# LOCAL INHIBITION OF MYOMETRIAL ACTIVITY: A POSSIBLE NEW METHOD FOR THE PREVENTION AND TREATMENT OF PRETERM LABOR

by

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A THESIS

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APPROVED: [redacted]

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Preterm birth due to preterm labor is the leading cause of infant death in the United States[1]. As in normal delivery, preterm labor involves strong, rhythmic and coordinated uterine contractions. Inhibition of such contractions is a pre-requisite for preterm labor prevention. This study introduces a novel method of uterine contraction inhibition based on local drug administration. Drugs studied included the local anesthetics ropivacaine, procaine, and lidocaine, and the anti-inflammatory indomethacin. These drugs were tested in vitro for their ability to inhibit contractions of isolated uterine tissue strips from pregnant wistar rats. Both the local anesthetics and indomethacin were effective in completely eliminating myometrial contractions at reproducible concentrations. These results show that local administration of these drugs in vivo may prove clinically useful in the prevention and treatment of preterm labor.

## RESEARCH NOTE

The research for this thesis was conducted in Boston, Massachusetts with Dr. Dario Fauza, a pediatric surgeon and Fellow at the Harvard Center for Minimally Invasive Surgery at Harvard Medical School, and Dr. Timothy Maher, Professor of Pharmaceutical Sciences at the Massachusetts College of Pharmacy and Health Sciences. The laboratory facilities and resources of the Massachusetts College of Pharmacy and Health Sciences were generously provided by Dr. Maher to conduct all experimentation presented here.

# ACKNOWLEDGMENTS

The author expresses sincere appreciation to the following people: Dr. Dario Fauza and Dr. Timothy Maher, who gave me the opportunity, resources, and support to do this research. Dr. V. Pat Lombardi, and Dr. Frances Cogan, who have given me help here at the U of O. My best friend Bill Bankhead and my awesome roommate Melissa Lambright, who have kept me sane, helped me enter last-minute data, and edited in the wee hours. My family, who have been so supportive, and also fed me, clothed me, and provided great help with data entry. Ben Scarlet, who provided much needed mathematical and computer assistance. And, of course, all the other people at HCMIS and MCP who provided help during my time in Boston. Thank you all!

# DEDICATION

This thesis is dedicated to the rats.

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# CHAPTER 1

# INTRODUCTION

Premature birth is the leading cause of infant death in the United States[2]. Premature birth is the result of preterm labor, a process which is poorly understood. Current treatments for preterm labor focus on administration of drugs which inhibit uterine activity systemically to the mother. The current approach has limited effectiveness and a high incidence of side-effects. This study introduces a new approach to the prevention of preterm labor. Rather than apply drugs systemically to the mother, drugs could be applied locally to the uterine (myometrial) tissue to prevent contraction. To test this hypothesis, several drugs were tested *in vitro* for their ability to prevent contraction in the isolated myometrial tissue of pregnant rats. These drugs included the local anesthetics ropivacaine, procaine, and lidocaine, as well as the anti-inflammatory indomethacin. All of the above drugs were effective in completely inhibiting myometrial activity at reproducible concentrations. These results show that local application of such drugs may prove clinically useful in the prevention and treatment of preterm labor.

## The Process of Labor

Labor is a process which is poorly understood. We know that as a normal pregnancy progresses, the uterine muscle becomes progressively more excitable[8]. Slow, weak uterine contractions (Braxton-Hicks contractions) occur periodically throughout a normal pregnancy and become progressively stronger toward the end of pregnancy. Unknown factors then cause exceptionally strong, rhythmic labor contractions to begin. Labor contractions first stretch the cervix, then eventually push the fetus through the birth canal to delivery[8]. In a normal pregnancy, this process of labor occurs after about 40 weeks of pregnancy.

Two factors which may contribute to the increased uterine excitability throughout pregnancy are progressive hormonal changes and increasing mechanical stresses on the uterus[8], though the exact causes are unknown. Hormonal effects include an increasing ratio of estrogens to progesterone, increased uterine sensitivity to oxytocin, and increased levels of oxytocin during labor. Mechanical factors include stretching of the uterine musculature and the stretch or irritation of the cervix. Stretching of the cervix is also known to cause a neurogenic response which leads to increases in oxytocin secretion[8].

## Preterm Labor and its Prevention

Preterm labor is defined as labor which commences after the 20$^{th}$ week but before the 37th week of gestation[6]. Delivery before the 20$^{th}$ week is termed a miscarriage. Without intervention, preterm labor usually leads to premature delivery. It is estimated that 8 percent of births occur prematurely due to premature onset of labor[6].

The mechanisms underlying the inducement of preterm labor have yet to be determined. Considering that the processes underlying the initiation of normal labor are poorly understood, the causes of preterm labor are even more of a mystery. Many risk factors for preterm labor are known, including poor maternal health, congenital abnormalities of the fetus, and both surgical and non-surgical trauma[6]. In the majority of cases, however, the exact cause of premature labor cannot be clearly identified.

Premature birth presents numerous severe complications for the infant; most significant among these is inadequate development of the lungs[7]. Complications often are so severe that they lead to the death of a premature infant. In fact, about 75 percent of perinatal deaths are due to complications associated with prematurity[6], making premature birth the leading cause of infant death in the United States[2]. Those infants who do survive prematurity often have lasting developmental problems and disabilities. Freeman and Pescar have said that "Although great strides have been made in saving many premature babies and increasing the quality of life for countless others, the real answer to the problem of prematurity is the prevention of preterm labor (225)".

Not only is preterm labor a serious problem in general, but it is also the foremost limiting factor in the development of fetal surgery. Surgery conducted on a fetus while still in the womb may help correct birth defects in neonates. Development of such technique shows extraordinary promise for improving the lives of infants and children with developmental abnormalities. One severe and limiting side effect of prenatal surgery, however, is the inducement of preterm

labor post-operatively, which often leads to premature birth[9]. This inducement of preterm labor is the foremost limiting factor to prenatal therapeutic interventions, especially fetal surgery. A recent review suggested that "preterm labor is to the infantile field of fetal surgery what rejection was to the field of transplantation[5]". If preterm labor were eliminated, it could prevent the deaths of thousands of infants every year, plus allow the expansion of a field of surgery which could improve their lives.

Current treatments for preterm labor focus on the administration of certain drugs, known as tocolytic agents, which inhibit the ability of uterine muscle to contract. Currently, these drugs are applied systemically to the mother. Such drugs include β-adrenergic sympathomimetic agents, magnesium sulfate infusion, prostaglandin synthesis inhibitors, calcium channel blockers, and nitric oxide donors[6,12]. Bed rest may also be effective in some cases. The commonly used sympathomimetic agents Ritrodrine and Terbutaline have the highest success rate, around 70 to 80 percent[12]. These drugs, however, may induce maternal tachycardia and hypotension, as well as fetal tachycardia[12]. Both sympathomimetic agents and magnesium sulfate infusion carry a threat of pulmonary edema, though the latter treatment has fewer side effects overall[6]. Prostaglandin synthesis inhibitors, such as indomethacin, also have maternal side effects, and they are rarely used because they have been associated with potentially serious fetal problems[6]. Calcium channel blockers show limited effectiveness, and they carry the threat of maternal and fetal hypotension.

Finally, use of nitric oxide donors presents an increased risk of fetal intra-cranial hemorrhage.

## A New Approach

Systemic administration of the drugs described above has limited effectiveness and a high incidence of potentially serious side effects. The research presented in this thesis is the first step toward a possible new approach to the prevention and treatment of preterm labor. This new approach could potentially be safer and more effective than the treatments described above. Rather than treat preterm labor with drugs given systemically to the mother, drugs could be applied directly to the uterine muscle tissue. Local application of drugs which inhibit uterine contraction could prevent or eliminate labor contractions. If contractions are eliminated, labor is halted and delivery cannot occur.

This approach has some potential benefits over systemic treatment. With local application, the drug should be largely confined to the area to which it is applied. Drug concentrations outside of the local area of application would be much lower than those in the local area. With this approach, a high effective drug concentration could be achieved in the uterine muscle while sparing the rest of the mother's body the effects of the same high concentration. Thus, drug concentrations could be achieved in the uterine muscle which could be toxic to the mother were they applied systemically. Maternal side effects can thus be minimized or avoided. Additionally, since drug concentrations in the uterine muscle could be higher, uterine contractions could be inhibited more effectively.

## Physiology of Smooth Muscle Contraction

There are three general types of muscle in the human body: skeletal muscle, cardiac muscle, and smooth muscle. Skeletal muscles contract voluntarily and enable bodily movement. Cardiac muscle is the specialized muscle of the heart. Smooth muscle contracts much more slowly than skeletal or cardiac muscle, but can sustain contractions for longer periods of time. Smooth muscle also operates involuntarily (without conscious input) to carry out basic functions in the body. For these reasons, smooth muscle is specialized for "squeezing" functions in the body, such as maintaining the correct amount of tension in blood vessels, facilitating movement of food along the digestive tract, and pushing a baby through the birth canal during labor. Accordingly, uterine muscle is composed of smooth muscle cells.

When resting, all muscle cells are electrically polarized. That is, the inside of the cell is negatively charged relative to the outside of the cell. This polarization is maintained by movement of ions (charged particles) across the cell membrane. One of these ions is the positively charged sodium atom, $Na^+$. When a muscle cell is resting, sodium ion concentration is higher on the outside of the cell than on the inside. When a muscle cell receives an impulse to contract, however, sodium ions move rapidly into the cell through sodium channels in the cell membrane. Since the sodium ions are positively charged, and the inside of the cell is usually negatively charged, the influx of sodium ions *depolarizes* the muscle cell. Depolarization produces an electrical impulse called an *action potential*. The action potential triggers release of calcium ions, $Ca^{2+}$,

from both the sarcoplasmic reticulum (a cellular organelle) and the extracellular fluid into the intracellular fluid through calcium channels. Calcium ion influx causes the muscle to contract. Following contraction, calcium and sodium ions are pumped back out of the intracellular fluid and the cell repolarizes.

## Pharmacology of Local Anesthetics

Local anesthetics are drugs commonly used to inhibit pain responses in a localized area. Local anesthetics work because they are sodium channel blockers[11]. When the channels which allows sodium to enter the cellular fluid are blocked, depolarization and action potentials are prevented. Sodium channel blockers are effective anesthetics because nerve cells cannot transmit stimuli without action potentials. In addition, without an action potential a muscle cell cannot contract. It is reasonable, therefore, to expect that if a local anesthetic is applied directly to uterine tissue, it will inhibit uterine contractions.

