial tone are incompletely understood. Smooth muscle contraction requires actin-myosin interaction. In vascular and airway smooth muscle, the actin cytoskeleton is dynamically regulated and is important for maintaining contraction. In these muscles actin reorganization uses a signaling pathway involving the monomeric G protein Rho A, leading to downstream activation of Rho-kinase and calcium sensitization (1). We therefore questioned whether oxytocin induces actin reorganization in human cultured myometrial cells and if so, we sought to identify the signaling pathway.

Cell cultures from human myometrium were plated on eight-well microscope slides and grown to near confluency. After serum deprivation, cells were treated with oxytocin (10 μ M) for 5 min, fixed with 3.7% paraformaldehyde, permeabilized, and F-actin and G-actin pools were stained with FITC-phalloidin and Texas Red-DNase I, respectively. In some experiments, either Tyrphostin A23 (150 μ M), a tyrosine kinase inhibitor, or Y-27632 (10 μ M), a Rho-kinase inhibitor (2), were applied before the addition of oxytocin. Fluorescence microscopy was performed and images analyzed using Metamorph software. After measuring fluorescence intensities the F- to G-actin ratios were calculated as an indicator of F-actin polymerization. Statistical analysis was performed using paired t-test. A p-value < 0.05 was considered significant.

Pretreatment with oxytocin increased the F/G-ratio from 2.3 ± 0.2 to 3.2 ± 0.2 ($p=0.001$), which was partially inhibited by Tyrphostin A23 (3.1 ± 0.3 vs. 2.57 ± 0.2 , $p=0.03$), and completely inhibited by Y-27632 (3.2 ± 0.2 vs. 2.0 ± 0.1 , $p=0.0003$).

Oxytocin induces a reorganization of the actin cytoskeleton in human myometrial cells via a pathway involving Rho-kinase. If this pathway plays an important role in modulating uterine tone, Rho A and/or Rho-kinase are potential target proteins for uterine relaxation.

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References: 1. Science 273:245-248, 1996 2. Nature 389:990-994, 1997

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Use of umbilical flow velocimetry in the assessment of the pathogenesis of fetal bradycardia following combined spinal epidural analgesia in parturients.

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Introduction: The administration of spinal opioids as part of the combined spinal-epidural (CSE) technique for labor analgesia may be associated with transient changes in fetal heart rate (FHR). It has been suggested that uterine hyperstimulation associated with an imbalance of endogenous catecholamines may be responsible for this phenomenon. The purpose of this study was to establish the role of uteroplacental blood flow and uterine tone in the pathogenesis of fetal bradycardia following CSE using Doppler velocimetry and uterine tonometry.

Methods: Following IRB approval and after obtaining informed consent, a prospective evaluation of maternal, fetal and uteroplacental parameters was performed in 50 parturients having a CSE. All patients received intrathecal sufentanil 10 mcg as part of their CSE technique. Baseline FHR was measured before CSE and at 5, 10, 20 and 30 minutes following spinal injection. Maternal heart rate, intrauterine pressure, blood pressure, SpO_2 , FHR, fetal systolic/diastolic (S/D) ratio, and pulsatility index (PI) were assessed at similar timepoints.

Results: Of the 50 parturients evaluated, 2 (4%) developed fetal bradycardia following administration of intrathecal sufentanil. There were no significant differences in measured parameters at any time between parturients whose fetuses developed fetal bradycardia and those whose fetuses did not. In addition, there was no evidence of decreased uteroplacental perfusion or tetanic uterine contraction in any patient. *In-utero* resuscitation including the administration of terbutaline or nitroglycerin was successful in the two patients who developed fetal bradycardia, despite unaltered PI and SD measurements and neither required cesarean delivery.

Conclusions: These preliminary findings demonstrate that decreased uteroplacental blood flow and tetanic uterine contractions may not always be the cause of fetal bradycardia following administration of intrathecal opioids. Rather, other mechanisms, including supraspinal opioid effects, fetal sympathetic alterations, or systemic fetal absorption may also contribute. With the reduced doses of spinal sufentanil currently being used, it is possible that the incidence of this complication will decrease.

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Effect of Posture Prior to Spinal Anesthesia for Cesarean Section on Maternal Angiotensin II, Aldosterone, and Blood Pressure

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Introduction: Hypotension occurs nearly universally after spinal anesthesia for cesarean section, but the mechanisms are not fully understood. Angiotensin II (AII) may be an important mediator maintaining BP during regional anesthesia (1). Posture affects endogenous release of AII (2). We hypothesized that standing and ambulation would reduce hypotension by increasing AII release.

