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Effect of vaginal distension on blood flow and hypoxia of urogenital organs of the female rat

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Effect of vaginal distension on blood flow and hypoxia of urogenital organs of the female rat. J Appl Physiol 98: 1884–1890, 2005. First published December 10, 2004; doi:10.1152/japplphysiol.01071.2004.—Vaginal delivery of children causes traumatic injury to tissues of the pelvic floor and is correlated with stress urinary incontinence; however, the exact mechanism of organ and tissue injury leading to incontinence development is unknown. The purpose of this project was to test the hypothesis that vaginal distension results in decreased blood flow to, and hypoxia of, the urogenital organs responsible for continence, which would suggest an ischemic and/or reperfusion mechanism of injury. Thirteen female rats underwent vaginal distension for 1 h. Thirteen age-matched rats were sham-distended controls. Blood flow to the bladder, urethra, and vagina were determined using a microsphere technique. Hypoxia of these organs was determined by immunohistochemistry. Blood flow to all three organs was significantly decreased just before release of vaginal distension. Bladder blood flow decreased further immediately after release of vaginal distension and continued to be significantly decreased 15 min after the release. Blood flow to both the urethra and vagina tripled immediately after release, inducing a rapid return to normal values. Vaginal distension resulted in extensive smooth muscle hypoxia of the bladder, as well as extensive hypoxia of the vaginal epithelium and urethral hypoxia. Bladders from sham-distended rats demonstrated urothelial hypoxia as well as focal hypoxic areas of the detrusor muscle. We have clearly demonstrated that vaginal distension results in decreased blood flow to, and hypoxia of, the bladder, urethra, and vagina, supportive of hypoxic injury as a possible mechanism of injury leading to stress urinary incontinence.

Stress urinary incontinence (SUI) is defined as involuntary urine leakage on effort or exertion or on sneezing or coughing (1). It occurs when intravesical pressure exceeds urethral resistance as a result of increased intra-abdominal pressure in the absence of a detrusor contraction. SUI is a common condition, particularly among elderly women, affecting ~35% of women over the age of 40 yr (27).

Vaginal delivery of children causes traumatic injury to tissues of the pelvic floor and is strongly correlated with later development of SUI (20, 33); however, the exact mechanism of pelvic floor organ and tissue injury leading to incontinence development is not known. Large baby weight and long duration of second stage of labor have both been correlated with incontinence development (12, 32), suggesting that tissue injury due to ischemic or reperfusion injury of pelvic floor organs and tissues could contribute to development of incontinence.

We have previously investigated the urodynamic effects of vaginal distension in rats and have demonstrated that increased duration of distension leads to greater tissue damage and increased symptoms of urethral dysfunction (7). The purpose of this project was to use this animal model to test the hypothesis that vaginal distension results in decreased blood flow to, and hypoxia of, the urogenital organs responsible for continence. A decrease in blood flow and increase in tissue hypoxia would suggest an ischemic and/or reperfusion mechanism of injury leading to incontinence development.

Materials and Methods

This project was approved by the Institutional Animal Care and Use Committee of Albany Veterans Affairs Medical Center, Albany, NY. Twenty-six female virgin Sprague-Dawley rats (200-g body weight) were anesthetized (80 mg/kg ketamine and 10 mg/kg xylazine ip). A tracheotomy was performed, a tracheal tube (PE-240) was inserted, and the rat was placed on a respirator. Both the femoral and carotid arteries were then catheterized (PE-50) for vascular access, as previously described (16).

Vaginal distension. Vaginal distension was performed as previously described (7, 11). In brief, lubricated urethral dilators of increasing size (24-Fr to 32-Fr) were inserted and removed to accommodate the vagina to larger capacities. A 10-Fr Foley catheter was then inserted into the vagina and secured with a single stitch. In 13 rats [vaginal distension (VD) group], the balloon was inflated with 3 ml of water for 1 h, chosen based on our previous work (7). Thirteen rats (sham group) underwent vaginal accommodation and catheter placement, but the Foley catheter balloon was not inflated.

Microsphere infusion. Just before the end of the 1-h vaginal distension, 1,200,000 red microspheres in 0.48 ml of water were infused into the carotid artery. Microspheres lodge in perfused tissue and can be used to measure instantaneous blood flow to a tissue via a comparison to the number of spheres in the blood (37). Blood was simultaneously withdrawn from the femoral artery and analyzed for microsphere content as a reference sample for use in calculating organ blood flow. The vaginal balloon was then deflated and removed. An identical number of pink microspheres were infused immediately after distension (VD: n = 4; sham: n = 4), 15 min after distension (VD: n = 5; sham: n = 5), or 1 h after distension (VD: n = 4; sham: n = 4). Blood was simultaneously withdrawn at the time of microsphere infusion, as above.