To test this hypothesis, three local anesthetics were tested for their ability to inhibit myometrial contractions. The drugs tested were procaine HCl, lidocaine HCl, and ropivacaine. Procaine is a short-acting anesthetic, lidocaine acts for an intermediate amount of time, and ropivacaine is a long-acting anesthetic[11]. Procaine is considered to be the prototype local anesthetic, and is used as the potency standard[10]. Therefore, the potency value assigned for procaine is one. Lidocaine is four times more potent than procaine, so it has a potency value of four. Ropivacaine has a potency value of sixteen. Ropivacaine is referred to as having the highest "therapeutic index" because with its high potency, the same

level of anesthesia can be achieved with a less toxic dose than with other local anesthetics.

Not only can we expect local anesthetics to inhibit uterine contractions, but a further possible benefit of their use arises from the fact that application of local anesthetics should prevent the transmission of stimuli, as described above. It is thought that stimulation of the uterus is a possible reason why fetal surgery often leads to the inducement of preterm labor. If anesthetics are applied locally, such stimuli cannot be transmitted, and a possible factor contributing to preterm labor is prevented. It should be noted, however, that regardless of the stimuli presented, labor still cannot occur if the muscle cannot contract.

## Pharmacology of Indomethacin

Indomethacin is a nonsteroidal anti-inflammatory drug, analgesic, and anti-pyretic. The above actions appear to be associated with inhibition of prostaglandin synthesis[1]. Prostaglandins are a group of chemically related molecules found throughout the tissues of the body. Among a myriad of other functions, prostaglandins are known to increase the amplitude and frequency of uterine contractions in pregnant women[1]. Administration of a prostaglandin synthesis inhibitor, then, may reduce the ability of the uterus to contract. Indeed, it is known that administration of indomethacin systemically late in pregnancy can prolong gestation by inhibiting uterine contractions[1]. Prostaglandins are also known to play a part in cervical dilation, another prerequisite of labor. As mentioned earlier, indomethacin has been used systemically as a treatment for preterm labor. Indomethacin is, however, currently contraindicated during

pregnancy due to possible fetal effects and the existence of better medications for preterm labor prevention[15,6]. These effects may be avoided, however, if the drug is applied locally rather than systemically. It is reasonable to expect that if indomethacin is effective at inhibiting uterine contractions when applied systemically, it should also be effective locally. To test this hypothesis, indomethacin was tested for its ability to inhibit myometrial contractions in vitro.

## A Glossary of Terms for the Layperson

abortion (scientific definition) – the termination of a pregnancy. Abortion can occur naturally without intervention.

agonist – something which promotes the activity of something else – the opposite of antagonist.

analgesic – an analgesic is a pain reliever

antagonist – something which inhibits the activity of something else

anti pyretic – a drug which reduces or eliminates fevers

basal – the base or normal amount of something

estrogen – a female hormone

force transducer – an instrument which measures pulling forces

gestation – the time period needed for a fetus to mature

*in situ* – Latin for in the natural or normal place

*in vitro* – Latin for in glass – commonly referred to as "test tube" experiments

*in vivo* – Latin for in a living being

IP, intraperitoneal – inside the lining of the abdominal cavity. An intraperitoneal injection is an abdominal injection

labor – the process by which a baby is naturally born

myometrium – the muscular tissue of the uterus

neurogenic – generated by the nervous system

nonsteroidal anti-inflammatory – (NSAID) a drug such as Advil or Aleve

outbred – an outbred strain is the opposite of an inbred strain. A strain that is outbred has been bred to maintain genetic variability.

oxytocin – a hormone involved in pregnancy, labor, and lactation

perinatal – near birth, can be just before or just after birth

progesterone – a female hormone involved in menstrual cycles and pregnancy

sympathomimetic – a drug which mimics the actions of sympathetic nervous stimulation

systemic, systemically – throughout the whole system (body)

tissue – a group of cells which performs a specific function

# CHAPTER 2

# MATERIALS AND METHODS

## The Experimental Model

While the potential beneficiaries of this research are humans, it is generally considered unethical (not to mention illegal) to conduct research on humans without substantial prior testing in a non-human model. In pharmaceutical research, the model is usually an animal. The animal model chosen should be consistent with those used by other researchers in the same or similar fields. When possible, *in vitro* (test-tube) experiments are conducted prior to *in vivo* (live animal) experiments in order to ensure scientific accuracy, reduce waste, and minimize impact on animals.

The model chosen for this research was the isolated myometrial tissue of the pregnant female wistar rat, studied *in vitro*. Wistar rats are a common outbred strain used for research, and the use of rat myometrial tissue *in vitro* is well established for experiments regarding contractility of uterine muscle.

The majority of preterm labor incidents in humans occur during the second or third trimester of pregnancy[2]. In order to mimic this time period in the rat model, tissues were harvested and studied on the approximate date of the $2^{nd}/3^{rd}$ trimester border. The rat has a gestational period of approximately 21 to 23 days, so this date corresponds to the $14^{th}$ day of gestation.

## A Note on Animal Research

It should be noted that the idea of using an animal for research is distasteful to many people, myself included. Careful thought must be given to the reasons why research is being conducted, and the potential benefits must be weighed against animal loss of life. I believe the use of animals was justified in the case of this research because the benefits could be great while the discomfort of the animals was very small. I, as well as the institutions involved, made every effort to minimize the discomfort of each animal to the greatest possible extent.

## The Experimental Apparatus

Setup of tissue samples *in vitro* allows researchers to study them in an apparatus where their activity can be precisely measured. Isolated biological samples – such as the myometrial tissue strips used in this experiment – can be kept alive for very long periods of time (several hours) outside the body if kept in the proper conditions. These conditions include a watery environment at biological temperature and biological pH (acidity/basicity level), an adequate oxygen supply, an energy (food) source, and appropriate levels of biological salts.

The four tissue bath apparatuses used in this experiment met all of the conditions needed to keep tissue samples alive (figure 1). The 10 mL tissue baths were filled with a biological DeJalons solution (see appendix) containing glucose ($C_6H_{12}O_6$) and the biological salts NaCl, KCl, $CaCl_2$, and $NaHCO_3$. The DeJalons solution was adjusted to a biological pH of 7.4 with the addition of

minute amounts of concentrated HCl. The solution was heated with an external water bath to a biological temperature of 32° C * and bubbled with a 95% oxygen/5% carbon dioxide mixture.

With four tissue baths available, four different uterine samples could be tested at one time. Each of the four tissue baths had a force transducer located above it. The force transducer measures tension force, such as that produced by contracting uterine muscle. The height of each force transducer could be adjusted so that a base level of tension could be applied to the tissues in the baths. Data from the force transducers was sent to and collected by a calibrated four channel Grass model polygraph machine. The polygraph machine then produced graphical datasheets of tension vs. time.

## Drug Preparation

The drugs procaine, lidocaine, and ropivacaine were available in bottled injectable solutions in pH adjusted saline. Indomethacin solution was made in the lab from oral indomethacin capsules and measured saline. All drug solutions were current and stored according to manufacturer's instructions. Drug solutions were diluted to desired concentration with DeJalons solution. Dilution measurements were carried out with 5 ml and 1 ml micropipets, and were mixed in the test tube by vibration.

## Experimental Procedures

Pregnant wistar rats were euthanized on the 14 day of gestation by a lethal IP (intraperitoneal) injection of pentobarbital (100 mg/Kg). Four samples of

* Standard Biological temperature is 37° C, but many tissues do better in solution at a lower temperature.

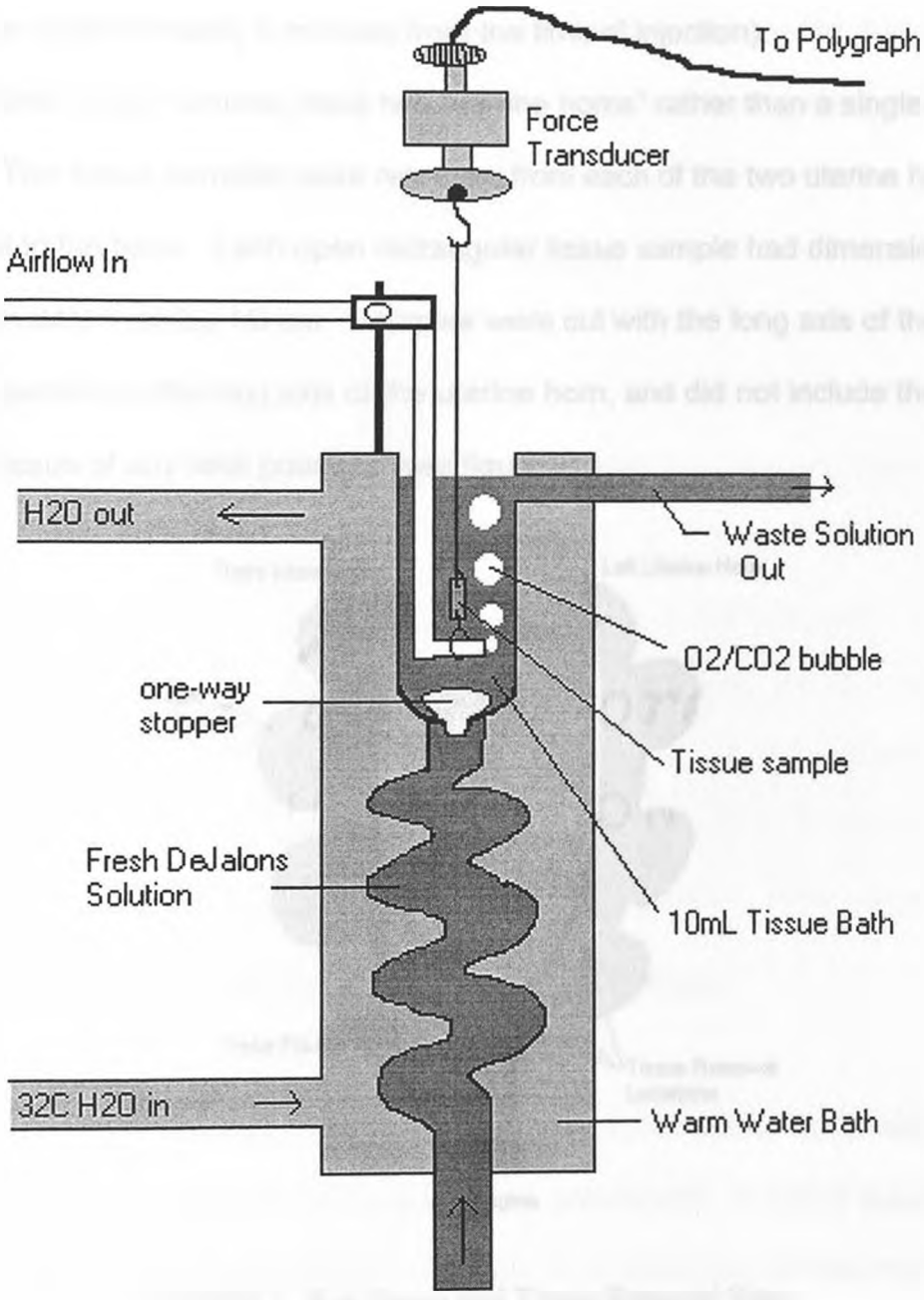


FIGURE 1. Schematic Diagram of a Tissue Bath Used in This Study.

myometrial tissue were surgically removed after the rat failed to exhibit a pain response (approximately 5 minutes from the time of injection).

