The role of the cholinergic anti-inflammatory pathway and nicotinic receptor in the remodeling of the cervix and preterm birth as shown through a novel treatment method

Daniel J. Barrero
University of Redlands
The role of the cholinergic anti-inflammatory pathway and nicotinic receptors in the remodeling of the cervix and preterm birth as shown through a novel localized treatment method

By Daniel Barrero

Academic Advisor: Dr. Lisa Olson

Research Advisors: Dr. Steve Yellon; Dr. Caryl Forristall;
Dr. Dustin VanOverbeke

March, 2016
Abstract

Premature birth is a global problem, affecting both developed and undeveloped countries including the United States. A crucial aspect of both preterm and term birth is remodeling of the cervix. Despite the large scale of the problem, factors leading to the initiation of remodeling are largely unknown. The present study examined the role of the cholinergic anti-inflammatory pathway, with particular focus on the nicotinic receptor, and its role in driving remodeling and preterm birth. Mice were treated with either vehicle control, mecamylamine (a nonspecific nicotinic receptor antagonist), RU486 (a progesterone receptor antagonist) as a preterm control, or progesterone (a hormone involved in the timing of birth) as a delayed birth control, using a novel thermogel treatment delivery method (TIM). Animals were sacrificed post partum and their cervixes were stained to analyze cell nuclei density and macrophage content, two factors expected to change during remodeling and parturition. Though the TIM was effective in delivering the drug treatments, mecamylamine did not cause preterm birth or a change in macrophage content and cell nuclei density. The results of this experiment indicate that the nicotinic receptor does not play a role in driving remodeling or preterm birth. Though the treatment did not yield significant results, the effectiveness of the TIM means that it may be developed into the first minimally invasive direct to cervix treatment in humans.

Introduction

Preterm birth is an all too common occurrence in the United States. In 2012, 1 in 9 babies was born prematurely (March of Dimes et al. 2012). Globally the U.S. ranks 131st out of 184 countries in the number of babies born prematurely, with significantly higher amounts of preterm birth than other developed nations (Hellwig 2012). Babies born prematurely, defined as before
the 37th week of pregnancy, are at significantly higher risk for respiratory distress, cerebral palsy, vision and hearing problems, and developmental delay, with symptoms becoming more severe the earlier the birth (World Health Organization 1970; Institute of Medicine 2007). Because of this, preterm babies are also more likely to die within their first 28 days (neonatal period) as well as first year than those born at term (Institute of Medicine 2007). The shortened development time affects a broad range of biological systems, but especially the lungs and respiratory system. It is not uncommon for babies born before 33 weeks to experience respiratory distress syndrome, and those born before 30 weeks to lack alveoli entirely, and thus experience significantly hindered breathing, and often develop chronic lung disease that is associated with growth, health, and neurodevelopmental problems (Institute of Medicine 2007). Additionally, because of the immediate severity of early birth, the majority of these babies must be admitted to neonatal care at great emotional and financial cost to the family. The financial burden of staying in the NICU for extended periods of time can often surpass $50,000, well outside the realm of feasibility for low income families or those with poor insurance (Institute of Medicine 2007). Babies that manage to survive past their stay in NICU are still at an increased risk for developmental issues later in life such as special educational requirements as well as fine motor skill development problems (Mackay et al. 2010; Hutton et al. 1997). In fact, special education needs (SEN) have been inversely correlated with gestation time, such that shorter gestation shows an increased proportion of SEN (Mackay et al. 2010). Critically, many adolescents and young adults who were born preterm continually demonstrate decreased cognitive ability compared those born at term (Institute of Medicine 2007).
Despite the large scale of the issue, the cause of preterm birth is not well understood. Often this process occurs spontaneously, however it is also possible for it to be caused by infection or early rupture of the fetal membranes (Timmons et al. 2010). Still some risk factors have been identified. Smoking for example is often cited as a cause of ectopic pregnancy, low birth weight, and increased infant mortality, but it has also been implicated in preterm birth (Office of the Surgeon General et al. 2004). Other studies have identified multiple births as well as short time between births (interpregnancy intervals) as possibly playing a significant role in increasing a woman’s chances of delivering early (DeFranco 2007).

