Smooth Muscle Pharmacology in the Isolated Virgin and Pregnant Rat Uterus and Cervix

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ABSTRACT

Uterine smooth muscle function is established, but comparatively little is known about cervical smooth muscle pharmacology. We performed a proof-of-principle experiment that smooth muscle was expressed in the cervix in both virgin and pregnant rats, using the uterus as a comparator. We tested whether all tissues were pharmacologically responsive to contractile and relaxant agonists. Immunohistochemistry revealed the expression of smooth muscle α-actin in all tissues. The isolated tissue bath was used to measure isometric contractility of uterine strips and whole cervixes from virgin and pregnant (day 11 ± 2) female Sprague-Dawley rats. We tested classic activators of uterine smooth muscle contraction and relaxation in both uterus and cervix. All tissues contracted to the depolarizing agent potassium chloride, prostaglandin F2α, muscarinic cholinergic agonist carbachol [2-[(aminocarbonxyl)oxy]-N,N,N-trimethylaminium chloride], and 5-hydroxytryptamine. Unlike other tissues, the pregnant cervix did not contract to oxytocin, but the oxytocin receptor was present. Both cervix and uterus (virgin and pregnant) had concentration-dependent, near-complete relaxation to the adrenergic agonist norepinephrine and adenylyl cyclase activator forskolin [(3R,4aR,5S,6S,6aS,10aR,10bS)-6,10–10b-trihydroxy-3,4a,7,10a-pentamethyl-1-oxo-3-vinyldecahydro-1H-benzof][chromen-5-yl acetate]. The β-adrenergic receptor agonist isoprotolenol was less potent in pregnant cervix versus virgin by ~10-fold. All tissues, particularly the cervix, responded poorly to the nitric-oxide donor sodium nitroprusside, relaxing ~20% maximally. These findings support the importance of smooth muscle in the cervix, the use of the isolated cervix in pharmacological studies, and a similarity between smooth muscle pharmacology of the nonpregnant uterus and cervix. This work highlights the unappreciated smooth muscle function of the cervix versus uterus and cervical changes in pharmacology during pregnancy.

INTRODUCTION

The uterus and cervix depend on passive elements (collagen, extracellular matrix proteins) to carry out their functions in female reproduction, but coordination of active smooth muscle function is also critical, which is illustrated by pregnancy, labor, and delivery. Pregnancy is defined by the quiescence of uterine smooth muscle and the nonpliant, load-bearing cervix. In contrast, in labor and delivery the uterus contracts forcefully to deliver the fetus and placenta, and to do so the cervix must be soft and compliant. In premature labor, this coordinated process that begins too early is one that would be beneficial to stop. In each situation, the function of smooth muscle of the uterus and cervix is important.

Although there is considerable knowledge about uterine smooth muscle reactivity to both contractile and relaxant stimuli (López Bernal, 2007; Wray, 2007), far less is known about the smooth muscle function of the cervix (Bryman et al., 1985). With the recognition that a short cervix may contribute to premature labor, the tensile strength and smooth muscle content of the human cervix have begun to receive attention (Oxlund et al., 2010a,b). The present study represents an initial comparative analysis of limited smooth muscle pharmacology between the uterus and cervix of virgin and pregnant rats. Most importantly, this is a proof-of-principle study that the smooth muscle reactivity of the isolated cervix can be measured and is pharmacologically modifiable.

Some of the seminal papers on cervical smooth muscle function date back to the 1940s (Adler et al., 1944) and 1960s (Rorie and Newton, 1967). Mitchell and Taggart (2009) point to the paucity of work done on cervical samples, which is understandable given the difficulty in obtaining human cervical samples on a regular basis. In the present study, we isolated a strip of the uterine horn (longitudinal muscle) and whole cervix of the same female rat and investigated the ability of these two separate tissues to respond to a series of pharmacological agonists in a classic isometric contractile assay. We used the rat pregnant between days 9 and 13 of...
gestation because this time range is a time sufficiently distanced from initiation of pregnancy. We combined this work with histological and immunohistochemical studies to identify smooth muscle and receptor expression.

Using the uterus as a comparator, we chose to use a group of compounds within the cervix based on: 1) their importance in modifying uterine smooth muscle tone, 2) their role as neurotransmitters within these reproductive tissues, and 3) their role in modifying smooth muscle function. We discovered that, like the smooth muscle of the uterus, that of the cervix is pharmacologically modifiable, and responsiveness to pharmacological agonists changes in pregnancy.