Rats, unlike humans, have two "uterine horns" rather than a single uterine cavity. Two tissue samples were removed from each of the two uterine horns proximal to the base. Each open rectangular tissue sample had dimensions of approximately 1 cm by 1/3 cm. Samples were cut with the long axis of the sample parallel to the long axis of the uterine horn, and did not include the curved tissue of any fetal pouches (see figure 2).

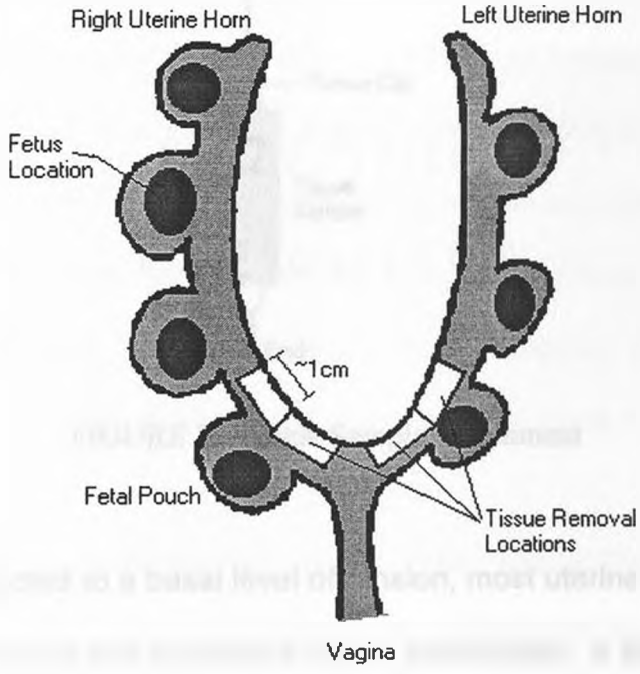


FIGURE 2. Rat Uterus and Tissue Removal Sites.

Tissue samples were placed in a petri dish with room-temperature biological solution after removal. Metal tissue clips were attached to the lengthwise ends of each tissue sample. Samples were then transferred to the

apparatus tissue baths, usually within 5 minutes of removal from the rat. In the tissue baths, one end of each sample was fixed with one tissue clip, while the other clip was tied to a thread attached to the force transducer (see figure 3). Each tissue sample was subjected to a basal tension of one gram.

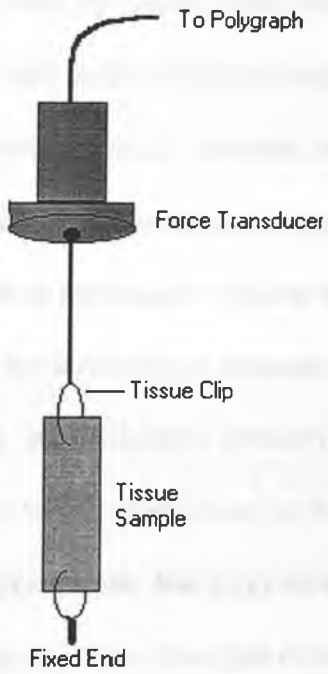


FIGURE 3. Tissue Sample Attachment

When subjected to a basal level of tension, most uterine tissue samples contract spontaneously and continue to do so periodically. A typical tissue sample under 1 gram basal tension contracts once every two to three minutes, achieving a maximum tension somewhere between 2 and 5 grams of tension. At first, these contractions can be erratic, so an equilibration period is needed to allow the contractions to become regular. The tissue samples in these experiments were allowed to equilibrate for 30 minutes. Once the contractions

became regular, a baseline of activity was recorded for 15 to 20 minutes. Samples that did not maintain regular phasic activity (consistent contraction amplitude and frequency deviating no more than 25%) during this period were discarded.

During equilibration (as well as those times in which no drugs were tested) the tissue baths were flushed with fresh biological solution every 15 minutes. Fresh solution ensures an optimal level of nutrients, salts, and pH[†].

Once a regular baseline of contractile activity was observed, drug dilutions were added to tissue baths with a micropipet (figure 4). Tension vs. time data for each drug dose was recorded for a minimum interval of 7 minutes. In "cumulative dose" experiments, an additional amount of drug was then added to bring the bath concentration up to a higher dose for the next data collection interval. In "discrete dose" experiments, the drug from a previous interval was removed from the bath by three or more changes of solution, after which a new drug dose was introduced. No data was recorded after three hours.

## Data Collection

The Grass polygraph machine produces data sheets (figure 5) which record tension data from the force transducer with respect to time. Four pens move in accordance with the tension measured by the four force transducers, and paper datasheets pass under the pens at a rate of .5cm per minute. The result is a data sheet which shows four dark wavy lines which correspond to data

[†] It should be noted, however, that since the volume of the bath (10 mL) was approximately 200 times greater than the volume of the tissue sample (~.05 ml), we can assume that it would take a very long time for the metabolism of the tissue sample to have a measurable effect on the makeup of the bath solution.

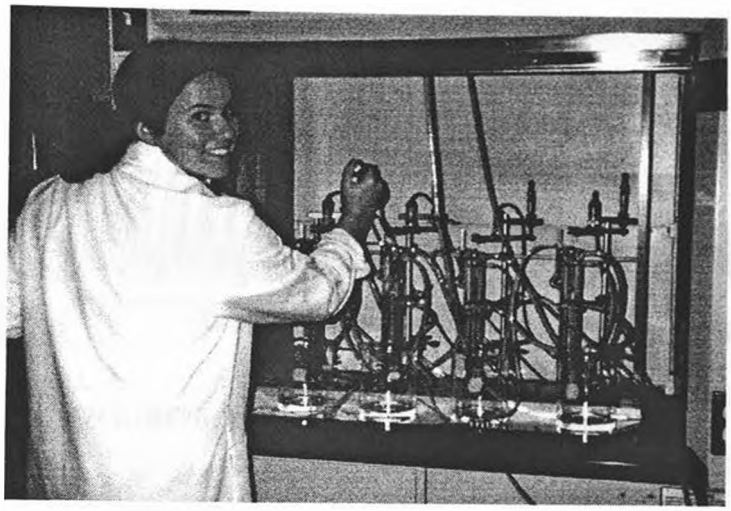

FIGURE 4. Adding drug to the 4 apparatuses.

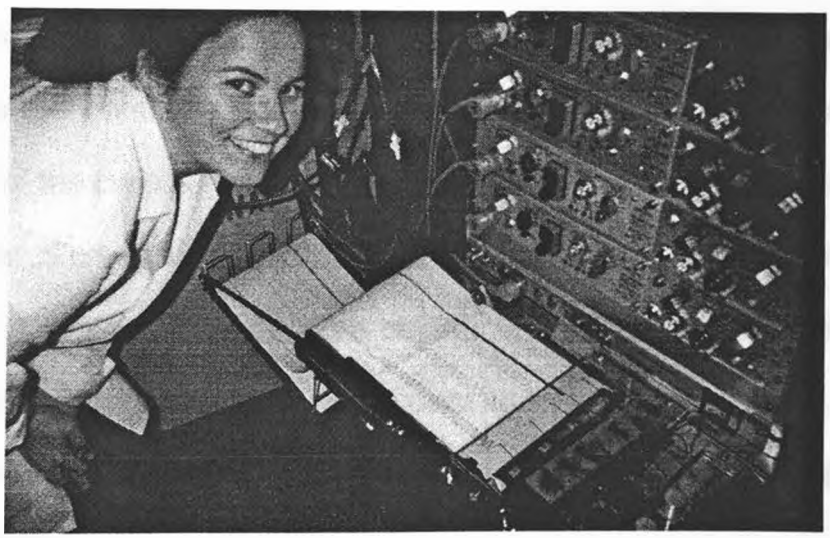

FIGURE 5. Grass polygraph machine producing a data sheet.

from the four separate tissue samples, plus one additional line which marks off the time. On such a datasheet, contractions of uterine muscle appear as peaks on the line (figure 6).

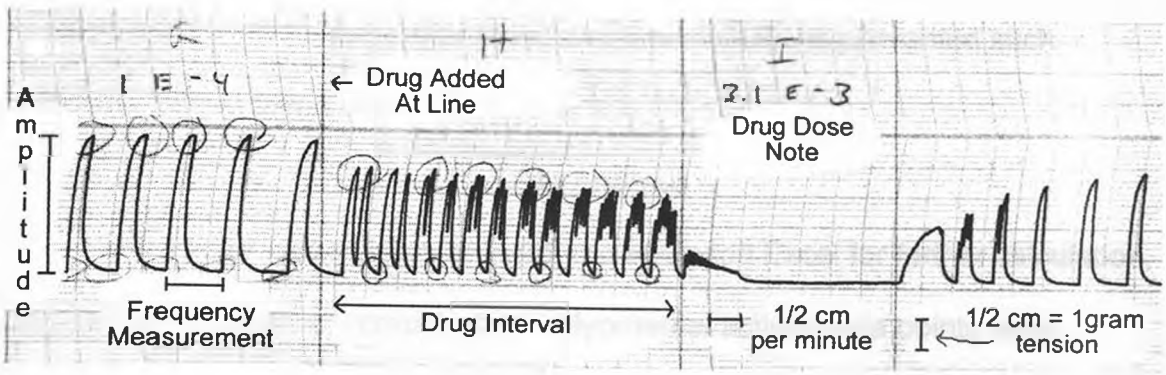


FIGURE 6: Portion of a data sheet - Annotated

The height of each peak, or "amplitude" corresponds to the maximum tension of the contraction. For these experiments the polygraph was adjusted so that .5cm in height corresponds to one gram of tension. The amplitude of a peak is a good measurement of contraction strength. Measuring the width of the peak allows calculation of the contraction frequency. On the data sheets for these experiments, .5 cm in width corresponds to one minute. Contraction frequency is a measure of how many contractions occur in one minute‡. Both amplitude and frequency data were collected for each data interval. In each interval, a minimum of three peaks and a maximum of six peaks were sampled and averaged for each data entry.

