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Internal Pudendal Artery from Type 2 Diabetic Female Rats Demonstrate Elevated Endothelin-1-Mediated Constriction

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Abstract

Introduction—Diabetes is a risk factor for female sexual dysfunction (FSD). FSD has several etiologies, including a vasculogenic component that could be exacerbated in diabetes. The internal pudendal artery supplies blood to the vagina and clitoris and diabetes-associated functional abnormalities in this vascular bed may contribute to FSD.

Aim—The Goto-Kakizaki (GK) rat is a non-obese model of type 2 diabetes with elevated endothelin-1 (ET-1) activity. We hypothesize that female GK rats have diminished sexual responses and that the internal pudendal arteries demonstrate increased ET-1 constrictor sensitivity.

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Methods—Female Wistar and GK rats were used. Apomorphine (APO)-mediated genital vasocongestive arousal (GVA) was measured. Functional contraction (ET-1 and phenylephrine) and relaxation (acetylcholine, ACh) in the presence or absence of the ET_A receptor antagonist (ET_AR; atrasentan) or Rho-kinase inhibitor (Y-27632) were assessed in the internal pudendal and mesenteric arteries. Protein expression of ET-1 and RhoA/Rho-kinase signaling pathway was determined in the internal pudendal and mesenteric arteries.

Main Outcome Measure—APO-mediated GVAs; contraction and relaxation of internal pudendal and mesenteric arteries; ET-1/RhoA/Rho-kinase protein expression.

Results—GK rats demonstrated no APO-induced GVAs. Internal pudendal arteries, but not mesenteric arteries, from GK rats exhibited greater contractile sensitivity to ET-1 compared with Wistar arteries. ET_AR blockade reduced ET-1-mediated constriction in GK internal pudendal and mesenteric arteries. Rho-kinase inhibition reduced ET-1-mediated constriction of GK internal pudendal but not mesenteric arteries; however, it had no effect on arteries from Wistar rats. RhoA protein expression was elevated in GK internal pudendal arteries. At the highest concentrations, ACh-mediated relaxation was greater in the GK internal pudendal artery; however, no difference was observed in the mesenteric artery.

Conclusions—Female GK rats demonstrate decreased sexual responses that may be because of increased constrictor sensitivity to the ET-1/RhoA/Rho-kinase signaling in the internal pudendal artery.

Keywords

Internal Pudendal Artery; Type 2 Diabetes; Female Sexual Dysfunction; Endothelin-1

Introduction

Female sexual dysfunction (FSD) is a complicated disease composed of several subcategories, including decreased desire and arousal, orgasmic disorder, pain, and distress [1,2]. The prevalence of FSD varies depending on age and menopausal status as well as instruments used to determine FSD [3]. The complexity and unique categories of FSD has led to changes in epidemiologic data, and a recent study reveals that approximately 43% of women suffer from some form of FSD [3]. FSD is strongly correlated with several cardiovascular diseases, including diabetes. Between 14% and 51% of women with type 2 diabetes report sexual dysfunction [4]. Type 2 diabetic women present reduced desire, diminished clitoral sensation, vaginal dryness, vaginal discomfort, and orgasmic dysfunction at a higher incidence than age-matched nondiabetic women [5]. Several FSD etiologies exist, including a vasculogenic component [6–8], which may be a common link between FSD and type 2 diabetes.

Diabetic complications include dysregulation of functional vasoconstrictive sensitivity and vascular remodeling [9]. The endogenous vasoactive peptide endothelin-1 (ET-1) is known to exert a variety of effects within the vasculature, including potent vasoconstriction, profibrotic, and proliferative consequences resulting in altered vessel architecture [10]. In addition, ET-1 levels are elevated in diabetic patients [11]. Similarly, in diabetic rats, elevated ET-1-mediated constriction of resistant mesenteric and cerebral arteries has been

reported [12,13]. The Goto-Kakizaki (GK) rat is an inbred, spontaneous model of type 2 diabetes that does not present the comorbid complications of obesity or hypertension [14]. The GK rat is a mild to moderate hyperglycemic model, similar to what is observed in the majority of type 2 diabetic patients presenting FSD [5]. It has been recently demonstrated that this diabetic condition results in erectile dysfunction [15]. Thus, due to the correlation between ET-1-mediated vascular pathologies, type 2 diabetes and sexual dysfunction, it is conceivable that ET-1 plays a role in vasculogenic diabetes/FSD.