Still, knowledge in this field is rapidly developing, and several studies have shown promising leads regarding certain biomarkers as predictors of preterm birth risk. A biomarker is simply a measurable substance that is indicative of a phenomenon. It may cause the phenomenon or simply be present in elevated levels during the event. Several proinflammatory and anti-inflammatory cytokines known as interleukins (IL) have been implicated as possible biomarkers. Molecules such as interleukin-10 have been examined as early indicators of preterm labor (Dubicke et al. 2010). Separately it has proven difficult to isolate biomarkers which can be solely indicative of preterm labor due to both the vast amount of potential biomarkers as well as variability in the expression of these factors (Holt et al. 2011). However, researchers have preformed analytics such as multifactor dimensionality reduction analysis on biomarkers found in tissues varying from maternal plasma and cord plasma to amniotic fluid in order to examine if the ratios of various molecules are better biomarkers than individual substances (Bhat et al. 2014). Through this analysis researchers determined that elevated levels of angiopoietin 2 and
tumor necrosis factor alpha (TNFα) can be used as possible predictors of preterm birth (Bhat, et al. 2014).

Additionally, researchers have proposed a correlation between the length of the cervix and risk for preterm birth. It seems that women whose cervixes are shorter run a higher risk of preterm birth than women with average cervix lengths (Feltovich et al. 2012). Though a clear and noticeable predictor for preterm birth has yet to be found, this development supports the concept that the cervix acts as an important barrier during pregnancy, possibly serving as a gatekeeper between the developing baby and the outside environment. This serves to both keep the baby safely developing in the uterus and prevents foreign bodies from reaching the baby.

There are two major organ structures involved in pregnancy and parturition, the uterus and the cervix. Though anatomically these may seem just separate regions of the same abdominal pelvic organ, they play vastly different roles throughout pregnancy. The baby develops in the uterus, which is composed of smooth muscle. This smooth muscle produces the contractions responsible for pushing the baby out in the final stage of parturition (Myers et al. 2015). The cervix is the structurally rigid, lower distal portion of the uterus consisting of the opening to the vagina, called the os (Myers et al. 2015; Timmons et al. 2010). In typical pregnancy the cervix acts as a protective barrier for both the baby and the uterine environment until parturition, remaining rigidly closed and sealed by a mucous plug (Feltovich, et al. 2015). Its rigidity is due largely to the dense extracellular collagen matrix that makes up the cervix, allowing it to withstand the pressure of the fetus developing in the uterus. At the time of parturition the cervix undergoes a remodeling process including a massive reorganization of its
collagen matrix, ultimately allowing for the passage and birth of the mature fetus (Uldbjerg, et al. 1983).

Ultimately the cervix and the uterus both respond to various signals to coordinate the timing of birth. One of the most studied signals is the hormone progesterone. In both human and mouse models, progesterone serves to maintain pregnancy. However, towards the end of pregnancy, the human cervix and uterus undergo progesterone withdrawal whereby receptors are unable to respond to the hormone. This prevents the pregnancy from continuing and begins parturition (Parizek et al. 2013; Zakar et al. 2007). Though many researchers focus on the uterus, every case of preterm birth is accompanied by premature cervical remodeling (Timmons et al. 2010). It is simply impossible for the baby to pass without remodeling the cervix and as such, this process merits more intense study (Word et al. 2007).

Remodeling of the cervix is not a singular event, but rather a process that occurs throughout pregnancy, which culminates in the dilation of the cervix and passage of the baby (Fig. 1; Word et al. 2007). Additionally, some researchers believe that due to apoptotic events early in pregnancy, the process of remodeling may be initiated as early as conception (Leppert 1998). Remodeling appears as an inflammatory response occurring in three major steps: softening, ripening, and dilation (Fig. 1) (Norman, et al. 2007; Christiaens, et al. 2008; Bollopragada et al. 2008).
Softening is the longest stage of remodeling, which can occur until the 32nd week of pregnancy (fig. 1). This stage is marked by swelling and hypertrophy as well as increased vascularity. This stage is also marked by innervation. During innervation, the amount of nerve fibers in the cervix is maintained and even increases. This change is unique to the cervix, as studies have shown that the uterus can lose nerve fibers during pregnancy (Tingåker et al. 2006). The major nerves that innervate the cervix and uterus during pregnancy are the parasympathetic fibers of both the pelvic and the vagus nerve (Clyde et al. 2010). These nerve fibers are crucial for both maintaining pregnancy as well as initiating the birthing process by controlling the release of either pro-inflammatory or anti-inflammatory cytokines, which become increasingly important in a later stage (Dubike et al. 2009; Collins et al. 2002).
Softening is followed by ripening, typically occurring between weeks 32 and 37 (fig. 1). This stage is marked by changes in the collagen rich extracellular matrix of the cervix along with an increase in hyaluronic acid (HA) and glucosaminoclycans (GAGs). This in turn decreases the structural integrity of the cervix, increasing distensibility (Word et al. 2007).