Measurement of both contraction amplitude and frequency presents an idea of the overall contraction strength in an interval. Ideally, however, the best measurement of overall contraction strength would have been gained through integration of the contraction peaks. Integration provides a measurement of the area under a curve, to which both amplitude and frequency contribute. Logistics

of integration from a paper data sheet and time limitations prevented such measurement in this study.

## Data Analysis

Data point values were entered into Microsoft Excel for further calculation, analysis and graphical interpretation. Myometrial activity data points were expressed as mean percentage deviations from the baseline for each drug concentration value. Statistical analysis was performed by Robin R. High of the U of Oregon Statistical Consulting Department. Statistical tests were done with PROC MIXED of the SAS system, a one way repeated measures analysis of variance. P values of 0.05 or less were considered significant.

‡ Usually frequency is a measure of cycles per second. In this study, frequency was recorded as cycles per minute because of the very long cycle period.

# CHAPTER 3

## RESULTS

### Ropivacaine

Seven experiments were run to test the effects of the local anesthetic ropivacaine on myometrial activity *in vitro*. In the seven experiments (consisting of seven rats) 21 tissue samples were tested with various concentrations of the drug. A portion of a ropivacaine data sheet is shown in figure 7.

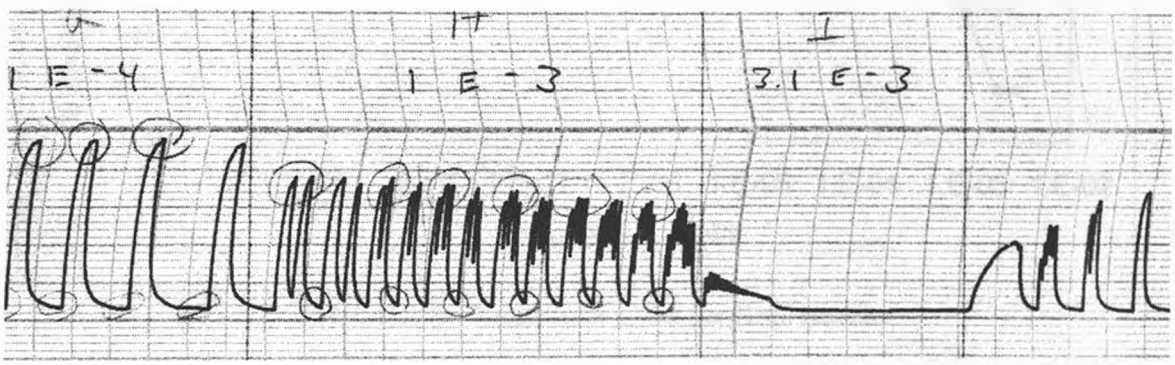


FIGURE 7: Response of Tissue Sample Treated With Ropivacaine.

For each drug concentration, values for contraction amplitude and frequency were measured as described in the methods section. Values for each drug concentration were then compared with the baseline values for each tissue sample. The mean percent change from baseline of contraction amplitude and frequency at each drug concentration tested is shown graphically in figures 8 and

9. Drug concentrations are shown on the X axis and are expressed as moles of drug per liter of solution (in scientific notation). The X axis is displayed on a logarithmic scale. The Y axis shows values for percent change from baseline. Data points represent the mean percent change from baseline at each drug concentration. Standard error bars are included for each data point.

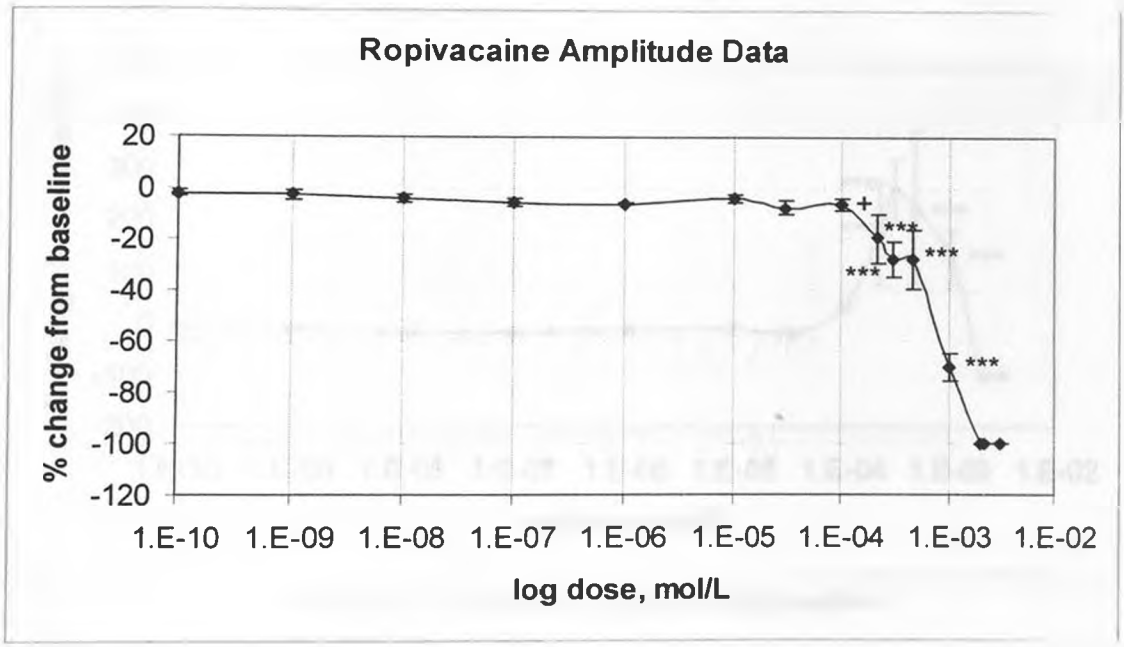


FIGURE 8: Amplitude Data for Ropivacaine

Symbols representing statistical significance should be read as follows:

| P<0.1 | P<0.05 | P<0.01 | P<0.001 |
|---|---|---|---|
| + | * | ** | *** |
| tendency | significant | more significant | most significant |

Results show that a ropivacaine concentration of 2.0E-03 mol/L was sufficient to completely eliminate uterine contractions. At this dose, both the amplitude of contractions and frequency of contractions were zero. As shown by the star

symbols in the above graph, at concentrations of 2.17E-04 mol/L (0.000217 mol/L) and greater, the amplitude values were statistically different than baseline. Drug concentrations from 2.17E-04 mol/L to 2.0E-03 mol/L show statistically significant depression of contraction amplitude.

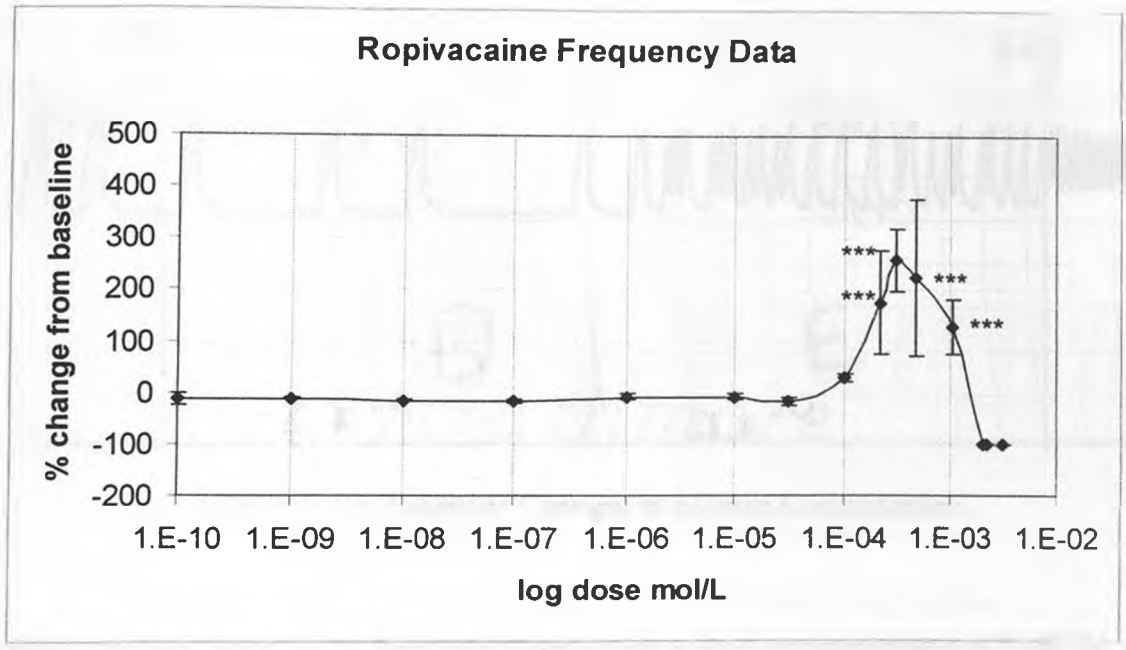


FIGURE 9: Frequency Data for Ropivacaine

| P<0.1 | P<0.05 | P<0.01 | P<0.001 |
|---|---|---|---|
| + | * | ** | *** |

As figure 9 shows, the frequency values were statistically different than baseline at a concentrations of 2.17E-04 mol/L and greater. It should be noted that the frequency *increases* significantly at these intermediate concentrations. However, the contraction frequency goes to zero at the higher concentration of 2.0E-03 mol/L.