Methods: With IRB approval 45 consenting healthy term parturients for C/S were randomly assigned to stand or remain supine with uterine displacement for 15 min prior to spinal anesthesia with bupivacaine/fentanyl. All patients received 10 ml/kg LR prior to induction, and 10 mg ephedrine was given by an anesthesiologist blinded to pre-block posture for SBP <100 or >20% less than baseline. Blood was analyzed for AII and aldosterone (ALD) prior to fluid bolus, after the period of standing or lying, and 20 min after the spinal was initiated. BP was recorded every minute from spinal induction to delivery.

Results: Patient demographics (height, weight, parity, age) did not differ between the groups. Blood pressure declined more in the supine group than the standing group (48 ± 23 vs. 31 ± 13 mm Hg, $P=.0027$) and the nadir SBP was lower in the supine group (82 ± 14 vs. 91 ± 11 mm Hg, $P=.03$). The change in ALD correlated with the SBP nadir ($R^2=.19$, $P=.08$). The pre-spinal (post-standing/lying) ALD, but not AII, was higher in the standing group (56.6 ± 24 vs. 28.7 ± 7.4 ng/dL, $P=.006$). However, neither absolute nor relative pre-spinal AII or ALD changes differed significantly between groups. Post-spinal AII and ALD did not differ between groups, but post-spinal ALD correlated inversely with the nadir of BP ($R^2=.263$, $P=.04$).

Conclusion: A brief period of standing prior to induction improves maternal BP during spinal anesthesia. Higher ALD in patients who stand may reflect postural release of the very short half-lived AII. Conversely, the higher post-spinal ALD in patients who experienced lower nadir SBP may reflect a compensatory response to hypotension. The relationship between the hormonal response to pre-block posture and hemodynamics during spinal anesthesia remains uncertain.

Refs: 1. Anesthesiology 1994; 80:1000-7. 2. J Lab Clin Med 1975; 86:754-63.

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The Minimum Local Analgesic Dose (MLAD) Of Intrathecal Bupivacaine In Labor And The Effect Of Intrathecal Fentanyl

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Introduction: Although various intrathecal local anesthetic/opioid combinations are used for CSE analgesia in labor, little is known regarding the individual contribution of these drugs to the overall efficacy of analgesia. We used a clinical model, originally applied to estimate the minimum local analgesic concentration (MLAC) of epidural local anesthetics¹ to determine the MLAD of intrathecal bupivacaine and the dose sparing effect of fentanyl.

Methods: Following ethics committee approval, 120 women receiving CSE analgesia for labor, at 2-6 cm cervical dilatation, were randomized into 4 groups to receive bupivacaine alone (Bup) or with 5, 15 or 25 μ g fentanyl (F) using a double blinded, up down sequential allocation technique. Analgesia was assessed with a 100mm VAPS at 5 minute intervals for 15 minutes after intrathecal injection. An effective dose, defined as a VAPS of 10mm or less within 15 minutes of injection, directed a decrement of 0.25mg bupivacaine for the next patient within that group. An ineffective dose directed a 0.25mg bupivacaine increase. MLAD was estimated using the method of Dixon and Massey.

Results: Obstetric and patient characteristics were similar in the groups. Although there was a significant reduction in MLAD for all Bup-F groups compared to the Bup control group, MLAD did not differ significantly between the Bup-F groups

Group (N=30)	MLAD*(mg) (95% CI)	Duration (mins) Mean (SD)	Pruritus (%)
Bup Control	1.99 (1.71,2.27)	43 (19)	0
Bup+F 5 μ g	0.69 (0.35,1.02)	56 (17)	40
Bup+F 15 μ g	0.71 (0.00,1.53)	68 (33)	60
Bup+F 25 μ g	0.85 (0.58,1.13)	77 (26)	73

[* Dunn's post hoc test $P<0.001$ for Bup vs Bup-F groups]

There was a dose dependent increase in pruritus and duration of spinal analgesia with fentanyl dosage (Chi square trend and Cuzick's trend respectively, $P<0.0001$). There were no significant differences in motor block or analgesic onset time.

Conclusion: The addition of intrathecal fentanyl 5 μ g offers a similar significant ($P<0.001$) dose sparing effect as 15 and 25 μ g but with less pruritus.

References: 1. Columb MO, Lyons G. Anesthesia & Analgesia 1995; 81: 833-7.