Materials and Methods

Animal Use. All animal use protocols were approved by the Michigan State University Institutional Animal Use and Care Committee. These experiments have been carried out in accordance with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 2011). Virgin female Sprague-Dawley rats (225 g, between 10 and 12 weeks of age; Charles River Breeding Laboratories, Portage, MI) were used in these experiments. Bedding from the cage of the male rat that the female rat was to be bred with was placed in the cage of the female 3 to 5 days before breeding. Female rats were staged in their estrus cycle, and then paired with a male rat for 5 days in a metabolic cage. This allowed for detection and collection of a vaginal mucus plug. Once this plug was discovered, males were removed from the cage, and this was considered day 1 of the pregnancy. This breeding protocol was approximately 90% successful. Rats (either virgin or days 9–13 in pregnancy; average 1 of the pregnancy. This breeding protocol was approximately 90% successful, males were removed from the cage, and this was considered day 0 of the pregnancy. We combined this work with histological and immunohistochemical studies to identify smooth muscle and receptor expression.

Isolated Tissue Bath Protocol. The largely avascular cervix (color white) was dissected away from the uterine horns. The cervix was placed on two L-shaped stainless-steel wires as a circular preparation. Bilateral uterine horns, and cervix were removed and placed in a dish containing physiological salt solution (PSS): 130 mM NaCl, 4.7 mM KCl, 1.18 mM KH₂PO₄, 1.17 mM MgSO₄·7H₂O, 1.16 mM CaCl₂·2H₂O, 14.9 mM NaHCO₃, 5.5 mM dextrose, and 0.03 mM CaNa₂EDTA, pH 7.2.

Histology and Immunohistochemistry. Some uterine sections and cervices were cleaned, formalin-fixed, paraffin-embedded, processed for sectioning, and taken through Masson trichrome staining. The largely avascular cervix (color white) was dissected away from the uterine horns. The cervix was placed on two L-shaped stainless-steel wires as a circular preparation. Bilateral uterine horns, and cervix were removed and placed in a dish containing physiological salt solution (PSS): 130 mM NaCl, 4.7 mM KCl, 1.18 mM KH₂PO₄, 1.17 mM MgSO₄·7H₂O, 1.16 mM CaCl₂·2H₂O, 14.9 mM NaHCO₃, 5.5 mM dextrose, and 0.03 mM CaNa₂EDTA, pH 7.2.

Histology and Immunohistochemistry. Some uterine sections and cervices were cleaned, formalin-fixed, paraffin-embedded, processed for sectioning, and taken through Masson trichrome staining. Within the Investigative Pathology laboratory at Michigan State University for identification of collagen (blue), keratin/muscle (red), and cytoplasm (pink). In separate studies, formalin-fixed, paraffin-embedded sections of the uterus and cervix were cleared, antigen-unmasked for 1 min (citrate-based antigen unmasking solution; Vector Laboratories, Burlingame, CA), blocked, and incubated with a primary antibody against smooth muscle α-actin (clone 1A4, EMD Chemicals, Gibbstown, NJ; or ab5694, Abcam Inc., Cambridge, MA) for identification of smooth muscle or primary antibody against the oxytocin receptor (rabbit, APO692; Acros Antibodies, San Diego, CA). A standard immunohistochemistry protocol was used as written by the manufacturer, Vector Laboratories, and 3,3-diaminobenzidine was used to visualize the binding of antibodies to the target of interest (allowing sections to develop for one min). In parallel sections, the primary antibody was left out of the reaction. Most sections were counterstained with hematoxylin. In some cases, counterstaining was not performed so we could view antibody-based staining with clarity. Sections were photographed on a Nikon (Tokyo, Japan) Eclipse inverted microscope, and images were captured within the program MMJ Cell Tools (Molecular Machines & Industries, Haslett, MI).

Contractile Agonists. Agonists were added to the bath, in a cumulative fashion, in 5-min steps. Tissue responses readily plateaued during this time, so this was sufficient to measure agonist-induced changes in contractility. Contractility includes changes in basal tone and increases in oscillatory contraction amplitude and frequency. When a final maximum was achieved, tissues were washed thoroughly for 1 h. A second contractile agonist was then investigated in the same manner. Agonists were randomized throughout the experiment and two were used in each tissue. Comparison of agonist response to first and second curves revealed no statistical differences (potency, maximums), thus these responses were combined in the graphs presented.