## Response to a Drug Concentration Can Change Over Time

Figure 10 shows the unusual result that the response of tissue samples to a given drug concentration can change over time.

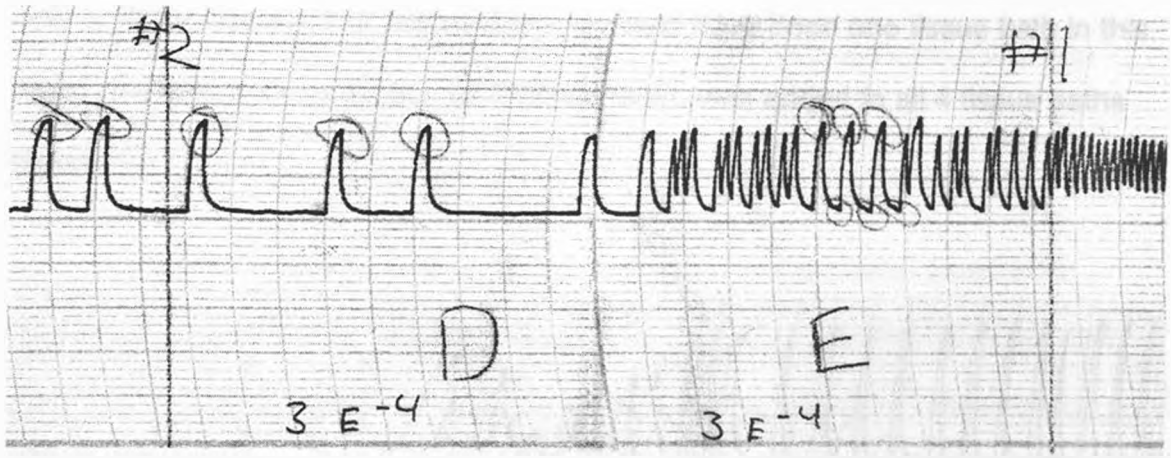


FIGURE 10: Response Changes at a Given Concentration.

Both interval D and E on the data sheet have a drug concentration of 3.0E-04 mol/L, yet the response is obviously different between D and E. About 9 minutes (.5 cm/min) after the drug has been added (vertical line labeled #2) contractions increase in frequency dramatically.

## Ropivacaine Can Induce Contractions

The experiments described above show that ropivacaine eliminates uterine contractions at a concentration of 2.0E-03 mol/L, significantly decreases the amplitude of uterine contractions at concentrations between 2.17E-04 mol/L and 2.0E-03 mol/L, and significantly increases contraction frequency at concentrations between 2.17E-04 mol/L and 2.0E-03 mol/L. A serendipitous

accident yielded the unexpected additional result that ropivacaine seems to induce contractions at an intermediate dose. An experiment was run in which only one tissue sample yielded a good baseline. The remaining three tissue samples had no good baseline and showed no activity after some initial irregular contractions – they had "flatlined" prematurely. Usually, drug dilutions are added only to those tissues that are usable – it would have been one tissue bath in this case. Instead, a drug dilution of 3.0E-04 mol/L was added to all 4 tissue baths (figure 11).

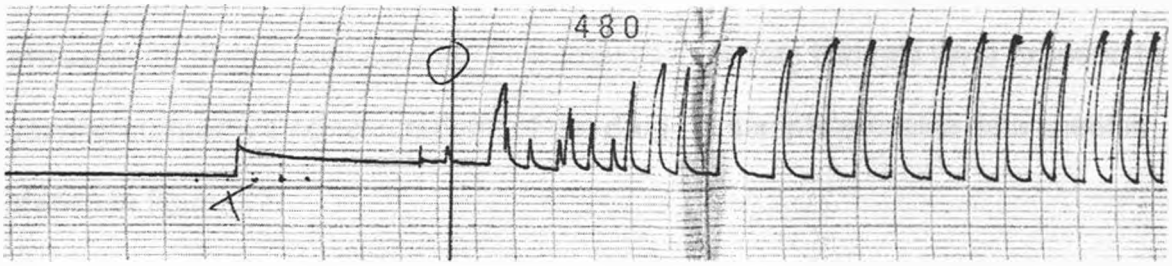


FIGURE 11: Contractions Begin After Addition of Ropivacaine.

The time of the drug addition is shown in the figure as a dark vertical line. While the one regularly contracting tissue sample (not shown) showed the typical response to the drug of decreased amplitude and increased frequency, the drug concentration caused the three tissue samples that had not been contracting to begin contracting. After several minutes these contractions became regular, arguably more regular than the contractions exhibited by the good-baseline tissue sample.

## Contractions Return after Ropivacaine is Removed

In all ropivacaine experiments run, uterine contractions returned as soon as ropivacaine was removed from the tissue baths by flushing with new solution. This effect can be seen at the end of the data sheet shown in figure 7. Drug is removed by flushing at the last vertical line after contractions have been eliminated. Contractions resume with levels of regularity comparable to that observed in the baseline.

## Procaine

Two experiments were run to test the effects of the local anesthetic procaine on myometrial activity. Five total tissues from two rats were exposed to varying cumulative concentrations of the drug. A sample procaine data sheet is shown in figure 12.

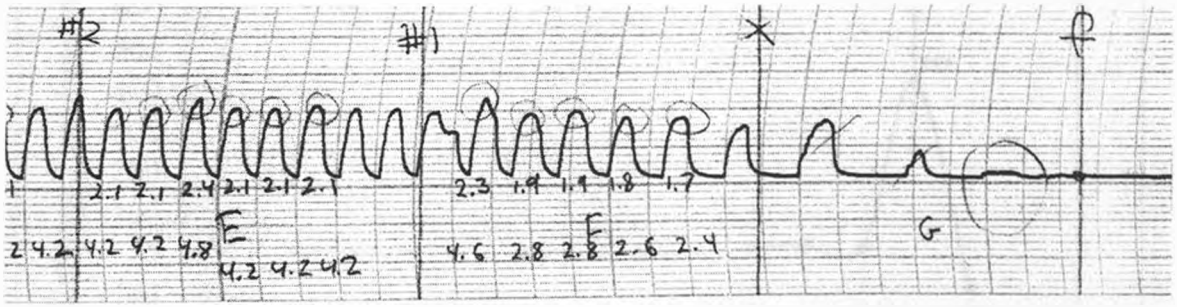


FIGURE 12: Response of Tissue Sample Treated With Procaine

Results for contraction amplitude (figure 13) and contraction frequency (figure 14) show that a procaine concentration of 1.0E-2 mol/L is sufficient to completely eliminate both.

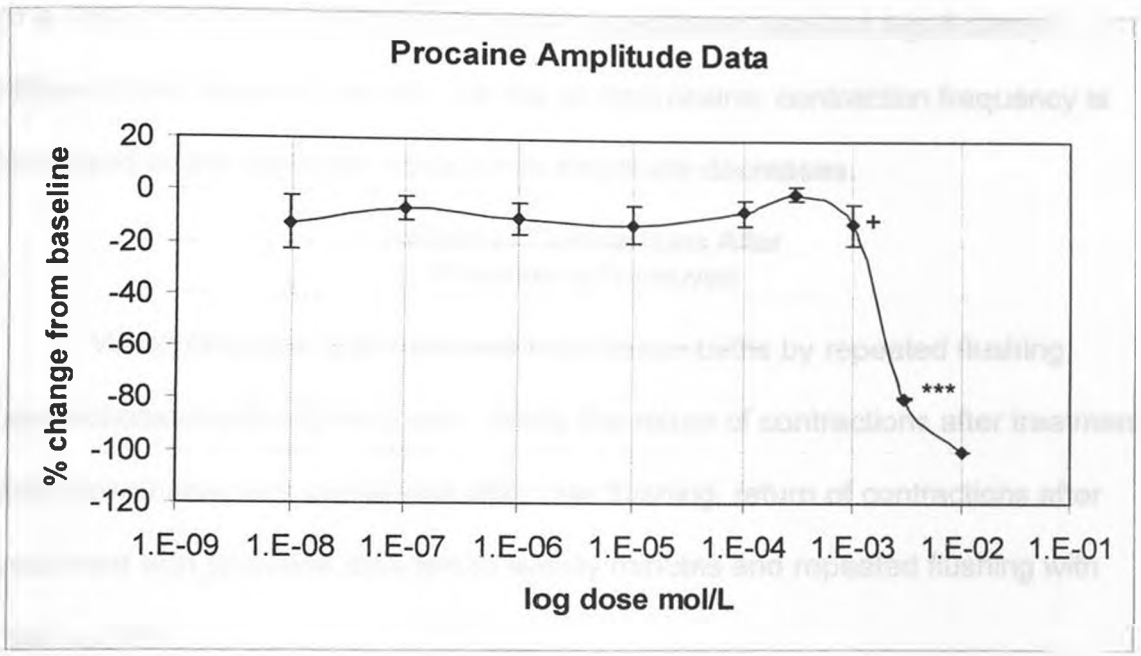


FIGURE 13: Amplitude Data for Procaine

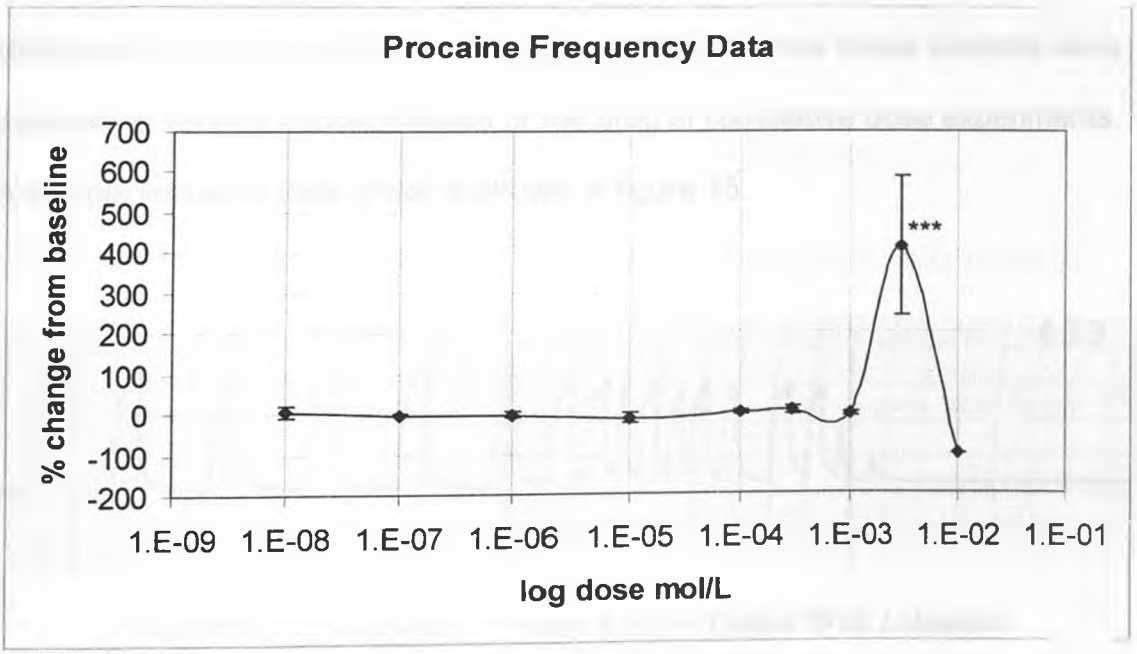


FIGURE 14: Frequency Data for Procaine

At a dose of 3.0E-03 mol/L amplitude and frequency data are significantly different than baseline values. Similar to ropivacaine, contraction frequency is increased above baseline levels while amplitude decreases.