The final stage of remodeling is dilation (fig. 1). Typically occurring from the 37th week until birth, this is the shortest stage and accounts for the greatest increase in distensibility. Though immune cells are present throughout remodeling, there is a massive infiltration by leukocytes during dilation. Both the release of proinflammatory cytokines and the withdrawal of anti-inflammatory cytokines signal immune cells, particularly macrophages, to migrate into the cervix. This migration of macrophages and other immune cells leads to increased hypertrophy of cervical tissue through the propagation of inflammatory cytokines and the release of various chemicals that weaken the structure of the cervix (Hamilton et al. 2012). Finally comes degradation and reorganization of collagen fibers in the cervix brought on mainly by the release of collagenase and nitric oxide synthase (NOS) from the macrophages. This process decreases the tensile strength of the cervix, allowing it to dilate. Additionally, hormonal signals throughout the remodeling process are crucial. As already briefly mentioned, progesterone is a hormone known to play a significant role in the maintenance of pregnancy. At a certain point during late pregnancy, its receptors under an isoform switching. This results in the coding and production of a slightly structurally different progesterone receptor that cannot bind progesterone, and in turn makes maintaining the pregnancy impossible, initiating the labor process (Parizek et al. 2013). Thus, though serum progesterone does not dip like in the mouse model, a similar affect is achieved. The process of remodeling occurs to some degree through the entirety of the
pregnancy; though its most dramatic changes occur typically during dilation in week 32, consisting of an extreme increase in cervical distensibility brought on by immune cell migration, NOS production, and reduced collagen concentration (fig. 1).

Figure 2. Rat cervixes samples taken at different days of gestation stained with methyl blue for cell nuclei and F4/80 for macrophages. NP= non pregnant; d15 & d18= post breeding days 15 and 18 respectively; PP=post partum. Black arrows point to cell nuclei, red arrows point to macrophages Scale bar represents 100 µm.

A clear illustration of how this immune infiltration progresses is demonstrated in figure 2. Increasing hypertrophy is shown by the apparent decrease in cell nuclei density between the non-pregnant and pregnant animals. Additionally there is a clear increase in macrophages present in post breeding day 18 compared to both day 15 and non-pregnancy, demonstrating the
macrophage infiltration which is key to the remodeling of the cervix. Mice are expected to give birth on day 19 of pregnancy, and as such day 15 and 18 are when cervical remodeling and dilation occur respectively. Figure 2 demonstrates that on those days, immune cell migration increases along with hypertrophy of other cells, shown by decreased apparent cell density.

Currently there are no effective treatments for preterm birth. While uterine contractions can be paused using treatments such as tocolytic prophylaxis, it only stalls birth for a short period of time (hours) when the baby would need weeks to fully develop (Simhan et al. 2007). Unfortunately, despite being a common treatment it seems that tocolytics alone seem to have no significant benefit to the baby, and when used in combination with steroids to mature the respiratory system has only minimally beneficial effects (Berkman et al. 2003). The core issue remains that once the remodeling process has been triggered, there is no way to stop parturition. For that reason an emphasis on preventative treatments is crucial. By understanding the processes that govern the initiation of parturition and remodeling, the door is opened for development of treatments that could prevent these processes, leading to more effective treatments with reduced impact on both short and long term well-being of the child.

This study uses mice as a model organism for parturition. Mice are consistently and widely accepted as animal models for preterm birth as they go through similar changes in cervical collagen content, cell nuclei density, and macrophage presence during cervical remodeling, as well as have similar hormonal regulation, the major difference being that in mice a drop in serum progesterone takes the place of a receptor isoform switch (Mitchell et al. 2009). For this reason, progesterone is able to induce delayed birth in mice. Additionally mice have a short 19 day gestation period and are relatively simple to care for.
With this in mind, this study asks the question: Do particular nerve pathways or neurotransmitters play a critical role in signaling the remodeling process? In order to begin to answer that question, this study examines neural regulation of immune function in the cervix through the vagus nerve, particularly focusing on the role of the neurotransmitter acetylcholine through the cholinergic anti-inflammatory pathway in the timing of birth, recruitment and activation of immune cells, as well as the remodeling process, which along with innervation of the cervix are crucial aspects of preterm and term birth (Yellon et al. 2003; Kirby et al. 2005).