Relaxant Agonists. An agonist that caused a stable contraction in all tissues (virgin and pregnant cervix and uterus) was chosen to stimulate muscle tone in these tissues, to which vehicle or a relaxant agonist could be used. Tissues were contracted with a half-maximal concentration of the cholinergic muscarinic agonist carbamylcholine (1–10 μM). Carbamylcholine-induced contraction remained sufficiently stable over the hour necessary to complete a cumulative concentration-response curve. A 5-min period of contraction to carbamylcholine was established, and then either vehicle or relaxant agonist was added in a cumulative fashion. Tissue responses readily plateaued over a 5-min period, so this was sufficient to measure agonist-induced relaxation. The ranges of contractions established by carbamylcholine during this 5-min period were for the five different agonists tested (in integrated units/mg KCl contraction): virgin uterus, 117 to 142; pregnant uterus, 152 to 214; virgin cervix, 117 to 142; and pregnant cervix, 121 to 161. The magnitude of carbamylcholine-induced contraction established before the addition of the different relaxant agonists was not statistically different compared within group (e.g., all agonists in pregnant uterus). When carbamylcholine-induced contraction was compared between a virgin and pregnant tissue for each agonist, all initial contractions were statistically similar except for contraction established before calcitonin gene-related peptide (CGRP) (virgin 83 ± 9 versus pregnant 235 ± 38) in the cervix and norepinephrine (NE) in the uterus (virgin 117 ± 17 versus 176 ± 17) (p < 0.05). We report these values such that we can interpret whether a different initial contraction would modify the outcome of the relaxant.

For experiments using aorta, tissues were contracted half-maximally with the adrenergic agonist phenylephrine (2 × 10⁻⁶ M) before being relaxed with the α₁-adrenergic antagonist prazosin (1 μM) in a cumulative fashion, in 5-min steps.
the addition of a relaxant agonist (sodium nitroprusside (SNP), CGRP).

Materials. Sigma (St. Louis MO) was the source of all agonists: acetylcholine chloride, CGRP, carbamylcholine chloride, forskolin [3R, A, R, 55, 6S, 6aS, 10S, 10aR, 10bS]-6,10–10–tri hydroxy-3, 4, 7, 7, 10a-pentamethyl-1-oxo-3-vinyl(dodehydro-1H-benzol[f] chroment-5-y1 acetate], 5-hydroxytryptamine (5-HT), isoproterenol, NE, oxytocin, SNP, and prostaglandin F2α (PGF2α).

Data Analysis. All data are reported as means ± S.E.M. for the number of animals indicated in parentheses in the figures. Adjustments to image data included only brightness and contrast settings, applied uniformly to all images in a figure. Uterine and cervical data from the same rat are graphed separately to insure that the response of the cervix, typically less in magnitude than the uterus, was well visualized and could be compared between virgin and pregnant groups. All data are reported as integrated units of uterine/cervical activity over a timed 5-min window. Integrated activity was measured by using the integral function of the program Chart 7.0 (ADInstruments Ltd.). This calculates the area under the wave form as the sum of the data points multiplied by the sample interval. Data points were taken at four per second for 5 min; Chart adds these values together and multiplies by the duration of the window (5 min). By using integrated activity, we take into account changes in basal tone, oscillatory magnitude, and frequency. For contractile experiments, 5-min windows were taken from baseline for each addition, and data are reported as these units of activity normalized to the initial KCl challenge in milligrams. This allows for normalization of tissue size and health from experiment to experiment. For relaxant experiments, a 5-min baseline period (carbamylcholine-induced contraction) just before the addition of relaxant agonists was measured; this was considered 100% activity. Changes in this integrated activity with addition of relaxant agonists are reported as a percentage of this initial baseline activity. Agonist potencies were calculated by using a nonlinear regression (curve fit) within Prism 5.0 (GraphPad Software Inc., San Diego, CA) and are reported as concentrations that caused a half-maximal effect. Maximums are reported as the maximal effect achieved. Where a maximal response was not achieved, the actual potency (EC50 value) was considered equal to or greater than the reported value. Maximums are reported as integrated units/KCl contraction (for contractile agonists), and maximums of relaxant agonists are reported as the percentage of carbamylcholine-induced contraction remaining. Student’s t test was used to compare EC50 values of agonist responses in the uterus versus cervix, and p < 0.05 was considered significant.

