Bladder and erectile dysfunctions in the Type 2 diabetic Goto-Kakizaki rat

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Bladder and erectile dysfunctions in the Type 2 diabetic Goto-Kakizaki rat. Am J Physiol Regul Integr Comp Physiol 306: R108–R117, 2014. First published December 4, 2013; doi:10.1152/ajpregu.00033.2013.—Despite the fact that urogenito-sexual complications significantly impact the quality of life of diabetic patients, a robust in vivo experimental model is lacking. Bladder and erectile function in the Type 2 diabetic Goto-Kakizaki (GK) rat and responses to standard-of-care treatments for each disorder have been assessed. GK rats (n = 25, 18-wk-old, GK/Par colony) and age-matched Wistar rats (n = 23), characterized for their metabolic parameters, were used. Bladder function was assessed by cystometry in conscious rats treated by intravenous solifenacin (1 mg/kg). Subsequently, erectile function was assessed under anesthesia following electrical stimulation of the cavernous nerve in presence of intravesical sildenafil (0.3 mg/kg). GK rats displayed detrusor overactivity with a significant increase in frequency/amplitude of nonvoiding contractions during the filling phase, together with an increase in bladder capacity, intercontraction interval, voided volume, and maximal pressure of voiding contraction. Solifenacin significantly decreased parameters characterizing voiding contractions without modifying voiding efficiency. Erectile function in GK rats was markedly impaired and remained so after sildenafil treatment despite a significant improvement. GK rats display both bladder and erectile dysfunctions and respond at least partially to standard-of-care treatments for each disorder, thus representing a suitable model to investigate the pathophysiology and assess the efficacy of new therapeutic agents for Type 2 diabetes-associated bladder and erectile complications.

urology; sexual dysfunction; phosphodiesterase type 5 inhibitor; antimuscarinic

diabetics live decades with the disease and suffer from numerous bothersome and costly complications. With diabetes mellitus reaching epidemic proportions, the recognition of urological and sexual dysfunction, i.e., diabetic bladder dysfunction (DBD) and erectile dysfunction (ED), as common and burdensome complications of this disease is critical since more than 80% of diabetics suffer from these conditions (19). These complications have a profound effect on the quality of life of men and women with diabetes and current treatments are not satisfactory in this population, at least with respect to ED therapy. Although phosphodiesterase type 5 (PDE5) inhibitors remain the first line treatment for ED due to diabetes, the nonresponders rate among diabetic patients is particularly elevated (37 to 56% in diabetic population versus 20% in general population) (32). Moreover, very limited information regarding the natural history and the physiopathology of DBD is currently available. Indeed, research into diabetic voiding dysfunction significantly lags behind other complications of DM, thereby preventing the development of suitable treatment for DBD.

Accordingly, there is a need to improve current treatments for both ED due to diabetes and DBD, and new valuable research tools are mandatory. Indeed, most of experimental in vivo studies of bladder/erectile dysfunctions caused by diabetes have used animal models of chemically induced Type 1 diabetes models such as the streptozotocin-induced diabetic rat for which bladder and erectile dysfunctions have been well characterized (14, 15). However, evidences for a robust model for Type 2 diabetes urogenito-sexual complications are scarce.

The Goto-Kakizaki (GK) rat model is one of the best characterized animal model of spontaneous Type 2 diabetes (4, 41). Despite the fact that this diabetic rat is nonobese, it presents many similarities with Type 2 diabetic patients in term of pancreas dysfunction such as impaired glucose-stimulated insulin secretion, reduction in β-cell mass, perturbed islets microenvironment, and multiple β-cell functional common defects (41). Impaired insulin sensitivity in the liver, skeletal muscle, and adipose tissues has also been reported in GK rats. Moreover, GK rats exhibit an increased fat mass compared with lean mass, despite a lower body weight (38). This increased fat mass in GK rats compared with Wistar rats is observed until 6–8 mo of age and fails to expand with age, thereby yielding lower body weight overall. Although the GK rat is not strictly speaking a model of metabolic syndrome, it exhibits several features of metabolic syndrome as they are hyperglycemic, insulin resistant even if they are not hypoinsulinemic, they have an increased fat mass versus lean mass even if they are not obese, abnormal cholesterol levels even if their triglycerides are decreased rather than increased and exhibit a slightly increased blood pressure (13, 51) and a defective cardiac function (21, 29). Finally, diabetic nephropathy, one of the most common complications of diabetes Type 2, characterized by increased urinary protein and loss of renal function, has also been reported in the GK rat (46, 47).
While ED has been previously reported in 10- and 18-wk-old GK rats (12), previous studies investigating DBD in GK rats did not evidence major in vivo functional modifications in terms of voiding behavior or urodynamic alterations at 10 wk of age (43). Conversely, Aizawa et al. (3) reported bladder underactivity in 46-wk-old GK rats and an increase in urine residual volume was evidenced in 70-wk-old GK rat (43). In vitro, increases in responsiveness to receptor agonist-mediated contractile responses (i.e., carbachol or ATP) in 12-, 16-, 32-, or 70-wk-old GK detrusor strips have also been reported. Such an increase was associated with an upregulation of M2 and M3 mRNA muscarinic receptors in 70-wk-old GK rat bladder (43, 55).

The aim of this study was to assess bladder and erectile functions in the same 18- to 19-wk-old GK rats and the effect of standard-of-care treatments for each condition; i.e., antimuscarinics for overactive bladder (OAB) and PDE5 inhibitors for ED.