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Hypoxyprobe injection and dissection. Two hours before euthanasia, the rats were injected intraperitoneally with a 10 mg/ml solution (60 mg/kg) of hypoxyprobe-1 (pimonidazole, Natural Pharmacia International, Research Triangle Park, NC) in saline (37). Hypoxyprobe-1 is a substituted 2-nitrominidazole (pimonidazole hydrochloride) that is freely water soluble and widely used to demonstrate hypoxic conditions in tissues (in vivo) and cultured cells (2, 10). Once injected into a laboratory animal or human, it is rapidly distributed to all tissues in the body but only forms adducts with proteins in cells having oxygen concentrations \( \leq 10 \) Torr at 37°C.

Euthanasia of all animals was performed by pentobarbital overdose (100 mg/kg ip) 1 h after the end of vaginal or sham distension. After euthanasia, the bladder, urethra, and vagina were dissected. One-half of each organ, including both mucosa and muscle, was weighed, frozen, and saved for microsphere content analysis. In addition, one kidney was dissected, weighed, and saved for microsphere content analysis as a control organ. The other one-half of the bladder, urethra, and vagina, as well as the other kidney, were immersion-fixed in 10% neutral buffered formalin for immunohistochemical analysis of hypoxyprobe-1. The microspheres did not interfere with immunohistochemical analysis. Four noncatherized controls were injected with hypoxyprobe-1 as above, and the tissues were dissected and immersion-fixed for immunohistochemistry.

**Microsphere analysis and blood flow calculation.** Quantification of fluorescent microspheres in each blood sample and tissue specimen was performed by Interactive Medical Technologies (North Hollywood, CA). The microspheres are too large to pass through the capillary bed, and they lodge into the tissues into which they circulate (37). Therefore, the microspheres in the tissue specimens were compared with the microspheres found in the reference specimen blood specimen from the same rat to calculate the blood flow to each tissue as milliliters per minute per gram tissue. The two different colors of microspheres were identified and analyzed independently.

**Immunohistochemistry.** The fixed tissues underwent routine dehydration and embedding in paraffin in preparation for immunostaining.

Fig. 1. Blood flow to the kidney (A), bladder (B), urethra (C), and vagina (D) as a function of time after release of vaginal distension. Data at a time point of less than zero are just before release of distension. ○, Data from the sham distension group; ●, data from the vaginal distension group. Each symbol represents the mean ± SE of data from 4–13 rats. *Significant difference compared with the sham distension group at the same time point, \( P < 0.05 \).

Fig. 2. Immunohistochemistry of the kidney from a rat in the control group with no vaginal catheter insertion (a) and vaginal distension group with vaginal catheterization and balloon inflation (b). Hypoxia of \( P_{O_2} \) <10 Torr is indicated by brown diaminobenzidine (DAB) staining in the cells of the distal convoluted tubules of the nephron. Counterstain is hematoxylin. Arrows indicate cells of the distal convoluted tubules. Bar = 250 μm.
Serial 5 μM full-thickness cross sections of bladder, urethra, vagina, and kidney were cut from paraffin blocks with a microtome, and the sections were attached to positively charged slides. The sections were then deparaffinized in xylene and then rehydrated through graded ethanols.

Immunohistochemistry was performed as done previously (15, 16, 24). In brief, the sections were treated with Protease I solution (Ventana Medical Systems, Tucson, AZ) for antigen retrieval and blocked with serum-free protein block (DAKO, Carpinteria, CA). Treatment with protease does not alter the hypoxyprobe-1 signal (24). One hundred microliters of hypoxyprobe primary mouse monoclonal antibody (1:50 dilution in PBS) supplied with the hypoxyprobe-1 kit (Natural Pharmacia International) were applied for 0.5 h. Antibody binding was detected following incubation with secondary goat anti-mouse antibody (Lab Vision, Fremont, CA) biotinylated with diaminobenzidine and diluted 1:10 with rat serum (DAKO) and reagents in the Vectastain immunohistochemical staining kit (Vector Laboratories, Burlingame, CA). Immunostained sections were then lightly counterstained with hematoxylin, dehydrated through graded ethanols, and mounted with Permount mounting medium for observation. We qualitatively evaluated at least four sections from each organ.