Results

Histology and Immunohistochemistry. Cross-sections of the isolated uterine horn and cervix from virgin and pregnant animals were stained with Masson trichrome to delineate the presence of cells/keratin (pink/red) and collagen (blue). Figure 1A demonstrates that the cervix has significant collagen composition (blue) but does have layers of cells that are irregularly located in the cervical tissue. A midzonal section that lies between the two cervical lumens shows cellularity as well, and the lumen itself demonstrates a robust epithelial cell layer. In pregnancy, this epithelial layer is lost but the cellularity in the stroma of the tissue remains. Staining of smooth muscle α-actin demonstrates the presence of smooth muscle in both the virgin (Fig. 1A, left) and pregnant cervix (Fig. 1A, right), indicated by the black/brown staining in deep stromal and outer cervical layers (Fig. 1B, top). Staining was absent in sections in which the primary antibody against α-actin was not present but tissues were counterstained with hematoxylin (Fig. 1B). This was true for two different α-actin antibodies used [antibody 1 from Cal- biochem (San Diego, CA); antibody 2 from Abcam Inc.]. Antibodies do not recognize other isoforms of actin. Thus, cervixes express smooth muscle and continue to do so in pregnancy. Figure 2 demonstrates that uterine tissue has two cellular layers (pink) consistent with the expression of smooth muscle α-actin (longitudinal, circular layers of smooth muscle). Smooth muscle α-actin continues to be expressed in pregnancy. Elongated uterine glands, indicated by a star in Fig. 2A, did not stain for α-actin; smaller glands showed some reactivity. Collectively, these data support the idea that all tissues should have the ability to generate smooth muscle tone.

Response to Contractile Agonists. The uterus and the cervix used in all experiments were from the same animal, providing for the ideal comparison between tissue reactivity. Both the uterus and the cervix are phasic tissues, meaning that tissues showed regular oscillatory activity from baseline. Figure 3A shows a representative tracing of spontaneous oscillation during the equilibration period; the tissue had not been exposed to any agonist but was under passive tension. Approximately 50% of both uterine (Fig. 3A, left) and cervical (Fig. 3A, right) tissues demonstrated spontaneous oscillations. Figure 3B demonstrates that all tissues contracted to cumulative additions of the depolarizing stimulus KCl (6–100 mM) in a concentration-dependent manner. Contraction was lost when KCl was washed out, verifying that all tissues are capable of producing active smooth muscle tone. All tissues contracted to KCl at the beginning and end of the experiment. KCl-induced contraction (in milligrams) did not change during the course of the experiment in the virgin
uterus (first challenge, 3700 ± 251; final challenge, 4539 ± 1096; p < 0.05), pregnant uterus (first challenge, 6844 ± 369; final challenge, 6839 ± 437; p < 0.05), pregnant cervix (first challenge, 1566 ± 80; final challenge, 1725 ± 256; p < 0.05). This finding allows us to use KCl as a normalizer throughout the experiment. However, the magnitude of first contraction to KCl in the pregnant uterus was greater than that of the virgin uterus (6844 ± 369 versus 3700 ± 251; p < 0.05), whereas that of the pregnant cervix was reduced compared with the virgin cervix (1566 ± 80 versus 2296 ± 154; p < 0.05).

Contractile agonists were added in a cumulative fashion, and Table 1 compiles the potencies and efficacies (maxima) of these agonists in the uterus and cervix, virgin and pregnant. Figure 4A, left shows a representative tracing of the virgin uterine (top) and virgin cervical (bottom) contraction to cumulative additions of an agonist (oxytocin). PGF2α (Fig. 5A; Table 1) was a robust contractant in both virgin uterus and cervix. Oxytocin induced contraction in all virgin tissues and was slightly, but not significantly, more potent and efficacious in the pregnant versus virgin uterus. By contrast, the pregnant cervix lost responsiveness to oxytocin such that no discernible concentration-response curve could be identified and potency and maximum values could not be calculated. All tissues contracted to KCl, so these tissues do not lack the ability to contract. The inability to contract to oxytocin is not because of a lack of oxytocin receptors, because the oxytocin receptor could be immunohistochemically detected in both the virgin and pregnant cervixes, with and without hematoxylin counterstaining (Fig. 6, A and B, respectively).

The uterus and cervix are innervated by the parasympathetic nervous system, and the muscarinic cholinergic agonist carbamylcholine (10⁻⁹ to 10⁻⁵ M) caused a concentration-dependent contraction, one that was particularly robust in the cervix, both virgin and pregnant (Fig. 7A; Table 1). A maximal response to carbamylcholine was not achieved in the uterine strips up to 10 μM. The primary amine 5-HT (10⁻⁹ to 10⁻⁵ M) contracted both uterus and cervix (Fig. 7B; Table 1). 5-HT was modestly, but not significantly, more potent in contracting the uterus versus the cervix. Overall,
The potencies of agonists were generally similar between the pregnant versus virgin tissues, and the general rank order of potency in both the uterus and cervix was oxytocin > 5-HT > PGF$_{2\alpha}$ > carbamylcholine (exception of loss of oxytocin contraction in pregnant cervix). The magnitude of contraction in the cervix was generally less than that of the uterus. Collectively, these data confirm for the uterus and supports for cervical smooth muscle the ability to contract to pharmacological agonists.