Return of Contractions After
Procaine is Removed

When procaine was removed from tissue baths by repeated flushing, contractions eventually returned. While the return of contractions after treatment with ropivacaine was immediate after one flushing, return of contractions after treatment with procaine took ten to twenty minutes and repeated flushing with new solution.

## Lidocaine

Three experiments were run to test the effects of the local anesthetic lidocaine on uterine contractions in vitro. A total of twelve tissue samples were exposed to varying concentrations of the drug in cumulative dose experiments. A sample lidocaine data sheet is shown in figure 15.

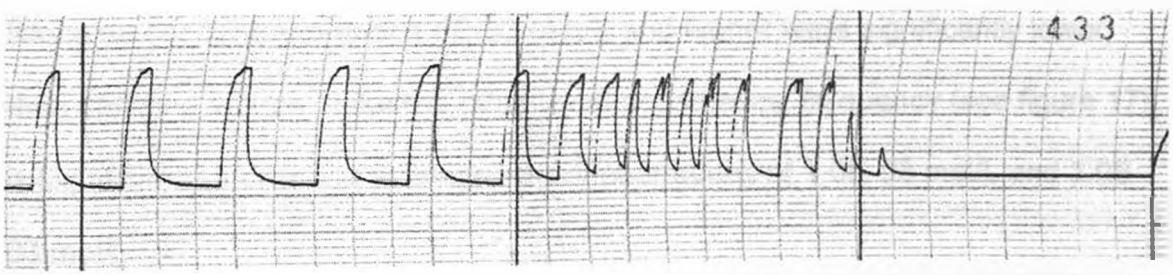


FIGURE 15: Response of Tissue Sample Treated With Lidocaine.

Results for contraction amplitude (figure 16) and frequency (figure 17) show that a drug concentration of 1.0E-2 mol/L was sufficient to eliminate contractions. A

concentration of only 6.0E-03 was sufficient to eliminate contractions in 75% of tissues tested at that dose, but the remaining 25% required the higher dose of 1.0E-02 mol/L.

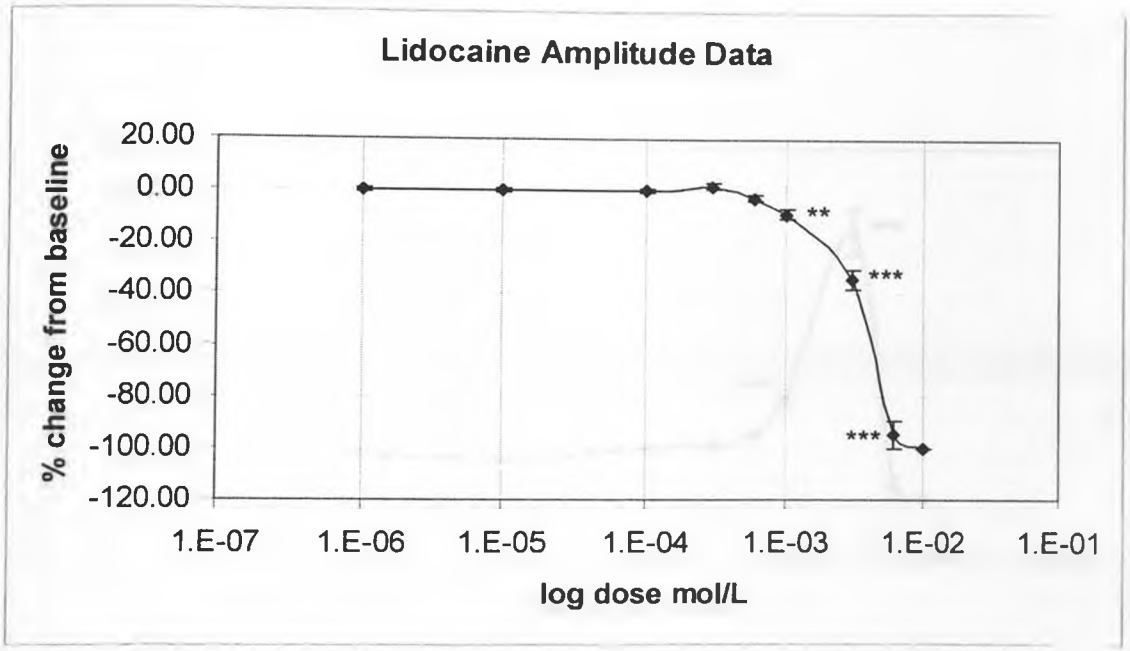


FIGURE 16: Amplitude Data for Lidocaine

As shown by the starred points in the above amplitude graph, data points at lidocaine concentrations of 1.0E-03 mol/L and higher were significantly lower than baseline values. Like the other local anesthetics, frequency (see figure 17) is significantly increased at lidocaine concentrations of 1.0E-03 mol/L and 3.0E-03 mol/L.

Return of Contractions After
Lidocaine is Removed

Like the results observed for ropivacaine, contractions return immediately after lidocaine is removed from the tissue bath solution by flushing with new

solution. This effect can be seen at the far right-hand side of figure 15, where a new contraction begins. The far right vertical line shows where the bath was flushed with new solution after contractions had been eliminated.

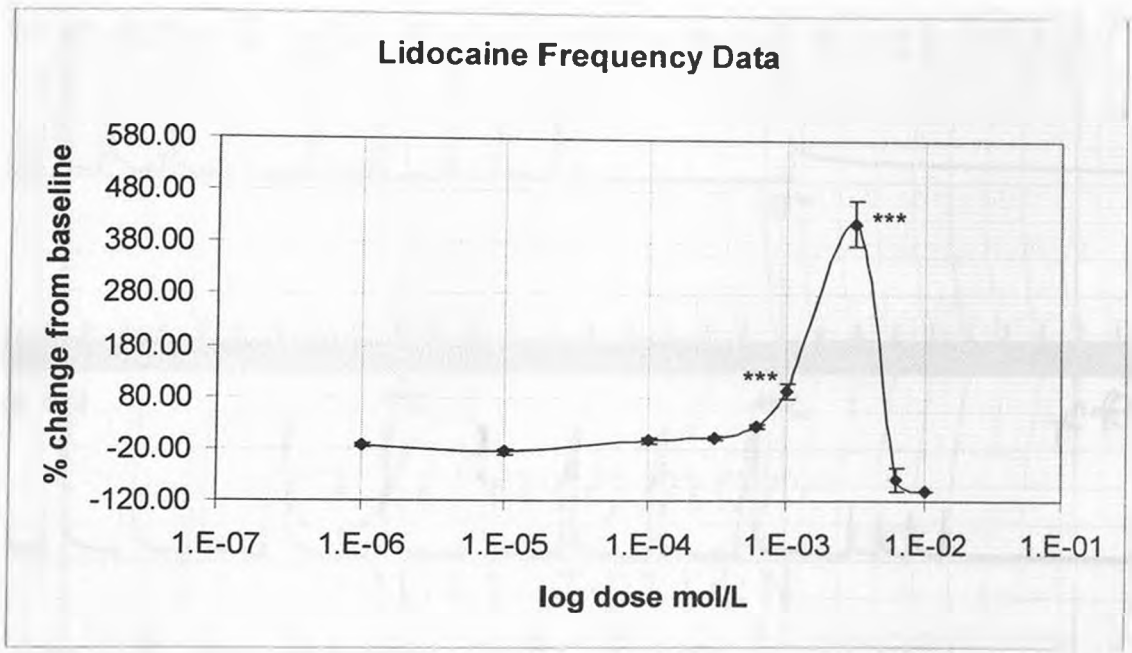


FIGURE 17: Frequency Data for Lidocaine

## Indomethacin

Nine experiments were run to test the effects of the anti-inflammatory indomethacin on uterine contractions. A total of 19 tissue samples were exposed to varying concentrations of indomethacin in both cumulative and discrete dose experiments. A sample indomethacin data sheet is shown in figure 18. The data from two tissue samples is shown in this example. Results show that a drug concentration of 1.0E-04 mol/L was sufficient to eliminate contractions in all samples, while a drug concentration of 1.0E-05 mol/L was sufficient in some.

This fact can be clearly seen in figure 18. Overall results for contraction amplitude (figure 19) demonstrate that contraction amplitude is significantly decreased at concentrations of 1.0E-05 mol/L and higher.