Data analysis. Blood flow data are presented as mean ± SE for each group at each time point. VD and sham groups at each time point were compared using a Students t-test, with P < 0.05 indicating a significant difference. Immunohistochemistry data were compared qualitatively.

RESULTS

Blood flow. As expected, blood flow to the kidney was unaffected by vaginal distension. There were no significant differences in renal blood flow between the VD and sham groups (Fig. 1A). In contrast to the kidney, blood flow to the bladder was significantly decreased just before release of vaginal distension compared with the sham group (Fig. 1B). Bladder blood flow decreased further immediately after release of vaginal distension such that there was a significant decrease compared with the sham group at the same time point. Bladder blood flow continued to be significantly decreased 15 min after the release of vaginal distension compared with sham distension, suggesting that bladder blood flow recovers slowly from vaginal distension. One hour after the end of vaginal distension, blood flow to the bladder remained lower in the VD group compared with the sham group, but the difference was no longer statistically significant, suggesting a return toward the normal range of blood flow in the VD group.

Blood flow to the urethra was significantly decreased just before release of vaginal distension compared with sham distension (Fig. 1C). Urethral blood flow tripled immediately after release of vaginal distension, suggesting a rapid reperfusion as blood flow returned to the urethra after distension. This value exceeded but was not significantly different from the sham group immediately after release of distension. Both 15 min and 1 h after release of vaginal distension, blood flow to the urethra was not significantly different from sham distension at the same time points.

Blood flow to the vagina was significantly decreased just before release of vaginal distension compared with sham distension (Fig. 1D). Similar to the urethra, a tripling of vaginal blood flow occurred immediately after release of vaginal distension, suggesting a rapid reperfusion as blood flow returned to the vagina after release of distension. This value was not significantly different from the sham group immediately after release of distension. As with the urethra, 15 min and 1 h after

Fig. 3. Immunohistochemistry of the bladder from a rat in the control group with no vaginal catheter insertion (a), sham group 1 h after vaginal catheterization without balloon inflation (b), and 1 h after vaginal distension (c). Hypoxia of PO2 <10 Torr is indicated by brown DAB staining. Counterstain is hematoxylin. D, detrusor smooth muscle; U, urothelium. Bar = 250 μm.
release of vaginal distension, blood flow to the vagina was not significantly different compared with that in the sham group at the same time points.

**Hypoxia.** Kidneys from noncatheterized control animals as well as both those from rats that underwent vaginal distension and those that underwent a sham catheter insertion demonstrated hypoxia only in the distal convoluted tubules of the nephron (Fig. 2), as our laboratory has previously demonstrated in normal rats (15) and rabbits (24). The kidney was, therefore, used as a positive tissue control for immunohistochemistry experiments. The lack of a qualitative difference in hypoxyprobe staining, along with the lack of a significant difference in renal blood flow between the VD and sham groups, indicates that vaginal distension had no effect on perfusion or oxygenation of the kidney.

Bladders from noncatheterized control rats demonstrated no hypoxyprobe staining and, therefore, no or only limited hypoxia (Fig. 3a). In contrast, bladders from rats that underwent sham distension demonstrated urothelial hypoxia, as well as focal hypoxic areas of the detrusor muscle (Fig. 3b), indicating that dilating and accommodating the urethra as well as placing a catheter in the vagina, even in the absence of vaginal balloon inflation, can disrupt bladder oxygenation. Bladders from rats that underwent vaginal distension demonstrated extensive smooth muscle hypoxia (Fig. 3c), indicating that inflation of the vaginal balloon increases the hypoxic effect on the bladder.

The urethra and vagina of noncatheterized control rats demonstrated no hypoxyprobe staining and, therefore, no hypoxia (Fig. 4a and b). Sham-distended animals demonstrated hypoxia of the stratum spinosum of the vaginal epithelium and little hypoxia of vaginal smooth muscle (Fig. 4c and d). Rats that underwent vaginal distension demonstrated increased hypoxia of the stratum spinosum of the vaginal epithelium and little hypoxia of vaginal smooth muscle (Fig. 5). The urethra, in contrast, demonstrated hypoxia of the urothelium, as well as focal hypoxia of the urethral submucosa, which was greater on the posterior side than the anterior side (Fig. 5). High magnification of immunohistochemistry results reveals focal hypoxic areas between the striated muscle fibers of the external urethral sphincter (Fig. 5c).