### Response to Relaxant Agonists

Tissues were contracted half-maximally to carbamylcholine (1–10 $\times$ 10$^{-6}$ M). This caused a measurable increase in activity but was not maximal such that additions to the bath had the potential to either decrease or increase tone. Figure 4B, right shows representative tracings of virgin uterine (top) and virgin cervical (bottom) relaxation to cumulative addition of the adrenergic agonist NE, and Table 2 compares the potency and efficacy of this and four other relaxant agonists. Figure 8 demonstrates the effects of the most active vehicle used for relaxant agonists, DMSO. This vehicle was taken through the experiment with the same timing as agonist additions but with vehicle additions (same volume) instead. As a control for vehicle additions, 10-fold dilutions of neat DMSO (considered the final addition in parallel to 10$^{-5}$ M agonist) were used for parallel additions to contracted tissues (final cumulative concentration 0.111% DMSO). Contraction induced by carbachol [2-[(aminocarbonxyl)oxy]-N,N,N-trimethylammonium chloride] remained largely stable during these additions but was reduced overall ~25% in all tissues over the course of the experiment.

The sympathetic nervous system innervates both cervix and uterus, and Fig. 9 shows the potent and efficacious ability of the adrenergic agonist NE (Fig. 9A) to relax both tissues, as did the $\beta$-adrenergic receptor agonist isoproterenol (10$^{-10}$ to 10$^{-5}$ M; Table 2; Fig. 9B) and the adenylate cyclase activator forskolin (10$^{-10}$ to 10$^{-5}$ M; Table 2; Fig. 9C). Isoproterenol was potent in relaxing both the uterus and cervix, so potent in the normal cervix that relaxation was not calculable.

### Table 1

<table>
<thead>
<tr>
<th>Contractant</th>
<th>Uterus Virgin</th>
<th>Uterus Pregnant</th>
<th>Cervix Virgin</th>
<th>Cervix Pregnant</th>
<th>$-\log IC_{50}$ Uterus</th>
<th>$-\log IC_{50}$ Cervix</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGF$_{2\alpha}$</td>
<td>200.5 ± 13.7</td>
<td>169.9 ± 14.5</td>
<td>29.4 ± 3.8</td>
<td>33.6 ± 5.3</td>
<td>6.73 ± 0.19</td>
<td>6.68 ± 0.28</td>
</tr>
<tr>
<td>Oxytocin</td>
<td>229.0 ± 15.6</td>
<td>301.5 ± 10.7</td>
<td>70.0 ± 14.8</td>
<td>N.C.*</td>
<td>8.81 ± 0.26</td>
<td>9.03 ± 0.17</td>
</tr>
<tr>
<td>Carbachol$^{**}$</td>
<td>262.2 ± 10.9</td>
<td>255.7 ± 89.3</td>
<td>162.1 ± 19.7</td>
<td>233.9 ± 29.7</td>
<td>5.62 ± 0.085</td>
<td>5.47 ± 0.45</td>
</tr>
<tr>
<td>5-HT</td>
<td>118.1 ± 9.5</td>
<td>145.7 ± 7.5</td>
<td>49.4 ± 6.3</td>
<td>33.7 ± 4.4</td>
<td>7.09 ± 0.25</td>
<td>7.33 ± 0.17</td>
</tr>
</tbody>
</table>

N.C., not calculable.

* Significantly different from virgin value.

$^{**}$ IC$_{50}$ are estimates because maximums may not have been obtained.

![Fig. 4](image-url) Representative tracings of the contraction of virgin tissues to oxytocin (A) and relaxation to NE (B), demonstrating how cumulative concentration response curves were carried out. Representative of more than 100 experiments.
already observed at $10^{-11}$ M isoproterenol. However, isoproterenol was $\sim$10-fold less potent in the pregnant versus virgin cervix, although the contractions established by carbamylcholine were statistically similar in both groups (virgin, 124 ± 24 integrated units/mg KCl contraction; pregnant, 123 ± 24 integrated units/mg KCl contraction; $p > 0.05$). These data suggest that, although the β-adrenergic receptor function may be reduced, the adenylate cyclase/adrenergic system is largely intact. We examined another smooth muscle relaxant system, NO. The NO donor SNP ($10^{-10}$ to $10^{-5}$ M; Fig. 10A) caused a modest relaxation in the uterus (pregnant and virgin), but this is only $\sim$10 to 20% maximum relaxation when vehicle-induced effects are taken into account. No discernible relaxation could be detected in carbamylcholine-contracted cervices. In contrast, the same solution of SNP (10 μM) completely relaxed isolated thoracic aorta half-maximally contracted with the adrenergic agonist phenylephrine (data not shown). CGRP is a sensory neuropeptide, and it caused a concentration-dependent relaxation in the uterus (to $\sim$40% carbamylcholine contraction remaining; Fig. 10B). This was less clear in the cervix, where in both the virgin and pregnant tissues high concentrations (10 and 100 nM) were necessary to observe relaxation. CGRP (100 nM) also caused significantly relaxation ($\geq 50\%$) in the half-maximally phenylephrine-contracted rat thoracic aorta. Collectively, relaxant agonists produced similar responses between the uterus and cervix with a potency order of isoproterenol > CGRP > SNP > NE = forskolin where this order is most strongly validated for the uterus.