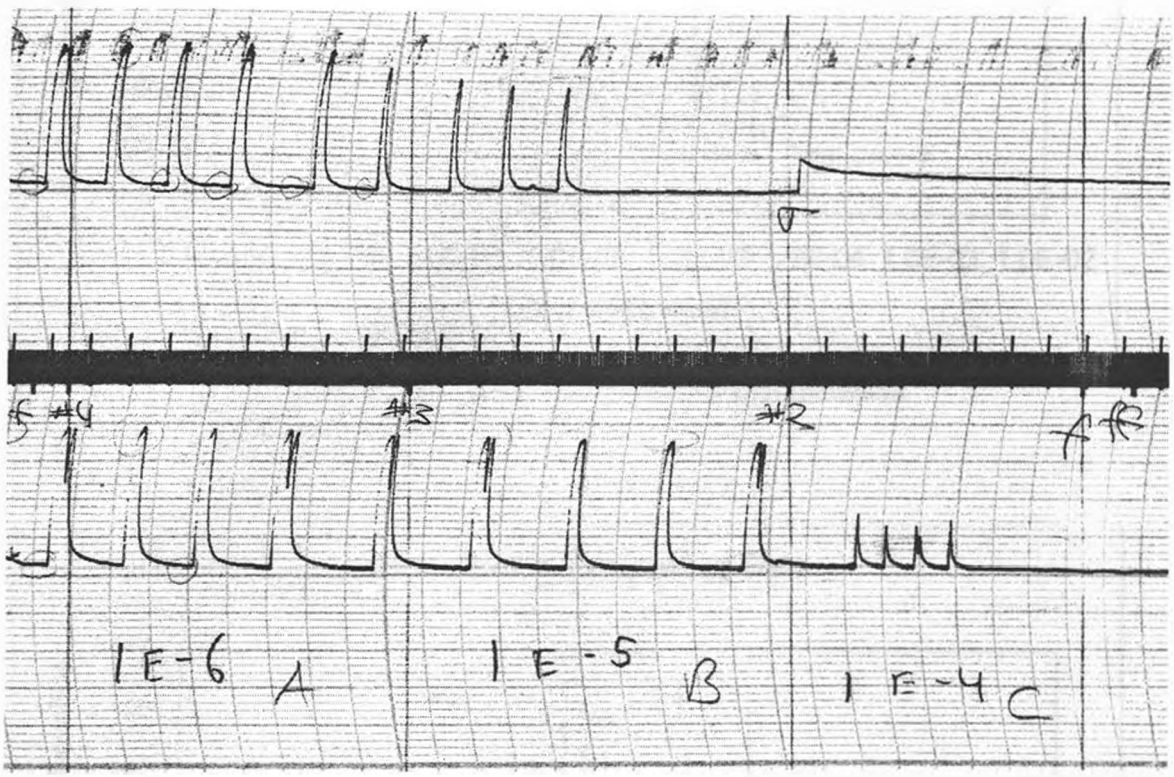


FIGURE 18: Response of Two Tissue Samples
to Treatment With Indomethacin.

Results for contractions frequency (figure 20) show a significant decrease at a drug concentration of 1.0E-05 mol/L. Strangely however, a significant decrease is not observed at the concentration of 3.0E-05 mol/L. This is probably due to the very large standard error value for this data point.

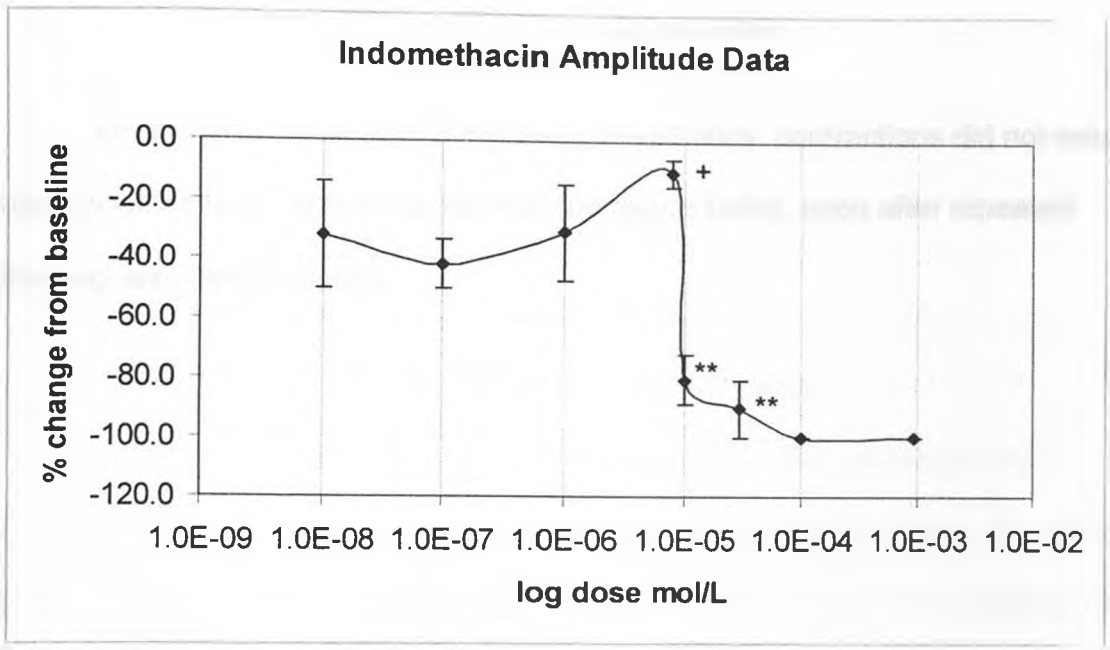


FIGURE 19: Amplitude Data for Indomethacin

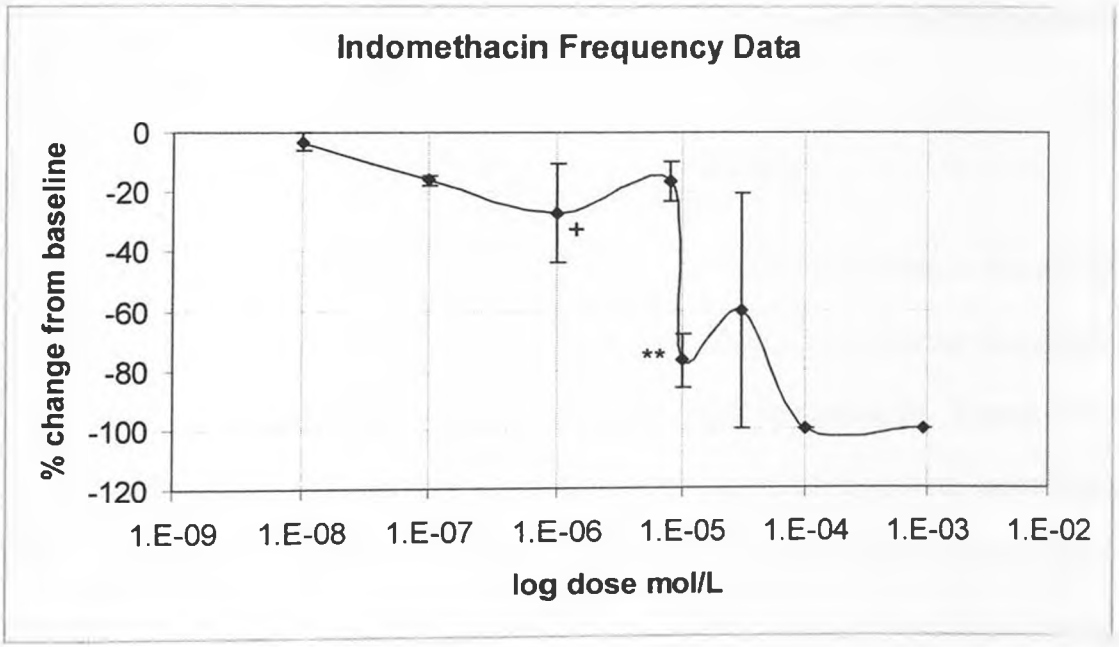


FIGURE 20: Frequency Data for Indomethacin

## Return of Contractions After Indomethacin is Removed

Unlike data observed for the local anesthetics, contractions did not return after indomethacin was removed from the tissue baths, even after repeated flushing with fresh solution.

# CHAPTER 4

# DISCUSSION

## Inhibition of Myometrial Activity

Results show that the drugs ropivacaine, procaine, lidocaine, and indomethacin can completely eliminate myometrial tissue activity in vitro. These drugs eliminate uterine contractions at concentrations of 2.0E-03, 1.0E-02, 1.0E-02, and 1.0E-04 mol/L respectively. This data supports the hypothesis that these drugs could be used to eliminate uterine contractions, and thus prevent preterm labor if applied to myometrial tissue in vivo. Further in vivo experimentation is warranted.

## Application for the Prevention of Preterm Labor

The hypothesis is that local application of the drugs tested in this study will prevent preterm labor by inhibiting uterine contractions. Of interest is a paper by Fauza, Berde, and Fishman published in 1999 (see reference 3). Fauza et al. demonstrated that local application of the drug bupivacaine (a local anesthetic) to the myometrial tissue prevented preterm labor after fetal surgery in a live leporine (rabbit) model. The study showed that application of a local anesthetic directly to the uterine muscle can prevent preterm labor, at least in rabbits. This provides reason to believe that the drugs tested in this study, especially the local anesthetics, should work as well.

There was, however, a high fetal mortality rate in the groups of the Fauza study which were treated with the drug, possibly due to transplacental transfer of the local anesthetic. This is, obviously, a serious problem, and similar effects seem likely with the local anesthetics tested in this study. Fauza et al. point out that the lipid solubility, and hence the placental transferability of local anesthetics can be manipulated, a possible solution to the problem.

A silver lining to the cloud of possible fetal mortality is the fact that in the Fauza study, no preterm labor was observed in the group with the high fetal mortality rate. A dead fetus is known to be a very strong stimulus toward the abortion of a pregnancy, so the fact that abortion was prevented by the local anesthetic is fairly impressive.

In addition to fetal mortality, potential problems with local application of drugs in vivo include the possibility of maternal and fetal side effects. Though the drugs are applied to a local area rather than systemically, a certain amount of drug will get into the bloodstream[10]. Once in the bloodstream, the local anesthetics can cause side effects such as neurotoxicity , hypotension, and cardiac effects[15]. Indomethacin can have undesired effects on the central nervous system in addition to gastrointestinal upset[15]. If these drugs cross the placenta, they can have similar undesired effects on the fetus.

The Fauza paper (see reference 3) is also of interest because of the novel vehicle used to deliver the drug. In the test group, the myometrium was injected with a suspension of biodegradable microspheres loaded with bupivacaine. These microspheres release the drug in a continuous, controlled manner. This

suspension has been shown to provide peripheral nerve blockade in rats for up to 5.5 days. Results show that this drug delivery system may also be appropriate for the drugs tested in this study.

## Agonist Effect of Local Anesthetics on Contraction Frequency.

As stated above, all of the drugs tested were effective at completely eliminating myometrial activity at reproducible concentrations. Clearly, at the higher concentrations these drugs are antagonists of myometrial activity. All of the local anesthetic drugs tested, which include ropivacaine, procaine, and lidocaine, significantly increase contraction frequency at concentrations just below those which eliminate contractions. This is known as an agonist effect. The local anesthetics then, are antagonists of contraction frequency at certain doses and agonists of contraction frequency at others.

