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Characterization of the vasculature supplying the genital tissues in female rats

Johanna L. Hannan, PhD^{1,3}, Geoffrey L. Cheung, MSc¹, Mark C. Blaser, BEng¹, Judith J. Pang, Med Tech (Dip)¹, Stephen C. Pang, PhD¹, R. Clinton Webb, PhD³, and Michael A. Adams, PhD^{1,2}

¹Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada

²Department of Urology, Queen's University, Kingston, Ontario, Canada

³Department of Physiology, Medical College of Georgia, Augusta, Georgia, USA

Abstract

Introduction—The internal pudendal arteries are the key resistance vessels controlling the peripheral circulatory component of sexual responses in both male and females. Previous studies in the male rat demonstrated that this vessel has markedly heightened susceptibility to vascular damage compared to other vessels in the body. Evidence suggests that the female may also be susceptible to vascular pathologies contributing to sexual dysfunction.

Aim—To characterize the anatomical, morphological and functional properties of the pudendal artery in female rats.

Methods—The pelvic arteries in young Sprague-Dawley female rats were dissected to generate a composite representation of the vascular gross anatomy. Morphometry was performed on perfusion-fixed pudendal arteries whereas others were mounted in a wire myograph to assess responses to vasoactive drugs. These measures were contrasted with a previous study examining male rats.

Main Outcomes Measured—Gross anatomy, lumen diameter, wall thickness, cross sectional area and contractile responses in the internal pudendal artery.

Results—The gross anatomy of the pudendal artery in female rats appears to parallel that found in male rats; acting as the primary feeder vessel of the clitoral, labial and vaginal tissue. Compared to the male rat, the female pudendal artery has a smaller lumen diameter (169 ± 5.7 vs $303 \pm 13.8 \mu\text{m}$), wall thickness (14 ± 0.7 vs $47 \pm 2.2 \mu\text{m}$) and cross-sectional area (8 ± 0.4 vs $52 \pm 3.4 \times 10^3 \mu\text{m}^2$). These structural differences also translate into a decreased contractile capacity of the pudendal arteries from female rats vs. males (8.1 ± 2.7 vs $20 \pm 1.4 \text{mN}$).

Conclusions—Although the gross anatomical features of the vasculature tree supplying the genital tissue in male and female rats appears to have similarities, the tissue specific properties of the vessel itself has a very different structure-function balance. We hypothesize this discordance likely reflects the very different sex-specific roles of this vessel in regulating blood flow during arousal.

Corresponding Author: Johanna Hannan, jhannan2@jhmi.edu, jlhannan@hotmail.com.

Conflict of Interest: None

Introduction

Female sexual dysfunction (FSD) is a complicated condition involving both psychological and physiological components that severely impact the quality of life in over a third of women between 18-60 years of age [1-3]. Surprisingly, more women suffer from sexual dysfunction than men, and yet less research into understanding FSD has been performed compared to erectile dysfunction. FSD can be subdivided into different classifications based on diagnostic categories of desire, arousal, orgasm, and pain [4]. Of interest to this study is female sexual arousal disorder (FSAD) which is defined as “the persistent or recurrent inability to attain or maintain sufficient sexual excitement that causes personal distress” [5,6]. Symptomatically, these women may present with complaints of diminished vaginal lubrication, increased time for arousal, diminished vaginal and clitoral sensation or vasocongestive engorgement, and difficulty with orgasm [7,8].

Components of the sexual arousal responses in females are very similar to that in males. In particular, an erection or vaginal /clitoral engorgement is a central nervous system driven event leading to increases in blood flow to the genital organs; an event that precedes arousal. This increased blood flow to the vagina, clitoris and labia is responsible for the vasocongestion, engorgement and lubrication in the sexual arousal response [9,10]. This component response is acknowledged to require a functioning neural, endocrine and vascular system. Many studies have demonstrated in both sexes that chronic disease states such as hypertension, diabetes, obesity, and atherosclerosis, which deleteriously impact the delivery of blood to the genital tissues, contribute to the incidence and severity of sexual dysfunction [11-15].

Many studies have focused on the physiological functions and structures of the end organs such as the penis, clitoris and vagina; however few have examined the vasculature supplying these tissues. In both males and females, the internal pudendal artery is the primary supplier of genital blood flow. Recently we have demonstrated the importance of the internal pudendal artery in male rats [16,17]. With aging, the pudendal artery undergoes significant vascular remodeling leading to increases in wall thickness and decreases in lumen diameter that severely impairs blood flow and causes erectile dysfunction [16]. These structural pathologies are exacerbated in hypertension as demonstrated by the marked neointimal formation confirmed in pudendal arteries of aged spontaneously hypertensive rats [17].