**DISCUSSION**

Vaginal delivery of children injures the tissues of the pelvic floor and is strongly correlated with development of SUI (20, 33). In one study of over 15,000 women (32), 12.2% of women who delivered vaginally developed SUI. In contrast, only 4.7% of nulliparous women and 6.9% of women who delivered by cesarean section developed SUI (32). In addition, vaginal childbirth injures the connective tissue and muscular structures that physically support the urethra and other organs of urinary continence as well as the innervation of these organs (3, 8, 13, 23). A similar pattern of pelvic floor injuries is correlated with SUI development (3, 26), suggesting a mechanism for SUI development in parous women: vaginal delivery results in injuries to the organs, muscles, supportive tissues, and innervation of the pelvic floor, resulting in incontinence, particularly SUI. However, the mechanism of pelvic organ and tissue injury leading to incontinence development is not known.

Large baby weight and long duration of second stage of labor have been correlated with both pelvic floor injuries and SUI development (12, 20, 32). This similarity in correlative clinical events suggests a mechanism of pelvic organ and tissue injury: ischemic and/or reperfusion injury of pelvic floor organs and tissues incurred during vaginal delivery contribute to later development of SUI. Supporting this theory are data that
vaginal pressures occurring during vaginal delivery can exceed 200 mmHg, with average pressures during contractions around 100 mmHg (30, 31), exceeding the threshold pressure of 80 mmHg for ischemic damage to peripheral nerves (28, 34).

The purpose of this project was to use an animal model to test the hypothesis that vaginal distension results in decreased blood flow to and hypoxia of the urogenital organs responsible for continence. Previous work with this animal model has demonstrated that increased duration of vaginal distension leads to both increased SUI symptoms and greater tissue damage (7), suggesting an ischemic mechanism. Similar rat models of simulated vaginal delivery produce symptoms of SUI as well as injury to the striated muscles of the urethra (11, 22), decrease of ganglion cells in the vaginal neural plexuses (25), and decrease in neuronal nitric oxide synthase in the striated muscle of the urethra (38). Interestingly, pregnancy and delivery of rat pups, which have a lower baby head-to-birth-canal ratio than humans, has also been demonstrated to affect the bladder and urethra (4, 38), suggesting that these organs are particularly sensitive to the events of delivery. Decreased blood flow and increased tissue hypoxia would suggest that ischemia to the organs of the pelvic floor occurs during vaginal distension and may contribute to SUI development.

Blood flow to the bladder was significantly decreased after 1 h of vaginal distension and was maintained at significantly low levels for at least 15 min after release of distension. Bladders from rats that underwent vaginal distension also demonstrated extensive smooth muscle hypoxia, visibly more than in rats that underwent either sham distension or no catheterization. Urothelial hypoxia was also present. Interestingly, bladders from rats that underwent a sham distension demonstrated greater hypoxia than noncatheterized control rats, indicating that oxygenation of the bladder is sensitive to urethral dilation and vaginal catheter placement.

Work by Kershen et al. (21) has demonstrated that blood flow to the bladder in people is decreased by 36% at capacity, suggesting that bladder microvasculature is compressed in a thin-walled full bladder. Brading and colleagues have made similar observations in the pig, suggesting the universality of this effect (5, 19). Gabella and Uvelius (14) observed congestion of all intramural blood vessels after 6 h of complete bladder outlet obstruction in female rats, suggesting that blood flow decreases further in conditions of acute complete outlet obstruction. Blood flow decreases further with age, as does bladder contractile function (35), which likely contributes to incontinence development in the elderly.

We have gathered anecdotal evidence in this as well as previous experiments that the rats do not void or leak urine during a 1-h vaginal distension and that a flood of urine is released from the bladder when the catheter is removed from the vagina. Similarly, women do not void during labor and sometimes go into urinary retention (29, 39). This suggests that vaginal distension in rats creates a short-duration severe outlet obstruction of the bladder, resulting in ischemia and hypoxia similar to those created with a short-duration direct complete acute outlet obstruction. This can be tested by draining the bladder during vaginal distension.