The internal pudendal artery is a critical vasculature bed responsive for delivering blood to the penis [16] as well as the clitoris and vagina [17]. Insufficient blood supply to the clitoris and vagina may contribute to the vasculogenic component of FSD [7]. We have previously shown that the internal pudendal artery is sensitive to ET-1 and that constriction is mediated through activation of the ET_A receptor (ET_AR) and the RhoA/Rho-kinase pathway [17]. The objective of this study was to determine if the female GK rat displays an impaired sexual arousal response. Additionally, this study aimed to investigate if ET-1-mediated constriction of internal pudendal arteries from diabetic rats would be elevated. We hypothesize that diabetic GK rats develop sexual dysfunction and that the internal pudendal artery displays greater ET-1-mediated constriction, via ET_AR and RhoA/Rho-kinase activation.

Materials and Methods

Drugs

The following drugs were used: ET-1, Y-27632 (Tocris Bioscience, Ellisville, MO, USA), phenylephrine (PE), acetylcholine (ACh), apomorphine (APO), ascorbic acid (Sigma Aldridge), and atrasentan (generous gift from Dr. Adviye Ergul).

Animals and Use

All experiments were performed on 22- to 26-week-old female Wistar (Harlan, Indianapolis, IN, USA) and diabetic GK (in-house bred, derived from the Tampa colony) rats [18]. All procedures were performed in accordance with the Guiding Principles in the Care and Use of Animals, approved by the Medical College of Georgia Committee on the Use of Animals in Research and Education. The animals were housed four per cage on a 12-hour light/dark cycle and fed a standard chow diet with water *ad libitum*. Blood glucose levels were collected from a small drop of blood taken from a toe-nail clip and measured with a blood glucose monitoring system (FreeStyle Lite, Alameda, CA, USA). Blood samples for hormone measurements were collected through the inferior vena cava, transferred to a heparin lined vacutubes. Rats were killed by removal of the heart and right and left ventricles were separated and weighed, and the ratio of the left ventricle/body weight was used as an index of change in cardiac structure.

APO-Induced Genital Arousal Response

Sexual arousal response followed the protocol originally reported by Beharry et al. [19]. Briefly, APO was prepared at 80 µg/mL in 100 µg/mL ascorbic acid in saline. Animals were individually housed in small, clear containers to allow for digital video recording from below. Rats were allowed 20 minutes to acclimate to darkened, quiet environment prior to

testing period. APO was injected subcutaneously (80 µg/kg), and animals were recorded for 30 minutes to observe genital vasocongestive arousal (GVA) responses, characterized by significant swelling of tissue surrounding the vagina in addition to increased introitus dimensions that persisted for 3 seconds, genital grooming and pelvic thrusts. APO additionally triggers a yawning response, an involuntary mouth opening accompanied by associated respiratory movement, which was recorded. Data are reported as number of GVAs and yawns during a 30-minute period.

Vaginal Smears

Estrous cycle state was determined by vaginal smear where characteristic cells of each stage of the cycle were examined under a light microscope after Papanicolaou staining [20].

Measurement of Serum Estradiol Levels

Blood from anesthetized rats was transferred to a heparin-vacutainer (BD) that was spun at 3,000 RPM for 10 minutes. Plasma was collected and stored at -80°C . Estradiol, progesterone, and testosterone levels were determined in duplicate in a single plate by a commercial enzyme-linked immunosorbant assay (Genway, San Diego, CA, USA).