Multiple studies have looked into the signaling pathways and structural integrity of the vaginal wall and clitoral bodies; however only two studies have examined the physiological responses of the female pudendal artery. The pudendal artery's response to endothelin-1 and its signaling pathway was recently characterized [18]. Additionally, pudendal arteries from female type 2 diabetic rats exhibiting diminished vasocongestive arousal responses had increased constrictor sensitivity to endothelin-1 via the downstream RhoA/Rho-kinase signaling pathway [19]. The current study sought to characterize the internal pudendal artery anatomically and morphologically in the female rat. A better understanding of the pudendal artery in relation to other pelvic and perineal structures is essential in ascertaining potential sites for structural or regulatory differences [20]. The vascular structure of the artery was also examined and compared to other arterial beds. To begin to understand some of its functional properties, adrenergic-mediated contractions were assessed and compared to male pudendal contractions.

Methods

Animals

Female Sprague-Dawley rats, weighing 250-300g and 15 weeks of age (n=24) were housed in pairs in a room set at 22–24°C with a 12-hour light/dark cycle. All rats were provided with standard rodent chow and tap water *ad libitum*. All procedures were performed in accordance with the guidelines of the XXXXXXXXXX on Animal Care, the XXXXXXXXXX Animal Care Committee and the XXXXXXXXXX Committee on the Use of Animals in Research and Education.

Anatomical dissections

Female rats (n=12, Charles River Laboratories, Montreal, Quebec, Canada) were anaesthetized with sodium pentobarbital (65mg/kg, i.p.), administered heparin (1000 units/kg, i.v.), and perfused via the abdominal aorta with Coomassie blue dye to allow visualization of the pelvic and perineal vessels. The arteries in the pelvic area were carefully dissected and observations were recorded and compiled to create illustrations.

Tissue preparation and morphometry

A separate group of rats (n=5, Charles River Laboratories, Montreal, Quebec, Canada) were anaesthetized with sodium pentobarbital (65mg/kg, i.p.) followed by intravenous administration of hexamethonium and heparin (30mg/kg and 1000 units/kg, Sigma Aldrich Chemical Co., St Louis, Missouri, USA). The animals were perfused at 70mmHg through the thoracic aorta with saline (0.9% Na⁺, Baxter Corp., Mississauga, On, Canada) containing sodium nitroprusside (300mg/L, Sigma Aldrich Co.) and perfusion fixed with 2% paraformaldehyde, 2% glutaraldehyde, 4% sucrose, and 0.05% calcium chloride in a 0.1M cacodylate buffer. Segments of the thoracic aorta, first-order mesenteric branches, pudendal and clitoral arteries were carefully excised. The pudendal artery was divided into proximal (between the inferior rectal artery and perineal artery branches) and distal (between the perineal artery and lower branches to the labia and vestibule) segments and the clitoral artery was removed from within the body of the clitoris. The vessels were stored in fixative overnight, post-fixed and processed as previously described and embedded in Epon 812 [16]. Sections (1µm thick) were cut using glass knives and stained with 0.1% azure methylene blue.

Quantification of blood vessels was performed with Image-Pro Plus 6.0 (Media Cybernetics). Wall thickness and lumen diameter was measured for every octant and quadrant, respectively. Total wall cross-sectional area (CSA) was calculated by using manual and automatic edge detection in Image-Pro to produce an area of interest around the vessel wall.

Myograph experiments

Adrenergic-mediated contraction in segments of the pudendal artery was assessed in a wire myograph (Danish Myograph Technology 610M). The rats (n=7, Harlan, Indianapolis, Indiana, USA) were killed by carbon dioxide exposure, followed by diaphragm incision and the pudendal arteries were carefully removed and the distal segments were cut into 2 mm rings. These rings were mounted in a wire myograph (with 25 µm tungsten wire and bathed in physiological Krebs solution (130mM NaCl, 4.7mM KCl, 1.6mM CaCl₂, 1.17mM MgSO₄, 1.18mM KH₂PO₄, 14.9mM NaHCO₃, 5.5mM dextrose) maintained at 37°C and aerated with a mixture of 95% O₂ and 5% CO₂. The vessels were then stretched to an optimal resting tension of 1.2-1.5mN based on a passive length tension curve and equilibrated for 30 minutes. Concentration response curves to phenylephrine (PE) were recorded using Chart 5 software and Powerlab acquisition hardware (Model ML866, ADI

Instruments, Colorado Springs, CO, USA). The subsequent concentration of drug was administered once the contraction reached a plateau with the previous dose. All compounds and drugs were obtained from Sigma Chemical Co.