In contrast to acute complete outlet obstruction, chronic partial outlet obstruction in both rats and rabbits leads to a temporary increase in blood flow to the bladder for 2 wk or 3

Fig. 5. Immunohistochemistry of the vagina (a and b) and urethra (a and c) from a rat 1 h after vaginal distension. Hypoxia of PO2 <10 Torr is indicated by brown DAB staining. Counterstain is hematoxylin. EUS, external urethral sphincter. Arrow indicates focal hypoxic area between striated muscle fibers of the EUS. a: bar = 250 μm; b and c: bar = 100 μm.
days in rats or rabbits, respectively (16). Despite the increased blood flow, extensive areas of hypoxia occur (15, 16, 36), suggesting a redistribution of perfusion in the bladder: a long-term compensatory effect not seen in our acute studies. Chronic outlet obstruction in the pig leads to decreased blood flow and oxygen tension at capacity (19), as well as increased duration of the ischemic period at capacity and during voiding (18), suggesting that the interrelationship between chronic bladder outlet obstruction, bladder blood flow, and resultant ischemia or hypoxia is a complex one.

An alternative possible mechanism is that vaginal distension temporarily obstructs the blood supply to the bladder. Blood to the bladder in rats arrives via the vesical arteries, which course near the bladder neck before entering the bladder at the trigone (9). It is possible that vaginal distension could create an obstruction of this vascular structure, causing ischemia to the bladder; however, it is located proximal to the location of vaginal distension and would not easily be obstructed by the distension. On the other hand, blood to the urethra in rats arrives via the pudendal artery (9), which could be obstructed by vaginal distension based on its anatomical location distal to the bladder. This hypothesized vascular obstruction mechanism is unlikely to explain the relatively long-lasting decrease in bladder blood flow after vaginal distension, which is more likely due to the complete outlet obstruction of the bladder caused by the vaginal distension. Blood flow to the urethra and vagina was significantly decreased by vaginal distension but recovered quickly after release of the distension, in concert with the hypothesized vascular obstruction mechanism suggested above. Immunohistochemical hypoxia data corroborated this result, demonstrating hypoxia of the stratum spinosum layer of the vaginal epithelium but little hypoxia of the vaginal submucosa and smooth muscle. It is not clear why stratum spinosum cells were more hypoxic than straturn basale cells. Both of these cells receive nutrients via diffusion, and the stratum spinosum cells are further away from the blood supply, potentially making them more susceptible to hypoxic injury.

In contrast to the vagina, the urethra demonstrated focal hypoxia of the urothelium, as well as the submucosa, smooth muscle, and potentially the striated muscle of the external urethral sphincter. There was greater hypoxia on the posterior (vaginal) side than the anterior (pubic bone) side of the urethra, suggesting that the urothelium and the urethral lumen provide some protection to the anterior urethra. The focal hypoxic areas between the striated muscle fibers of the external urethral sphincter are in the same area where our laboratory has previously observed distal nerve fascicle injury after vaginal distension (11), suggesting a hypoxic or reperfusion injury mechanism to the nerve injury.

In vitro ischemia work by Bratslavsky et al. (6) demonstrated that the rat urethra is more sensitive to ischemia than the bladder; a brief ischemic period (30 min) causes dysfunction in the urethra but not the bladder. Work with a pig model demonstrated that dysfunction due to urethral hypoxia has both a rapid component, resulting from a decrease in vascular filling of the lamina propria, and a slower phase, resulting from hypoxia-induced smooth muscle relaxation (17). This suggests that, even if the urethra undergoes less ischemia or hypoxia than the bladder, it may, nonetheless, contribute to urethral dysfunction.

In contrast to the bladder, blood flow to both the urethra and vagina tripled immediately after release of vaginal distension, suggesting that rapid reperfusion may have prevented extensive hypoxia in the urethra and vagina. Reperfusion injury, such as that seen in animal models of ischemia (6), may provide the mechanism for dysfunction in these organs that leads to symptoms of SUI in this animal model (7, 11, 25). Interestingly, although sham distension did not appear to affect mean blood flow to any of the organs studied, it induced mild hypoxia of the bladder and vaginal epithelium. This demonstrates the sensitivity of these organs to mild distension and shows that a lack of significant difference in mean blood flow does not necessarily imply that hypoxia is not present.

In conclusion, we have demonstrated decreased blood flow to, and hypoxia of, the bladder, urethra, and vagina due to vaginal distension in a rat model of simulated childbirth. This model could be used to further investigate the mechanisms of ischemic and reperfusion injury to pelvic floor organs and tissues. In addition, it could be used to investigate methods to ameliorate these effects.

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GRANTS

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