Discussion

Our goal was to investigate the individual contractile and relaxant function of the cervix and uterus in the normal, nonpregnant virgin female rat as a means to establish baseline contractility, using the uterus as a comparator. This represents a proof-of-principle experiment to determine whether the cervix could be routinely studied in this way. We also examined whether the pharmacology of the smooth muscle in these two tissues would be different basally and during...
a time in pregnancy that is not close to labor. Although the group of compounds studied was not exhaustive, they represent well known vasoactive substances, neurotransmitters, and substances with recognized effects in uterine smooth muscle. In the normal virgin female, the uterine and cervical smooth muscles are largely similar in their qualitative response to contractile and relaxant agonists. In pregnancy, the smooth muscle of both the uterus and cervix continues to contract and relax to stimuli, but the cervical loss of contraction to oxytocin supports the unappreciated fact that changes in smooth muscle responsiveness of these reproductive tissues may be critical to the normal progression of pregnancy.

We confirmed the significant expression of smooth muscle α-actin in the uterus. In contrast, the cervix from the same rat seemed less muscular but did possess smooth muscle, validated by use of two different α-actin antibodies (Fig. 1).

Estimates of the percentage of smooth muscle in the human uterus are ~35% (Petersen et al., 1991), and in the human cervix they have been reported as low as 6 to 8% (Rorie and Newton, 1967; Oxlund et al., 2010a,b) or as high as 45% (Danforth, 1954). This discrepancy needs to be resolved. We speculate that the cervical sampling is different in these studies, with samples taken closer to the proper uterus having a higher level of musculature compared with those nearer the vagina. The outer layer cervical layer of smooth muscle has been described as continuous with the outer myometrial layer but cervical electromyographic activity suggested that the smooth muscle cells of the outer myometrial and outer cervical layer are differently regulated (bovine tissue; van Engelen et al., 2007) and may operate independently. The smooth muscle cells within the deep stromal layer of the cervix are even less well defined, and their function has been

**TABLE 2**

<table>
<thead>
<tr>
<th>Relaxant</th>
<th>Maximum Relaxation</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Virgin</td>
<td>Pregnant</td>
<td>Virgin</td>
<td>Pregnant</td>
<td>Virgin</td>
<td>Pregnant</td>
<td>Virgin</td>
</tr>
<tr>
<td>NE</td>
<td>21.2 ± 8.4</td>
<td>2.5 ± 1.2</td>
<td>10.8 ± 6.9</td>
<td>38.6 ± 11.8</td>
<td>6.50 ± 0.23</td>
<td>6.45 ± 0.11</td>
<td>5.98 ± 0.56</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>1.3 ± 1.0</td>
<td>0.3 ± 0.3</td>
<td>10.4 ± 6.1</td>
<td>20.8 ± 5.5</td>
<td>9.51 ± 0.15</td>
<td>9.37 ± 0.11</td>
<td>9.95 ± 0.37</td>
</tr>
<tr>
<td>Forskolin</td>
<td>1.2 ± 0.8</td>
<td>3.4 ± 3.3</td>
<td>12.8 ± 2.5</td>
<td>17.2 ± 6.8</td>
<td>6.26 ± 0.22</td>
<td>5.62 ± 0.18</td>
<td>5.73 ± 0.21</td>
</tr>
<tr>
<td>SNP</td>
<td>66.8 ± 14.7</td>
<td>60.4 ± 12.1</td>
<td>N.C.</td>
<td>N.C.</td>
<td>9.01 ± 0.84</td>
<td>9.23 ± 0.99</td>
<td>N.C.</td>
</tr>
<tr>
<td>CGRP**</td>
<td>36.9 ± 10.6</td>
<td>37.6 ± 11.1</td>
<td>30.4 ± 8.9</td>
<td>60.4 ± 10.4</td>
<td>9.35 ± 0.53</td>
<td>9.10 ± 0.55</td>
<td>8.11 ± 0.49</td>
</tr>
</tbody>
</table>

* N.C., not calculable.
** EC₅₀ are estimates because maximums may not have been obtained.

* Significantly different from virgin value.