Data analysis and statistical procedures

The original female data in this study were compared with internal pudendal arteries from age-matched male Sprague-Dawley rats from a previous study [16]. All morphological measurements were presented as the mean $\pm$ standard error of the mean (SEM). The predicted wall thickness was determined from the line of best fit equations achieved from the relationships of the aorta, renal, mesenteric and clitoral arteries in Figure 6A. Predicted wall-to-lumen ratios were then presented as a percentage by using the predicted wall thickness and actual lumen diameter data. Contractions induced by PE were examined by measuring the maximum response to each dose and EC50 values were calculated by nonlinear regression analysis in Graph Pad Prism 5. The values were reported as mean and SEM, and compared using Student's unpaired t-tests.

Results

Characterization of the pelvic vasculature

The internal pudendal artery originates within the first half-centimeter of the medial surface of the internal iliac artery (Figure 1-4). The pudendal artery travels inferiorly above the abductor caudae internus and below flexor caudae brevis in a straight path in a caudal direction (Figure 2). This vessel is situated on the medial side of the ischium, within the ischiorectal fossa, and follows the bone to the ischial tuberosity where it turns ventrally. The pudendal is wrapped in a sheath of connective tissue along with other vessels such as the superior and inferior gluteal vessels. As the pudendal artery courses ventrally at the level of the ischial tuberosity, it is situated medial to the obturator internus muscle and lateral to the abductor caudae externus or levator muscles (Figure 1). The vessel remains ensheathed in a broad fascia which extends ventrally towards the midline where it fuses with the inferior fibers of the abductor caudae externus and in turn fuses onto the surface of the vagina. Finally, the internal pudendal artery bifurcates into two terminal branches: a posterior labial branch and an anterior clitoral branch (Figure 3). The posterior branch initially courses posteromedially before it arcs back towards the midline, remaining lateral to the anterior branch. The vessel then adopts a tortuous appearance and buries itself posteriorly, piercing what appears to be the fat and fascia surrounding the anterolateral vaginal opening.

The anterior or clitoral branch meets up with its contralateral counterpart at the midline immediately posterior to the pubis symphysis and continues ventrally to supply the clitoris (Figure 1). Both the labia and clitoral branches give off a number of smaller vessels that supply the lower vaginal wall and vestibule by dispersing into the surrounding fascia (Figures 3,4). The larger and more apparent of these vessels branch form the caudal surface of the clitoral artery and form a small plexus providing blood to the ventral surface of the vaginal wall.

The vessels that branch later from the internal iliac artery are the vaginal arteries (Figure 3). Generally, there is one major superior and inferior vaginal artery and they are immediately distinguishable by their complex branching system. The inferior vaginal branch, as its name implies, supplies the ventral wall and lateral wall of the vagina. The artery continuously bifurcates into a meshwork or plexus of arteries on the surface of the vaginal wall from the level below the cervix down to the vestibule.

The branches that travel towards the midline provide blood supply to the urethra. The more posterior branches near the vestibule anastomose and become indistinguishable from the

vessels that arise from the clitoral branch of the internal pudendal mentioned above. The laterally located branches anastomose with branches from the superior vaginal artery along the lateral vaginal wall. The superior vaginal artery supplies the dorsal wall of the vagina by giving off a number of daughter branches as well. Some of the more cranial branches of the superior vaginal artery appear to also supply the rectum which lies immediately superior to the vagina.

Morphological assessment of the internal pudendal artery

The female internal pudendal artery was determined to have a smaller lumen diameter, wall thickness and cross-sectional area compared to a first-order mesenteric artery; however possessed a greater wall-to-lumen ratio (Table 1). The mesenteric and pudendal arteries had similarly oriented smooth muscle cells that were arranged in 4-6 smooth muscle cells layers within the medial cross-sectional area. As the pudendal travels distally and becomes the clitoral artery it begins to taper slightly in both lumen diameter and wall thickness (Table 1). The smooth muscle cell orientation does not differ between the pudendal and clitoral arteries although the pudendal appears to have 2-3 more smooth muscle cell layers (Figure 5). The relationship between the wall thickness and lumen diameter in different arteries (aorta, mesenteric, clitoral) in the female rat has a linear relationship (Figure 6A). The pudendal artery from female rats has a low wall-to-lumen ratio (8.6%, Figure 6A). In females, the pudendal has a slightly higher wall-to-lumen ratio compared to other vascular beds but falls within the same slope as the relationship for other vessels. The male-female differences in the pudendal artery are emphasized when using the equations from the vascular relationships in multiple vessels to predict the wall-to-lumen ratio in the pudendal arteries (Figure 6B). The female prediction is very close to the actual wall-to-lumen ratio (7.8 vs 8.3%); conversely the male pudendal is not like the other vessels and the prediction is much smaller than the actual wall-to-lumen ratio of the pudendal (8.8 vs 15.5%).