Acetylcholine is the main neurotransmitter in the cholinergic anti-inflammatory pathway (Rosas-Ballina et al. 2009), which inhibits immune cell migration and cytokine production, two crucial factors for the remodeling process (Collins et al. 2002; Winkler 2003). Nicotinic cholinergic receptors bind to acetylcholine from the vagus nerve, and initiate the Jak2-STAT3 signaling pathway. Through this pathway, transcription factor STAT3 binds and activates DNA, which codes for anti-inflammatory cytokines such as interleukin 10 (IL-10) (de Jonge et al. 2005). Because acetylcholine can act on macrophages through several groups of receptors, this study focuses specifically on nicotinic receptors. In other regions of the body nicotinic receptors have been shown to have a key role in ameliorating inflammation (Nemethova et al. 2013). Mecamylamine was used to perform a chemical neurectomy, delivered by novel treatment delivery method directly to the cervix. Treatments were achieved through the development of a injectable transport media (TIM) formulated from Pluronic F-127 thermogel and agar, loaded with drug treatment and injected directly into the vaginal canal. This transport media was found to be biologically inert and effective in pilot studies for this experiment. Mecamylamine is a nonspecific antagonist for the nicotinic receptors of the cholinergic pathway, and as such it’s binding should prevent the Jak2-STAT3 pathway from propagating. As a result, the typical anti-
inflammatory effects are predicted be absent in treated mice. This would demonstrate if this pathway is crucial for remodeling of the cervix (Nemethova et al. 2013).

It is expected that blocking of the anti-inflammatory pathway would promote macrophage infiltration, encouraging remodeling of the cervix and ultimately preterm birth. Thus, treatment with mecamylamine can be used to understand the role of acetylcholine and the nicotinic receptor in the remodeling of the cervix. In addition to providing a better understanding of crucial innervation during pregnancy, this study could provide groundwork for the first method to effectively treat preterm birth. If crucial neurotransmitter pathways are identified to cause preterm birth, antagonists for these pathways can be theoretically given to human patients. This would effectively be the first treatment to prevent the crucial step of remodeling of the cervix instead of just blocking contractions. By tackling an earlier stage in parturition, treatments as proposed by this study have the potential to effectively delay birth until the baby is fully developed by maintaining the structural integrity of the cervix for a much longer period of time. Conversely, in the event that birth needs to be medically induced, an agonist to this pathway could be used as a treatment. This would remove the need for cesarean section surgeries and the inherent risks that come with them.

Methods

Animals
CD-1 non-transgenic mice were used. Mice were ordered as day 13 post-breeding and allowed to acclimatize for one day. Treatment started on post breeding day 14 (D14). All animals were humanly treated according to IACUC protocol.

Formulation of Transport Implantable Media

In order to effectively deliver drugs to the cervix, a novel method of delivery was required. An implantable transport media (TIM) was developed by combining the thermogel Pluronic F-127 (Sigma Aldrich) and the thickening agent agar (25% & 2% w/v respectively). PF-127 is a thermogel which is solid at 37°C, the body temperature of mice, yet remains liquid at room temperature, facilitating the dissolution of treatments into the media. It is biologically inert and has been used previously as a drug delivery system in other regions of the body with successful results (El-Kamel 2002; Das et al. 2010). Agar was used to increase structural rigidity. Solutions of PF-127 were created in water as per instruction by the manufacturer, making sure to mix slowly and on ice until completely dissolved. These solutions were mixed with cooling agar to the desired final concentrations. Doses of each treatment were measured and mixed in to batches of the TIM such that a 0.9 mL volume would contain a single dose. These treatment or control loaded TIMs were drawn into a modified syringe for use on treatment days. Drug loaded TIM batches were formulated as close to the date of treatment as possible and inspected before use to ensure the drug remained in solution. Pilot studies were performed using DiD fluorescent dye loaded TIM in non-pregnant mice in order to visually assess diffusion into the cervix (fig. 3).

Treatment of Mice
Mice were divided into 4 groups. One group received the experimental treatment mecamylamine \((n=3)\), an antagonist to the nicotinic receptor of the cholinergic anti-inflammatory pathway, at one half of the LD50 dosage \((15\text{mg/kg}; \text{Aceto et al. 1969; Young et al. 2001})\). The remaining mice were divided into several controls. RU486 \((150\ \mu\text{g}; \ n=4)\), a progesterone receptor antagonist, was used as an early birth control because it consistently and reliably induces birth within 24 hours of treatment. Progesterone \((1\text{mg}; \ n=4)\) was used as a delayed birth control, as mice treated with progesterone typically give birth a day late. Finally, vehicle controls \((n=3)\) received no drug and served to make sure the TIM was having no effect and provide an example of term pregnancy for comparison.