Arterial Isolation and Force Measurement Protocol

Contractile studies of internal pudendal and third order mesenteric arteries were conducted in diabetic GK and Wistar rats. Internal pudendal and mesenteric arteries were of similar internal diameter (approximately 140 µm). Arteries were removed and carefully cleaned of connective tissue then were cut into 2 mm segments while in cold physiological salt solution (PSS) containing: NaCl, 130; NaHCO₃, 14.9; dextrose, 5.5; potassium chloride (KCl), 4.7; KH₂PO₄, 1.18; MgSO₄·7H₂O, 1.17; and CaCl₂·2H₂O, 1.6 (mM). Arterial segments were mounted using a 25 µm tungsten wire in a myograph (Danish MyoTech, ••, Denmark) in 37°C PSS continuously gassed with 95% O₂–5% CO₂. The vessels were then stretched to an optimal resting tension based on a passive length tension curve of 1.5 mN (equivalent to 90% of L100) and equilibrated for 60 minutes [16]. Then arterial integrity was assessed by contracting the segments with a depolarizing concentration of KCl (120 mM) followed by administrations of PE (10⁻⁶ M) and ACh (10⁻⁶ M) as an assessment of endothelial integrity. Cumulative concentration-response curves (CRCs) to ET-1 (10⁻¹¹ to 10⁻⁷ M) and PE (10⁻⁹ to 10⁻⁴ M) were performed on each arterial segment. Each concentration of applied agonist was allowed to plateau and was considered the maximal response for that specific concentration. Some arterial segments were incubated with vehicle (distilled water), atrasentan (an ET_AR antagonist, 10⁻⁸ M), or Y-27632 (a Rho-kinase inhibitor, 10⁻⁶ M) 30 minutes prior to the start of CRCs. Relaxation to ACh (10⁻⁹ to 10⁻⁵ M) was determined in arterial segments constricted with PE (approximately 50% of maximal contraction).

Tissue Homogenization and Protein Expression

Internal pudendal and mesenteric arteries were dissected, snap frozen in liquid nitrogen, and stored at -80°C . Samples were homogenized in radioimmunoprecipitation buffer (containing 50 mmol/L Tris-HCl, 1% Nonidet P-40, 0.25% Na-deoxycholate, 150 mmol/L NaCl, 1 mmol/L phenylmethylsulfonyl fluoride, 1 µg/mL aprotinin, 1 µg/mL leupeptin, 1

μg/mL pepstatin, 1 mmol/L sodium orthovanadate, and 1 mmol/L sodium fluoride) and sonicated on ice for 2-second bursts. Protein concentration was measured using the BCA assay (Pierce Biotechnology). Protein (20 μg) was loaded onto an SDS-PAGE 10% gel and transferred to a nitrocellulose membrane. Membranes were blocked with bovine serum albumin at 5% in Tris buffered saline (TBS) or TBS Tween-20 and subsequently probed with rabbit polyclonal anti-ET_AR and anti-ET_BR (1:200; Alomone Laboratories), mouse polyclonal anti-RhoA (1:750; BD Transduction Laboratories), mouse polyclonal anti-Rho-kinase-α and anti-Rho-kinase-β (1:250; BD Transduction Laboratories) overnight (18 hours) at 4°C. The immunostaining was detected using horseradish peroxidase-conjugated anti-rabbit IgG (1:2,000; GE Healthcare) or anti-mouse IgG (1:2,500; GE Healthcare) for 1 hour at room temperature. Bands were revealed by the SuperSignal West Pico luminol/enhancer solution (Thermo Scientific, Rockford, IL, USA) and quantified by densitometry imaging software (Un-scan-it: Gel Analysis Software, Silk Scientific Inc.). All densitometric measurements were normalized using an antibody against β-actin (1:20,000; Sigma Aldrich) as a loading control.