This agonist effect was an unexpected result, and I cannot explain why it occurs. Considering the aim of this study was to eliminate or reduce contractions, an increase in contraction frequency is a potential concern. Thought must be given to the possible manifestations of this effect in the proposed eventual use of these drugs. Eventual use may include local application of these drugs in vivo, possibly with a slow-release microsphere system. If the method of drug application ensures that the drug concentration remains at or above the dose required to eliminate contractions, there should be no problem. If, however, drug application results in areas where the drug concentration is too low to completely inhibit contractions, but high enough to

have the agonist effect on contraction frequency, there could be a problem. In such areas contraction frequency could increase.

With local application, areas of differing concentration are a real possibility. Local anesthetics tend spread out from the area to which they are locally applied, meaning drug concentration will be highest at the point of application and progressively lower at peripheral locations[11]. If a concentration as high or higher than is needed to eliminate contractions is applied, at some point along the periphery of the application site the concentration will be that which has been shown to increase contraction frequency.

It should be noted that indomethacin, an anti-inflammatory rather than a local anesthetic, did not have an agonist effect on contraction frequency. The only significant effects observed for indomethacin were inhibition and elimination of both contraction amplitude and frequency. Use of indomethacin, therefore, could avoid potential problems with increased contraction frequency.

## Combined Effects of Amplitude and Frequency Changes

While increased contraction frequency is a potential problem with the use of local anesthetics, it may not be as serious as it first seems. This is because as contraction frequency increases at the agonist concentration, contraction amplitude decreases. In other words, even though the contractions are more frequent at intermediate doses, they are not as strong. This effect can be clearly seen in the portion of the ropivacaine data sheet shown below (figure 21). In the second interval, the contractions are spaced closer together, but they are smaller than in the previous interval.

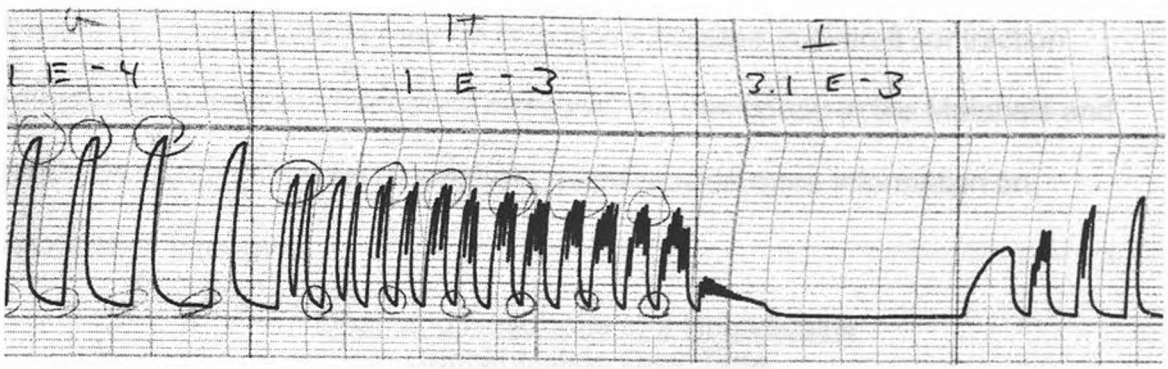


FIGURE 21: Increased Frequency and Decreased Amplitude

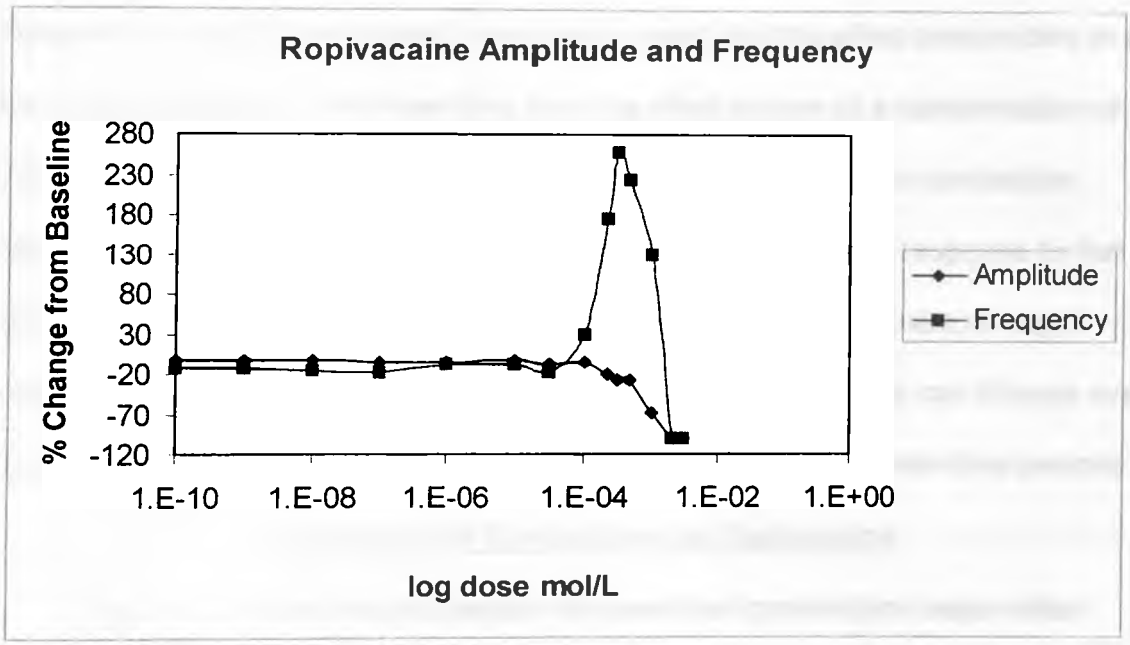


FIGURE 22: Ropivacaine Amplitude and Frequency

This effect can also be demonstrated graphically. Figure 22 shows a graph of percent change in frequency and amplitude in response to varying concentrations of ropivacaine (the graph is simply a combination of figures 8 and 9). It is clear that while frequency is increasing, amplitude is decreasing. This

decrease in amplitude may partially or completely mitigate the effect of increased frequency. Overall, there may be little or no increase in overall contraction effectiveness from the frequency increase. As mentioned in the Materials and Methods section, integration analysis might provide more information on contraction strength.

## Changing Response to a Ropivacaine Concentration Over Time

Figure 10 in the Results section showed that response to a given concentration of ropivacaine can change over time. This effect was only observed at one concentration in one experiment, but the effect was evident in all four tissue samples. It is interesting that the effect occurs at a concentration of 3.0E-04, a dose which corresponds to the maximum increase in contraction frequency (see figure 9). Perhaps the reason for the changing response to that dose is related to the reason for the contraction frequency increase, though I have no hypothesis. In any case, the fact that tissue responses can change over time calls into question the accuracy of data collected over shorter time periods.

## Inducement of Contractions by Ropivacaine

Figure 11 in the Results section showed that contractions began after addition of ropivacaine to three tissue samples that had not been contracting prior to drug administration. I cannot explain why this unusual result occurred in these tissue samples. Again, it is interesting to note that contractions were induced by the concentration of 3.0E-04, which corresponds to the maximum frequency increase and the changing response described above. This provides

further reason to believe that there is something special about the effect this concentration of ropivacaine has on myometrial activity.

This unusual result is certainly cause for concern when the possible application of this research is considered. If ropivacaine were used as a treatment or preventative agent for preterm labor in vitro, it is possible that the drug might induce contractions rather than eliminate them.

## Return of Contractions After Drugs Are Removed

After treatment with local anesthetics, contractions return when drug has been removed from the tissue bath. Resumption of myometrial activity after drug removal demonstrates that the contraction inhibition observed with drug was due to action of the drug. This rules out the possibility that the inhibition observed was due to tissue death or some other factor. The resumption of regular myometrial activity also implies that there is no lasting tissue damage induced by drug treatment.

While contractions return immediately after treatment with ropivacaine and lidocaine, a longer period is required for contractions to resume after treatment with procaine. This seems unusual because procaine is a supposedly a local anesthetic with a short duration of action, while lidocaine has a medium duration of action and ropivacaine has a long duration of action[10]. These classifications, however, may be irrelevant in vitro, since they are based on rates of metabolism in vivo. It is quite possible then that the longer period required for contractions to resume after procaine treatment will not be observed in vivo.

Unlike the local anesthetics, contractions did not return on their own after treatment with indomethacin. The tissue samples were observed for an hour or less after drug removal, and it is possible that contractions may have resumed after a longer time period had passed. To rule out the possibility of tissue death, two experiments were run in which basal tension was increased on each tissue sample after drug removal. An increase in basal tension usually induces a living tissue sample to contract. In all cases where this was tried, the tissue sample contracted, demonstrating that the tissue samples were still alive. While it is clear that indomethacin did not cause tissue death, it is unclear why contractions remain inhibited after it is removed.

## Summary

The drugs ropivacaine, procaine, lidocaine, and indomethacin were tested for their ability to inhibit myometrial activity in vitro. All were able to completely eliminate uterine contractions, suggesting a possible use in vivo to inhibit uterine contractions and thus prevent preterm labor. A possible delivery mechanism is slow-release microspheres. Potential problems include systemic side effects and fetal mortality. There is also the possibility that the local anesthetics may increase contraction frequency due to their agonist effect, though this effect may be mitigated by decreased contraction amplitude. The local anesthetic ropivacaine may also induce contractions were there previously were none. Contractions returned after treatment with local anesthetics, implying a lack of damage to the myometrial muscle. Overall, local inhibition of myometrial

contractions by the drugs ropivacaine, procaine, lidocaine, and indomethacin shows promise for a new method for preventing and treating preterm labor.

# APPENDIX A

## Modified DEJalons Solution

| SOLUTE | FORMULA WEIGHT | GRAMS/LITER |
|---|---|---|
| NACL | 58.44 | 8.99 |
| KCl | 74.56 | .42 |
| $CaCl_2/2H_2O$ | 147.02 | .32 |
| $NaHCO_3$ | 84.01 | .42 |
| Dextrose | 180.16 | 1.44 |

Reference: Eltze, 1979.

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