![Figure 3. Photomicrographs of DiD fluorescent dye and vehicle control TIM treated mice transitioning from the cervix-lumen interface to the uterus transition. DiD fluoresces red (665nm) and is highlighted by yellow circles.](image)

Treatment consisted of injections every two days over the course of the experiment (D14 and
D16 post breeding). Immediately prior to treatment mice were fixed with surgical collars in order to prevent vaginal grooming. To preform the treatments TIMs were drawn into a modified syringe and 0.9mL (one dose) of solution was injected through vaginal canal in order to ensure contact of TIM with the cervix. After each treatment animals were observed for two hours to monitor their well-being.

![Biopsy of the cervix stained for cell nuclei and macrophages by methyl blue and F4/80 respectively. Green boxes represent 417x300 µm photomicrographs (8 total) taken for analysis. Scale bar represents 3 mm.](image)

**Analysis**
Mice were sacrificed post partum by CO₂ asphyxiation. Animals were externally examined for blood on the vaginal pore, an indication of difficult birth. Implantations sites in the uterine horns were counted and recorded, as well as the amount of pups present in the cage at time of sacrifice.

Figure 5. Photomicrographs of post partum mice stained with methyl blue for cell nuclei and F4/80 for macrophages. Red arrows indicate example macrophages, black arrows indicate example non macrophage cell nuclei (Veh=vehicle; P4=progesterone; RU486=RU486; Mec=mecamylamine). Scale bar represents 100µm.

Pups were checked for viability after birth. Cervix and uterine tissues excised from mice were
placed in paraformaldehyde immediately after collection and transferred to 70% ethanol after 24 hours. Both tissues were processed for immunohistochemistry including macrophage and cell nuclei staining (fig 4; fig. 5)

Tissues were stained for cell nuclei using methyl blue and macrophages using F4/80, both general stains (fig. 5) Scans of the tissues were taken and snapshots of the cervix were taken using Aperio ScanScope (2 sets per animal, 8 snapshots per snap). An example of a tissue scan and snapshot sites can be seen in figure 4. Cell nuclei and macrophages were counted using the cell counter plug-in through the ImageJ program available through the National Institute of Health website (http://rsbweb.nih.gov/ij/plugins/cell-counter.html). Data collected were analyzed using 1-way ANOVA and post hoc Dunnett’s tests. All statistics were performed using the GraphPad Prism statistical package.

Results

In the pilot study, fluorescent microscopy of DiD loaded transport injectable media (TIM) treated mice (n=3) showed fluorescence in the cervix-lumen interface and the cervix itself, but not the cervix-uterus transition (fig 5). Additionally, the vehicle control TIM control group (n=3) showed no fluorescence.
In order to further determine the efficacy of the novel TIM, as well as overall treatment effects, day of delivery was compared between the vehicle and all other treatments. As figure 6 demonstrates, the day of birth for RU486 treated animals was significantly different from the vehicle treatment group (p=0.004). No other group significantly differed from the vehicle control day of birth as indicated by a Dunnett’s post hoc test.

Figure 6. Average day of delivery for mice. Bars represent standard error of the mean. Veh=vehicle (n=3); RU486=RU486 (n=4); P4=progesterone (n=4); Mec=mecamylamine (n=3). Stars indicate significant difference from all other groups shown by 1way ANOVA and from vehicle controls by post hoc Dunnett’s tests (*p=0.004).
No treatment group showed any significant difference in cell nuclei density, as demonstrated in figure 7. Macrophage counts, recorded as macrophages per cell nuclei per area, demonstrated a slight trend, though it failed to reach significance (p=0.3821). As shown in figure 8, the progesterone group had a seemingly lower macrophage count post partum compared to all other groups. However, despite the apparent trend, no group stood out as significant when compared to the vehicle control.
Discussion:

The results of this study seem to indicate that the nicotinic receptor is not significantly involved in preterm birth. This conclusion is drawn from the fact that treatment with mecamylamine, an antagonist to this receptor, failed to have any clear effect on the timing of birth, resulting in mecamylamine treated animals giving birth on average at the same time as vehicle controls (fig. 6). On a cellular level, there is no clear effect on the composition of the cervix caused by the blocking of this receptor. Cell nuclei and macrophage counts in mecamylamine treated animals did not differ significantly from those in vehicle controls, indicating that it is essentially the same as term birth (fig. 7; fig. 8).