Fig. 7. Concentration-dependent contraction of the uterus (top) and cervix (bottom) to carbachol (A) and 5-HT (B). Points represent means ± S.E.M. for the number of animals in parentheses.
described as anything from secretory to contractile (Tiltman, 1998). The few studies that investigate the isolated cervix pharmacologically underscore the paucity of work done in this area (Hollingsworth and Isherwood, 1978; Petersen et al., 1991).

**Uterus and Cervix: Similarity in Responses.** The contractile and relaxant agonists examined, including neurotransmitters, were similarly potent and qualitatively efficacious in the uterus and cervix (Houdeau et al., 2003; Dindyaev and Vinogradov, 2009; Gnanamanickam and Llewellyn-Smith, 2011). Carbamylcholine contracted both tissues from the virgin and pregnant rat, whereas NE, isoproterenol, and the direct adenylate cyclase activator forskolin abolished carbamylcholine-induced contraction. These findings highlight the physiological antagonism exerted by the two autonomic nervous systems and the similarity of response between uterine and cervical smooth muscle in the normal and pregnant state. Stimulation of the Gs/cAMP pathway facilitated a reduction in reproductive smooth muscle activity in both tissues, agreeing with other studies (Price and Bernal, 2001; Yuan and López Bernal, 2007). This differs from a report by Bryman et al. (1984) in which NE stimulated uterine smooth muscle activity, and isoproterenol was both stimulatory and inhibitory in cervical strips from early pregnant and nonpregnant women.

1 Adrenergic receptor coupling to phospholipase C is augmented in term myometrium (Dupuis et al., 2008), and 2 adrenergic receptors in the cervix have been described to mediate increases and decreases in cervical resistance (Gál et al., 2009). Terbutaline was reported to increase the cervical resistance of the isolated pregnant rat (Gáspar et al., 2005). The overall variable function of adrenergic receptor warrants future attention as to the specific role of the α and β adrenergic receptors.

Oxytocin, 5-HT, and PGF2α caused significant contraction in cervix and uterus. Oxytocin is an inducer of labor (Smith and Merrill, 2006; Arthur et al., 2007) as are prostaglandins (Goureau et al., 1992; Griffiths et al., 2006). Responsiveness of the human cervix to prostaglandins is one of the better-

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**Fig. 8.** Effect of vehicle additions (DMSO) on carbamylcholine-induced contraction in the virgin and pregnant uterus (top) and cervix (bottom). Points are the percentage of contraction remaining over a 5-min period after additions and represent means ± S.E.M. for the number of animals in parentheses.

**Fig. 9.** Effect of NE (A), isoproterenol (B), and forskolin (C) on half-maximal carbamylcholine-induced contraction in isolated rat uterine strip (top) and cervix (bottom). Points represent means ± S.E.M. for the number of animals in parentheses.
characterized responses for cervical smooth muscle. In cervical samples from pregnant and nonpregnant women, different prostaglandins (PGE2, PGI2, and 6-keto-PGF1α) inhibited muscle contraction (Bryman et al., 1985). We did not test the relaxant effects of these prostaglandins, but confirm the well known fact of PGF2α as an uterotonic and provide evidence that the cervix (virgin and pregnant) also contracts to PGF2α. In contrast, the effects of 5-HT on both uterine and especially cervical smooth muscle are less well known, and our reason for studying 5-HT was its well known actions as a vasoconstrictor. It is noteworthy that the ability of 5-HT to contract uterus is species-specific. The rat and human respond with contraction, whereas that of the pig relaxes (Cordeaux et al., 2009). In our hands, 5-HT increased smooth muscle tone in both the uterus and cervix.

Both the isolated uterus and cervix did not relax robustly to the NO donor sodium nitroprusside, although it abolished tone in contracted vascular smooth muscle. NO donors cause myometrial relaxation but this is independent of global elevation of cGMP (Buxton, 2004). NO has been tested as a treatment of threatened preterm labor (Leszcynska-Gorzelaik et al., 2001), suggesting that the uterus of the laboring pregnant rat responds significantly differently to NO donors than the nonpregnant or nonlaboring pregnant rat. Nitric-oxide synthases have been detected in human cervix of pregnant women at term, and the addition of the NO donor nitroglycerin or spermine NONOate at high concentrations (100 nM to 10 μM) reduced contraction (Ekerhovd et al., 2000). A similar report was made for uterine and cervical tissues from the rat (Okawa et al., 2004). Thus, pregnancy probably reshapes these mechanisms such that tissues from the pregnant rat gain responsiveness to NO as labor impends. We studied rats on days 9 to 13, a time that is still distanced from labor and did not observe robust relaxation to SNP, consistent with reports that NO donors were comparable with placebo as treatments for cervical ripening in first-trimester abortions (Promsonthi et al., 2009). Future studies should investigate the pharmacology of the labor-impending uterus and cervix (day 21), and we hypothesize that here smooth muscle responsiveness would yet again be markedly changed to support the process of labor. It is possible that different donors of NO would elicit a different outcome, but SNP has been a reliable tool for decades, used by many investigators, and was able to abolish agonist-induced contraction in the vasculature.