Data Analyses and Statistics

Statistical analyses of GVAs between Wistar and GK rats were performed using the Mann–Whitney rank sum test and Student's *t*-tests ($P < 0.05$) [21]. Arterial contractions are represented as a percentage of the maximum contraction to 120 mM KCl. Agonist concentration response curves were fitted using a nonlinear interactive fitting program (Graph Pad Prism 5.0; GraphPad Software Inc., San Diego, CA, USA). Agonist potencies are expressed as pD₂ (negative logarithm of the molar concentration of agonist producing 50% of the maximum response). Constraining curve-fit parameters were used to fit a sigmoidal curve and to determine pD₂ values for vehicle, atrasentan, and Y-27632 curves. Statistical analyses of pD₂ values were performed using *F*-test. The data are expressed as the mean ± standard error mean. A value of $P < 0.05$ was considered statistically significant.

Results

Metabolic Parameters

Female GK rats exhibited a lower body weight and elevated non-fasting blood glucose levels compared with Wistar (Table 1). No differences in the left ventricle weight (mg) to body weight (g) ratio between GK and Wistar rats was observed. Plasma estradiol was elevated in female GK rats; however, no difference was observed in progesterone or testosterone levels compared with Wistar rats.

GVAs were observed in Wistar but not in GK rats (Figure 1). APO-induced yawning responses were observed in both Wistar and GK groups, and it was significantly lower in the GK group (Figure 1, inset).

Functional Contractile Studies in Internal Pudendal and Mesenteric Arteries

Internal pudendal arteries from GK rats demonstrated an elevated constrictor sensitivity to ET-1 compared with Wistar rats, resulting in a significant leftward shift (pD₂: 9.2 ± 0.10 vs. 8.1 ± 0.11 , $P < 0.05$) in the concentration-response curve (Figure 2A). Interestingly, the GK

internal pudendal artery did not demonstrate elevated constrictor sensitivity to PE. In fact, the internal pudendal artery from female GK rats was less sensitive to PE, displaying a slight but significant rightward shift (Figure 2B; pD_2 : 7.1 ± 0.08 vs. 6.6 ± 0.10 , $P < 0.05$). In contrast, there was no hypersensitivity to ET-1 in the mesenteric arteries from GK rats (Figure 2C; pD_2 : 8.4 ± 0.09 vs. 8.2 ± 0.11). Upon stimulation with PE, mesenteric arteries from Wistar rats demonstrated augmented contraction at the highest concentrations, but no difference in pD_2 values were observed when compared with GK arteries (Figure 2D). The stage of the estrous cycle did not alter functional studies.

Atrasentan significantly reduced ET-1-induced contraction as indicated by a rightward shift in both Wistar (Figure 3A; pD_2 : 7.9 ± 0.06 vs. 7.3 ± 0.04 , $P < 0.05$) and GK internal pudendal arteries (Figure 3B; pD_2 : 9.2 ± 0.10 vs. 8.2 ± 0.19 , $P < 0.05$). Similarly, atrasentan significantly reduced ET-1-mediated contraction in the mesenteric artery from Wistar rat (Figure 3C; pD_2 : 8.4 ± 0.09 vs. 8.1 ± 0.10) as well as the GK rat (Figure 3D; pD_2 : 8.2 ± 0.11 vs. 7.5 ± 0.17 , $P < 0.05$).

Rho-kinase inhibition significantly caused a rightward shift in ET-1-mediated contraction of GK internal pudendal artery (Figure 4B; pD_2 : 9.2 ± 0.10 vs. 8.4 ± 0.10 , $P < 0.05$); however, Y-27632 did not affect ET-1 constriction in the Wistar internal pudendal (Figure 4A; pD_2 : 8.1 ± 0.11 vs. 7.9 ± 0.10) or mesenteric arteries from both groups (Figure 4C; Wistar, pD_2 : 8.4 ± 0.09 vs. 8.2 ± 0.08 and Figure 4D; GK, pD_2 : 8.2 ± 0.12 vs. 8.1 ± 0.13).