Physiological assessment of adrenergic reactivity of pudendal arteries

Female pudendal arteries readily contracted to the adrenergic agonist phenylephrine (Figure 7). In comparison to previous data in age-matched males, the female pudendal did not achieve the same maximal contraction (22.6 vs 13.7mN, $p < 0.01$). Nonetheless, the pudendal arteries did not differ in adrenergic sensitivity as the EC₅₀s did not differ between sexes.

Discussion

The current study describes and illustrates the gross anatomy and morphology of the internal pudendal and clitoral arteries in female rats. Similar to previous findings in the male rat, the female pudendal artery provides the primary source of blood flow to the genital tissues. Branches arising from this vessel include those supplying the lower portion of the vaginal wall, the clitoris, the vestibule and the labia. Structurally the pudendal artery has a smaller lumen diameter, wall thickness and much lower wall-to-lumen ratio compared to that of the male. The lumen of this artery also tapers as it travels distally and becomes the clitoral artery. Based on its smaller wall thickness, as expected, the female pudendal artery did not contract to the same magnitudes attained by the male pudendal. Furthermore, it was not surprising to find that the sensitivity to adrenergic-mediated contraction was not different.

Many of the differences between the male and female pudendal arteries can be explained by the hemodynamic demands of their genital organs. The volume of blood and inflow pressures required to fill the penis are much greater than the demands of the female genitalia. Furthermore, various clinical studies have confirmed the difference in the volume of blood as well as the pressures achieved by the genital organs during orgasm in both sexes. In fact, the volume of blood required to fill the clitoral tissue during a sexual response is one

tenth that required to fill the penis (10ml vs 100ml) [10,21]. Furthermore, the intracavernosal pressure within the penis reaches suprasystolic values during orgasm/ejaculation, whereas the vaginal, clitoral and labial pressures only increase approximately 30-40mmHg at peak sexual response [10,22,23]. Thus the male pudendal artery needs to be able to withstand greater inflow of blood at higher pressures and these requirements are reflected in the increased wall-to-lumen ratio of the pudendal artery.

The pudendal artery in the female rat is very similar anatomically to that of women. In women, the origin of the internal pudendal artery is also located on the internal iliac artery, but appears to arise much further down after the obturator, vesicular and inferior gluteal branches [24,25]. In both species, the internal pudendal artery gives off branches supplying the labia, distal vaginal wall and terminates as the common clitoral artery with branches forming the clitoral cavernous and dorsal clitoral arteries [24,26,27]. There is also evidence in both men and women of accessory pudendal arteries which arise off the inferior vesical, obturator and external pudendal arteries and supply the genital tissues [28]. In the dissections we have performed in male and female rats we have not seen any similar arteries. The proximal vaginal blood supply in women and rats comes from the vaginal arterial branches arising from the pudendal and uterine arteries. Due to these anatomical commonalities, the rat is an excellent model from which the vasculogenic components of female sexual dysfunction can be elucidated.

Given that a sexual response is highly vascular dependent event, risk factors that impact overall cardiovascular health can also lead to sexual dysfunction. In the last decade, many studies have revealed the vasculogenic link between cardiovascular disease and erectile dysfunction [29-31]. In fact, erectile dysfunction acts as a harbinger to underlying cardiovascular disease. There are now more studies implicating risk factors such as hypertension, atherosclerosis, diabetes and obesity, smoking, high cholesterol, and aging in female sexual dysfunction [15,32-35]. In spontaneously hypertensive rats, clitoral and vaginal blood vessels and clitoral cavernous spaces had an increase in wall-to-lumen ratio and marked fibrosis due to enhanced collagen deposition [36]. Clitoral cavernous fibrosis was also demonstrated in an atherosclerotic rabbit model which had significant occlusions in the common and internal iliac and internal pudendal arteries [13]. Additionally, these atherosclerotic animals displayed diminished vaginal and clitoral blood flow and pressure [37]. Type I and II diabetic rats and mice have shown degenerative changes in vaginal and clitoral integrity leading to impaired genital blood flow [19,38-40]. The type II diabetic Goto-Kakizaki rat has increased internal pudendal artery sensitivity to endothelin-1 via the RhoA/Rho-kinase pathway and is unable to achieve genital vasocongestive arousal [19]. These findings are also present in the human population. Clinically, FSD is more prevalent in women suffering from essential hypertension, diabetes and coronary artery disease [12,41-43]. Clitoral biopsies from diabetic and aged women demonstrated ultrastructural abnormalities in the smooth muscle cells, increased intercellular collagen fibers and a decrease in smooth muscle cell numbers [44]. Women with these risk factors have difficulty achieving arousal, are less satisfied and have sex less frequently. Further studies are necessary to elucidate the mechanisms leading to structural and pathophysiological changes that are happening to these key vessels and their end organs.