Cervical relaxation to CGRP was also difficult to measure, in that only the highest concentration of CGRP (100 nM) caused a relaxation that was above that caused by vehicle. Uterine relaxation to CGRP was clear and confirmatory (Anouar et al., 1998), but the potencies for CGRP are estimated because the maximum relaxation may not have been achieved by 100 nM, the highest concentration we could attain in the tissue bath. Why the cervix responds so irregularly to CGRP is a question, especially knowing that sensory innervation occurs in the cervix (Ghatei et al., 1985) and is a part of ripening (Collins et al., 2002).

**Uterus and Cervix: Differences in Responses.** There were a few notable differences between cervix and uterus. First, the absolute magnitude of contraction achieved in the cervix was less than that of the uterus, probably because of the relative lower content of smooth muscle. In pregnancy, contraction to KCl was reduced in the cervix, indicating a potential, but not total, loss of smooth muscle function. Cervical smooth muscle apoptosis in pregnancy has been suggested (Leppert, 1995). Second, the pregnant cervix lost contractile responsiveness to oxytocin, whereas the uterus did not. This finding was consistent with observations made by Hollingsworth and Isherwood (1978). Using term pregnant (day 22) strips of rat cervix, they demonstrated a reduced contraction to a given concentration (1 or 10 μM) of oxytocin; a concentration-response curve was not performed. Our experiments differ in that we kept the circular nature of the cervix intact and performed a whole concentration-response curve. Given that oxytocin is a major stimulant for labor, the loss of cervical response to oxytocin is logical. The mechanism of loss of contraction still needs to be determined, although it is unlikely this is because of oxytocin receptor loss given the significant staining for the oxytocin receptor in cellular sections of the cervix where α-actin is localized. Finally, the loss of sensitivity (reduced potency) to isoprotrenol in the pregnant cervix suggests that the β-adrenergic receptor is less effective in transducing the signal stimulated by isoprotrenol, either because of loss of receptor or loss of receptor-effector coupling. The lack of change in response to forskolin supports that any change in this adenylate cyclase-dependent system is not downstream of the receptor.

**Potential Clinical Ramifications.** A majority of the focus on the cervix in premature labor has been on the impact of short cervixes and cervical ripening, which includes a decrease in collagen, increased water retention, dilated blood vessels, and increased plasma extravasation (Ji et al., 2008). The result is a pliable, soft cervix. In premature labor, the cervix may be incompetent, short, or dilate (passively) inappropriately (Bergella et al., 2009; Mella and Bergella, 2009; Sinno et al., 2009; Abdel-Aleem et al., 2010; Timmons et al., 2010). Our work highlights a largely unstudied function of the cervix, and that is as a contractile tissue. If we can...
understand the importance of smooth muscle and identify pharmacological differences between the cervix and uterus we may find therapies that can be used in the treatment of premature labor.

Limitations and Conclusion. There are limitations to our study. First, we used the longitudinal smooth muscle of the uterus, not the circular smooth muscle. Use of the isometric contractile technique limits studying this tissue in a coordinated fashion. Second, studies suggest regional differences in the upper and lower uterus (Griffiths et al., 2006), and we have used a preparation that extends into both the upper and lower uterus (but excluding the cervical uterus). A third and final limitation is that we used only carbacholyline as the contractile agonist when investigating the effects of relaxant substances. This is a reasonable choice given the stable contraction elicited by carbacholyline in uterus and cervix. Use of oxytocin would have been ideal, but we could not use this because oxytocin-induced contraction does not occur in the pregnant cervix. In summary, we present that longitudinal and circular cervix preparations contracted and relaxed to similar substances, making the important observation that the cervix did contract and relax actively. The uterus had a greater maximum contractile response to the agonists examined compared with the cervix, except for carbacholyline. Both tissues continued to contract and relax in pregnancy, but oxytocin did not contract the pregnant cervix, and isoproterenol sensitivity was reduced in these same tissues. These studies lay a foundation for studying the pharmacology of female reproductive smooth muscle function in normal situations, pregnancy, and labor with the hopes of taking advantage of differences between the uterus and cervix in the treatment of premature labor.

Authorship Contributions

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References

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