The internal pudendal artery from GK rats demonstrated greater relaxation at the highest concentrations of ACh compared with Wistar rats; however, it did not present a shift in pD_2 (Figure 5A). No difference in ACh-mediated relaxation was observed between mesenteric arteries from GK rats and Wistar rats (Figure 5B).

Protein Expression

ET_AR expression was not significantly different in Wistar and GK for internal pudendal (Figure 6A; Wistar: 1.1 ± 0.41 vs. GK: 1.4 ± 0.30) and mesenteric arteries (Figure 6C; Wistar: 0.66 ± 0.03 vs. GK: 0.67 ± 0.04). ET_BR protein expression was not different between Wistar and GK for either internal pudendal (Wistar: 1.68 ± 0.31 vs. GK: 2.19 ± 0.61) or mesenteric arteries (Wistar: 1.4 ± 0.25 vs. GK: 1.6 ± 0.12).

RhoA expression in diabetic internal pudendal arteries was significantly increased (Figure 6C; Wistar: 1.39 ± 0.25 vs. GK: 1.55 ± 0.12 ; $P < 0.05$), whereas no difference was detected in mesenteric arteries (Figure 6D; Wistar: 0.31 ± 0.025 vs. GK: 0.39 ± 0.049). Differences were not observed for Rho-kinase- α in internal pudendal (Wistar: 1.0 ± 0.31 vs. GK: 1.1 ± 0.27) or mesenteric arteries (Wistar: 1.4 ± 0.51 vs. GK: 1.2 ± 0.12). Similar results were obtained for Rho-kinase- β expression in the internal pudendal (Wistar: 1.1 ± 0.47 vs. GK: 0.7 ± 0.39) and mesenteric arteries (Wistar: 0.5 ± 0.28 vs. GK: 0.5 ± 0.29).

Discussion

The present study is the first to assess APO-mediated sexual responses and examine the internal pudendal artery, both functionally and molecularly, in female diabetic rats. We

observed that APO-mediated sexual responses were completely abolished in the female diabetic rat. Additionally, the GK internal pudendal artery displayed elevated ET-1-mediated constrictor sensitivity; however, these vessels did not demonstrate elevated adrenergic sensitivity as tested by PE stimulation. In contrast to the internal pudendal artery, diabetic mesenteric arteries, a comparable artery with regards to internal diameter and resistance, do not show an elevation in ET-1-initiated constriction. This study also demonstrated that RhoA protein expression was elevated in the diabetic internal pudendal artery. Additionally, Rho-kinase appeared to play a critical role in ET-1-stimulated contraction of the diabetic internal pudendal artery. Interestingly, ACh-mediated relaxation of the GK internal pudendal artery was greater at the highest concentrations, suggesting that a compensatory mechanism in response to the elevated ET-1-mediated contraction.

APO-mediated GVAs were observed in the Wistar but not the GK rats, whereas both GK and Wistar rats demonstrated yawns. The presence of a yawning response in the GK rats demonstrates that the drug was successfully administered and that the deficit in sexual responses could be because of an underlying pathology. These results parallel similar observations by Beharry et al. where older female rats demonstrated a significant decrease in APO-induced GVAs when compared with young rats [22]. The APO-mediated yawning response that was observed in both Wistar and GK rats was significantly less in the diabetic animal, suggesting that central mediated sexual stimulation may also be altered. As well, our observations are supported by several recent clinical studies demonstrating that diabetic women suffer from FSD [23–26]. Interestingly, Giraldi and Kristensen recently reported that women with diabetes mellitus reported sexual problems that were more related to depression and psychological factors and less on organic factors [25]. APO-mediated sexual arousal responses are generated through the actions of dopamine; however, to date, no study has investigated dopamine in GK rat brains. Nevertheless, the exact mechanisms that lead to FSD in diabetes remain elusive and may include a variety of etiologies, including a combination of central and vasculogenic sources.