Though the results do not indicate that mecamylamine had any effect on the remodeling of the cervix, this is not due to an ineffective drug delivery method. Pilot studies using fluorescent dye demonstrated that the TIM could deliver chemicals of similar weight and charge...
into the cervix (fig. 5). Additionally, RU486 animals delivered preterm. RU486, also known as mifepristone, consistently induces birth within 24 hours of treatment (Dudley et al. 1996). For this reason it is used within the lab as an indicator of effective drug delivery. In this case the RU486 group delivered within 24 hours of treatment and significantly earlier than the vehicle control group, supporting the conclusion that the TIM was an effective method of delivery. Additionally, the trend toward reduced macrophage presence in the cervixes of progesterone treated mice compared with the uterus suggests that treatment was localized to the cervix, not affecting the uterus corpus, though further analysis could be performed to confirm this in future experiments. This is greatly significant because to the knowledge of this lab, there has never been an effective method of delivering drugs directly to the cervix in a minimally invasive fashion. Further testing using TIM as a starting point could serve to develop the first direct to cervix treatment method for humans. Vaginal treatments could be minimally invasive and simple to perform, and represents a huge potential for safe, effective cervix treatment, including both postponing and inducing birth.

The lack of significant difference in macrophages between mecamylamine and vehicle treatment groups indicates that the nicotinic receptor does not play a role in macrophage infiltration. Therefore it can be concluded that it is not significant in the initiation of remodeling and therefore preterm birth. However, the results may not be as clear as they appear. The biggest drawback of this study was the lack of animals. Though a minimum number of 3 animals was used per treatment, time constraints made it impossible to take macrophage and cell nuclei counts for all of them, dropping the amount of animals in all groups except progesterone (n=2 for all). This could have significantly contributed to the lack of significant differences in the cellular
analysis of the different groups. Even with the original group sizes, there was a surprising amount of variability, including a progesterone treated mouse giving birth significantly preterm (D15). This is unexpected and there is no clear explanation. Currently, the lab is exploring if stress caused by wearing grooming preventing collars could have contributed to this result. Mice groom themselves often and the inability to do so could have caused a stress response in this mouse that affected its day of delivery. However, this is unlikely, due to no other mice having such a drastic response. Replication with larger groups of animals is necessary to reduce the variability within this experiment and clarify if any trends may be significant.

Though mecamylamine dosages were based on treatments by other studies, this is the first time it has been delivered by thermogel directly into the cervix through the vagina (Aceto et al. 1969; Young et al. 2001). It is possible that to see significant effects, the dosages must be refined in further experiments. Studies indicate that several nicotinic receptors such as \(\alpha 4\beta 2\text{nAchR}\) play an important role in macrophage attenuation in the mouse gut (Nemethova et al. 2013; van der Zanden et al. 2009). As such it would be a logical next step to replicate this experiment with increased sample sizes and a variety of mecamylamine dosages to ensure that the lack of effect is not due to inappropriate dosage levels. However, operating under the assumption that the dosages were appropriate, nicotinic receptors can be considered unimportant for remodeling of the cervix.

Additionally, previous experiments within the lab have attempted to examine the role of the second main category of cholinergic anti-inflammatory receptor, the muscarinic receptor. However, the lack of an effective delivery method made it impossible to gather clear results.
Using TIM it is possible to retry this experiment and possibly eliminate both cholinergic receptors as important for remodeling.

Besides cholinergic, there are several other receptors have been noted as possible targets for future experiments. Several studies have indicated that nociceptors such the vanilloid receptor TRPV1 play a role in macrophage driven inflammation (Okada et al. 2011). In the case of this particular receptor, studies have also shown that TRPV1 nerve fibers exist in the cervix, but disappear from the uterus during pregnancy, suggesting it might play an important role in remodeling (Tingåker et al. 2008). This could be studied in much the same way that the nicotinic receptor was studied: by employing a drug which agonizes or antagonizes it and examining the subsequent effects on the timing of birth as well as cell nuclei density and macrophage infiltration. Because the TIM was effective, the methodology of this experiment can be repeated using different drug treatments in order to systematically eliminate receptors and neurological pathways until one that has a direct effect on preterm birth is found.
References


Barrero 25