Based on the similarities between the female rat and human internal pudendal vasculature, the rat appears to be a useful model to further decipher the pathophysiological mechanisms that may be leading to vasculogenic FSAD. Erectile dysfunction is an antecedent to cardiovascular disease and it is possible that FSAD could also predict undiagnosed cardiovascular disease in women. Currently there are no approved pharmacological treatments for women who suffer from FSAD. There is some off label use of hormone replacement therapies or phosphodiesterase 5 inhibitors but they have not been proven

efficacious in clinical trials. By revealing potential therapeutic targets within this key vascular bed supplying the genital tissues, a treatment to improve women's overall health and sexual function could be discovered.

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Figure 1. Illustration of the female rat pudendal artery in relation to major structures of the pelvic region. The pelvic bone was partially removed anteriorly to expose the pudendal artery.

Figure 2. Illustration of the female rat genital vasculature. The pelvic bone and abductor caudae externus muscle is cut to reveal the branches from the internal iliac artery.

Figure 3.
Illustration of the female rat genital vasculature. The abductor caudae externus muscle and the pelvic bone are cut and pulled back to reveal the vaginal vascular bed.

Figure 4.
Schematic of the left female rat pudendal artery and the vaginal arterial supply from its origin on the internal iliac artery to its destination.

Figure 5.
Light micrographs of glutaraldehyde-fixed, azure-methylene-blue-stained, pudendal and clitoral arteries in young female Sprague-Dawley rats.

Figure 6.

A) Comparison of the overall relationship (line of best fit) between wall thickness and lumen diameter in pudendal arteries (closed diamond) and the other vessels (aorta, renal and mesenteric arteries, triangles) in female (15 weeks, closed, thick dashed line) and male (open squares, solid line) rats. The wall-to-lumen relationship in male pudendals is demonstrated by the fine dashed line from a previous study [16]. **B)** Based on the equations derived from the line of best fit of the overall vessel graphs (aorta, renal, mesenteric arteries), the actual lumen diameter values of specific vessels were used to predict the wall thickness. This predicted wall thickness value was used to determine the predicted wall-to-lumen ratio and compared to the actual wall-to-lumen ratio average. Male data was taken from a previous publication [16].

Figure 7. Concentration response curves to phenylephrine (PE) in pudendal arteries from age-matched young male and female Sprague-Dawley rats. Male data from previously published results [16]. * $P < 0.01$ vs male.

Table 1

Morphometric analysis of aorta, mesenteric, pudendal and clitoral arteries of female Sprague-Dawley rats

	Aorta	Mesentery	Pudendal (Distal)	Pudendal (Proximal)	Clitoral
Lumen (μm)	1,563 $\pm$ 12.8	277 $\pm$ 23.6	153 $\pm$ 4.1	169 $\pm$ 5.7	136 $\pm$ 11.1
Wall thickness	64.6 $\pm$ 1.30	15.9 $\pm$ 0.98	12.2 $\pm$ 0.58	14.2 $\pm$ 0.68	12.4 $\pm$ 1.18
Wall : Lumen (%)	4.1 $\pm$ 0.09	5.8 $\pm$ 0.28	8.3 $\pm$ 0.40	8.6 $\pm$ 0.65	6.4 $\pm$ 1.03
CSA ($\times 10^3 \mu\text{m}^2$)	335.1 $\pm$ 10.17	14.8 $\pm$ 1.88	6.6 $\pm$ 0.38	8.2 $\pm$ 0.41	6.1 $\pm$ 0.13

Values are mean $\pm$ SEM. CSA = cross sectional area. n = 5 rats.