Many other studies have investigated diabetes induced sexual dysfunction in male and female rats. It was recently demonstrated that male GK rats display erectile dysfunction as measured by intracavernosal pressure/mean arterial pressure responses to cavernosal nerve stimulation [15]. In female rats with type I streptozotocin-induced diabetes, pelvic nerve-stimulated vaginal blood flow was significantly reduced [27]. Alloxan-induced female diabetic rabbits demonstrate a significant reduction in nerve-stimulated clitoral cavernous blood flow, clitoral fibrosis, and decreased clitoral cavernous smooth muscle [28]. Additionally, Giraldi et al. demonstrated an attenuated norepinephrine-mediated contraction and reduced electric field stimulated relaxation of isolated vaginal strips in diabetic rats [29]. Taken together, these studies demonstrate the deleterious effects of diabetes on the genitals; however, it did not investigate a vasculogenic component, which may as well be altered. The present findings demonstrate that the genital vasculature is vulnerable to diabetes; however, further experiments may be needed directly investigating vaginal and clitoral blood flow.

The present study demonstrated that there is a direct diabetic effect on the internal pudendal artery, a crucial vascular bed for blood supply to the genital tissue. Our investigation focused on the endogenous potent vasoconstrictor peptide, ET-1. It has been previously

reported that both the internal pudendal and the clitoral arteries from female Sprague-Dawley rats demonstrate ET-1-mediated contraction via the ET_AR [17]. We observed that ET-1-mediated constriction is augmented in diabetic internal pudendal arteries (Figure 2); however, PE concentration-response curves were unchanged. These data suggest that diabetic internal pudendal arteries display agonist specific upregulation of ET-1. Elevated ET-1 vasoconstrictor sensitivity has been previously reported in isolated mesenteric arteries from male GK rats [30]. Additionally, we observed that the elevated ET-1 response was specific to the internal pudendal vascular bed, as the mesenteric arteries did not display hyper reactivity to ET-1. A similar trend was previously reported in male GK rats that demonstrated different contractile responses to ET-1 between the mesenteric and basilar arteries [9]. When the ET_AR was inhibited, a reduction in ET-1-mediated constriction was observed in both internal pudendal and mesenteric arteries, as well as in both GK and Wistar rats. However, the effect of the ET_AR antagonist was strongest in the GK internal pudendal artery. Interestingly, we did not detect elevated ET_AR protein levels, suggesting that the increased constrictor sensitivity to ET-1 may be because of signaling components downstream of the ET_AR and not on increased levels of the receptor. Nevertheless, the hypersensitivity to ET-1 observed in the diabetic female internal pudendal artery suggests that this vascular bed may be more susceptible to developing vascular pathologies and deficits in sexual function may arise prior to the development of other secondary comorbidities.

Inhibition of Rho-kinase in ET-1-mediated constriction significantly decreased the sensitivity of diabetic internal pudendal arteries to ET-1; however, not to the level obtained in the Wistar internal pudendal arteries, it suggests that ET-1 activates other factors during contraction. Independent of this, this observation suggests that the Rho-kinase plays an important role in the elevated constrictor sensitivity to ET-1 in the GK internal pudendal artery. Interestingly, there was not an observed increase in protein expression of Rho-kinase subunits α and β in diabetic pudendal arteries; however, protein expression of the upstream activator of Rho-kinase, RhoA was increased. Wingard et al. demonstrated the combined constrictor effect of ET-1, and PE was associated with a fourfold increase of activated RhoA in the corpus cavernosum of male rats [31]. Although the present study investigated concentration response curves in the presence of a single agonist, we cannot say whether this would alter constrictor sensitivity or activated RhoA levels in the internal pudendal artery, in addition to the potential differences between genders. Overall, this suggests that in GK internal pudendal arteries, augmented RhoA and Rho-kinase activation contributes to elevated ET-1-mediated constrictor sensitivity resulting in a hypersensitive contraction. Thus, these increases in ET-1, RhoA, and Rho-kinase within the diabetic internal pudendal artery could compromise the ability of this artery to supply blood to the genital tissue and contribute to diabetes-induced vasculogenic FSD.

When GK internal pudendal arteries were stimulated with ACh, we observed a greater relaxation response when compared with Wistar (Figure 5A). Similarly, in male GK rats that demonstrated erectile dysfunction, relaxation of cavernosal tissue induced by the nitric oxide donor, sodium nitroprusside, and nonadrenergic, and noncholinergic-mediated relaxation was also enhanced [15]. This increased relaxation may be a compensatory mechanism in diabetic internal pudendal artery to overcome the hypersensitivity to ET-1 and limit damage

to the vasculature and end organs. Interestingly, other GK rat studies have shown a diminished endothelium-dependent relaxation in mesenteric, carotid, and aortic arteries [32–35]. Therefore, it is clear that this model of diabetes demonstrates dynamic vascular responses, which vary both on location and function, as well as between male and female rats. Importantly, the potentiated ET-1 contractile response in this diabetic model demonstrates the profound impact of this endogenous peptide, especially because of the unimpaired vasodilation response. The role of ET-1 in diabetes has been strongly established, particularly regarding cardiovascular health, but our observation suggests that it may be playing a strong role in female sexual response as well.

Finally, estradiol levels in GK rats were significantly elevated when compared with Wistar rats. Overall, the total estradiol levels reported in these animals were low compared with what others have measured. Cushman et al. reported recently that db/db mice had significantly lower estradiol levels compared with control and that estradiol supplementation ameliorates the diabetes-induced changes in vaginal structure [36]. When blood samples were taken at sacrifice, these rats were at different stages of their estrous cycle, which may have contributed to the observed measured variability.

There are some limitations of this study. The rats were at different stages of their estrous cycle, which may have influenced the estradiol levels detected as well as alter arterial responses to ACh. However, similar experiments using the internal pudendal artery from male GK rats demonstrated a similar enhancement in ACh-mediated relaxation, and therefore may be a compensatory mechanism to the elevated contractile responses observed [15]. The functional component of this study focused on contractile mechanisms potentially involved in diabetes-mediated vasculogenic FSD and not on relaxation mechanisms. Nevertheless, we did report that ACh-mediated relaxation of the GK internal pudendal artery was greater at the highest concentrations administered and may suggest that there was an alteration in nitric oxide synthase levels from the pudendal artery, which needs to be confirmed. Further investigation into mechanisms of relaxation from the female diabetic internal pudendal artery is still needed. Finally, our data demonstrated that this model of diabetes seems to have an accelerated contractile response with a concurrent relaxant response, possibly as a compensatory mechanism. We have only investigated functional responses, molecular changes, and APO-mediated GVA at one time point. However, there may exist additional time-dependent alterations in this model and may warrant investigation over several time periods to observe the adverse effect of diabetes on the internal pudendal artery. Despite these limitations, this work establishes basic functional and molecular mechanisms within the vasculature that may alter the sexual response in females with diabetes.

The complexity of FSD creates many challenges in diagnosis and treatment. As distress is now included in the definition of FSD [3], some evidence suggests that many women may experience arousal problems; however, only a few suffer from dysfunction. Nevertheless, it is well documented that several etiologies exist in FSD including a vasculogenic component [5,7,8]. Finally, our findings support the stated hypothesis and demonstrate elevated contractile sensitivity of diabetic internal pudendal artery.

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Figure 1.

Female GK diabetic rats do not exhibit APO-induced genital vasocongestive arousal (GVA) responses. The graph shows the average number of APO-induced GVA responses in female Wistar and diabetic GK rats during a 30-minute testing period. Inset represents the number of yawns during 30 minutes. Data expressed as mean $\pm$ standard deviation; * $P < 0.05$. NR = no response. $N = 7$. GK = Goto-Kakizaki; APO = apomorphine.

Figure 2.

Internal pudendal artery from GK rats exhibits augmented contractile responses to ET-1. Contractile response of arteries from Wistar rats (○) and GK rats (●). Internal pudendal arteries were submitted to concentration-response curves to (A) ET-1 and (B) PE. Mesenteric arteries were also stimulated with increasing concentrations of (C) ET-1 and (D) PE. Experimental values are expressed as a percentage of maximal contraction to KCl (120 mM). Error bars represent the standard error of the mean. N = 7–9; **P* < 0.05 vs. vehicle for right shift in pD₂ or max response (D). GK = Goto-Kakizaki; ET-1 = endothelin-1; PE = phenylephrine; KCl = potassium chloride.

Figure 3.

Contractile responses to ET-1 are mediated through ET_AR activation in internal pudendal artery. Contractile response to ET-1 in internal pudendal (A: Wistar; B: GK) and mesenteric (C: Wistar; D: GK) arteries in the presence (Wistar: □; GK: ■) or absence (Wistar ○; GK: ●) of the specific ET_AR antagonist, atrasentan (0.01 μM), administered 30 minutes prior to the first concentration of ET-1. Experimental values are expressed as a percentage of maximal contraction to KCl (120 mM). Error bars represent the standard error of the mean. N = 7–9; *P < 0.05 vs. vehicle for right shift in pD₂. GK = Goto-Kakizaki; ET-1 = endothelin-1; KCl = potassium chloride.

Figure 4.

Internal pudendal artery from GK rats exhibits augmented activation of the RhoA/Rho-kinase pathway. Contractile response to ET-1 in internal pudendal (A: Wistar; B: GK) and mesenteric arteries (C: Wistar; D: GK) in the presence (Wistar: $\square$; GK: $\blacksquare$) or absence (Wistar $\circ$; GK: $\bullet$) of the specific Rho-kinase antagonist, Y-27632 (1 μ M), administered 30 minutes prior to the first concentration of ET-1. Experimental values are expressed as a percentage of maximal contraction to KCl (120 mM). Error bars represent the standard error of the mean. $N = 7-9$; $*P < 0.05$ vs. vehicle for right shift in pD_2 . GK = Goto-Kakizaki; ET-1 = endothelin-1; KCl = potassium chloride.

Figure 5.

ACh-induced relaxation is augmented in internal pudendal artery from GK rats. Relaxation response of arteries from Wistar (○) and GK (●) rats. PE-contracted internal pudendal (A) and mesenteric (B) arteries were submitted to increasing concentrations of acetylcholine (ACh). Error bars represent the standard error of the mean. $N = 7-9$; $*P < 0.05$ vs. vehicle for maximal relaxation initiated by each concentration. GK = Goto-Kakizaki; PE = phenylephrine.

Internal Pudendal Artery

Mesenteric Artery

Figure 6.

RhoA expression is augmented in internal pudendal artery from GK rats. Internal pudendal and mesenteric arteries were probed for ET_AR (A and C) and RhoA (B and D) protein expression from Wistar (□) and GK (■) rats. Densitometry values reported have been normalized to β -actin levels in all samples to account for differences in loading (N = 6). * $P < 0.05$ vs. Wistar. GK = Goto-Kakizaki; ET_AR = ET_A receptor.

Table 1

Metabolic parameters of female Wistar and GK rats

	Wistar (N = 8)	GK (N = 12)
Body weight, g	258 ± 20.8	226 ± 11.6*
LV/BW, mg/g	2.0 ± 0.13	2.1 ± 0.12
Blood glucose, mg/dL	160 ± 29.3	213 ± 29.2*
Plasma estradiol, pg/mL	8.1 ± 2.26	14.7 ± 2.02*
Progesterone, ng/mL	39.8 ± 5.56	39.7 ± 4.39
Testosterone, ng/mL	0.11 ± 0.017	0.12 ± 0.079

* $P < 0.05$ vs. Wistar.

Values are expressed as mean ± standard error mean.

LV/BW = left ventricle/body weight; GK = Goto-Kakizaki.