MATERIALS AND METHODS

Animals and experimental design. Male GK rats (n = 25, GK/Par colony) and age-matched Wistar rats (n = 23) were used between 14 and 19 wk of age depending on the function evaluated. Animals were housed with free access to standard chow and water and maintained on an inverted 12-h dark/light cycle (10:00/22:00). Metabolic parameters of these nonobese diabetic GK rats have been studied at 14 wk of age so as not to interfere with functional experiments on bladder and erectile function. It is to be noted, however, that the GK rat is a stable model of Type 2 diabetes in terms of glycemic control and erectile function. It is to be noted, however, that the GK rat is a stable model of Type 2 diabetes in terms of glycemic control and insulin action, only mildly worsened with age, as it was shown from 2 to 18 mo with no change in hyperglycemia and insulin resistance between 14 and 18 wk of age (10). At 16 wk of age, rats were placed for 48 h in metabolic cages with refrigerated urine collection performed during the last 24 h (Diuresis cages, Tecniplast, France). Urine samples were then retrieved, and total urine volume was recorded. The 24-h urine samples were then centrifuged, and supernatant was immediately stored at −80°C (aliquot of 500 μl) until proteinuria evaluation.

Then both bladder and erectile function were investigated on the same rat at 18- and 19-wk-old, respectively, and the effect of standard-of-care treatments for OAB and ED has been assessed. Thus four experimental groups were considered and randomized to the following treatments: Wistar or GK rats treated with either vehicle or solifenacin during cystometry experiment and Wistar or GK rats treated with either vehicle or sildenafil during erectile function evaluation.

All procedures were performed in compliance with the legislation on the use of laboratory animals (NIH Publication No. 85-23, Revised 1996) and Animal Care Regulations in force in France as of 1988 (authorization from competent French Ministry of Agriculture–Agreement No. A91-471-109, May 2009).

Metabolic parameters. Plasma glucose and insulin were measured using commercial kits (Horiba Medical and Alpeco, France, respectively). Lipid parameters [total cholesterol, high-density lipoprotein (HDL) cholesterol, free fatty acid (FFA), and triglyceride plasma levels] were determined by using colorimetric assays (Horiba Medical, Roche diagnostics, Wako Chemicals and Horiba Medical, respectively). Only total testosterone plasma levels, which is a reflection of both free, sex hormone binding globulin-bound, and albumin-bound combined were determined using a commercial Elixa kit (Demeditec Diagnostics), since measurements of free bioavailable testosterone was not available at the lab at the time of testing. Urinary protein levels were measured by colorimetric method according to the manufacturer’s instructions (Bio-Rad Protein Assay based on the method of Bradford, Bio-Rad).

Oral glucose tolerance test. After 3 h of fasting, corresponding to the postabsorptive state, blood samples were collected before and after glucose administration (2 g/kg body wt) for determination of plasma glucose and insulin. Blood samples withdrawn from the tail vein were immediately centrifuged, and the plasma was stored at −20°C until glucose and insulin assays were performed.

Intravesical and intravenous catheters implantation. Two days before the cystometry experiment, the rats were anesthetized with isoflurane (1.5–2.0%, Centravet), and their body temperature was maintained at 37°C using a homeothermic blanket. The bladder dome was exposed via a midline abdominal incision. A polyethylene catheter (PE-50: 0.965 mm OD), filled with saline solution, was then inserted within the bladder through the apex of bladder dome and secured in place with a 4-zero cotton purse-string suture. The left jugular vein was catheterized with a polyethylene catheter (PE-10: 0.28 OD) filled with heparinized saline (25 μl.ml) for intravenous treatment administration. The free ends of both catheters were tunneled subcutaneously, exteriorized at the back of the neck, and sutured between the scapulas. Each rat was maintained individually in a cage with food and water ad libitum until cystometry experiment.

Cystometry experiments. Cystometry was performed in conscious rats. The animal was placed in a metabolic cage, which enables direct measurements of micturition volumes by means of a weighing device (Sartorius BP2215 Coubart) placed underneath. The free tip of the bladder catheter was connected to a pressure transducer for bladder pressure monitoring and to a syringe pump for bladder perfusion. After a 30-min acclimation period, the bladder was continuously perfused (50 μl/min) with saline until reproducible micturition cycles were obtained. Micturition cycles were considered reproducible when the variations in micturition volumes and in intervals between micturitions did not exceed ±10%. After stabilization, solifenacin (1 mg/kg) or its vehicle was intravenously delivered (250–300 μl during 1 min), and intravesical pressure was recorded during a 60-min period. Intravesical pressure was recorded continuously using a dedicated data acquisition software (Elphy, CNRS).

The following parameters were analyzed: micturition pressure (mmHg), duration (s), and AUC (mmHg × s) of voiding contraction; basal pressure (mmHg); pressure threshold at which voiding was initiated (mmHg); intercontraction interval (s); bladder capacity (μl), infusion rate × intercontraction interval), voided volume (μl), and voiding efficiency (% as the ratio of voided volume/infused volume × 100). The amplitude (mmHg) and the frequency (contraction per minute) of the nonvoiding bladder contractions during the filling phase with an amplitude of >3 mmHg were analyzed as well as the volume threshold at which nonvoiding contractions (NVC) are elicited (% as the percentage of total bladder filling volume) as previously described (9). Effects of solifenacin are presented as percentage of control period before the administration of solifenacin or vehicle to better isolate the effect of treatment, as previously described (11).

Erectile function evaluation: electrical stimulation of the cavernous nerve. At the end of cystometry experiments, the animals were kept for an additional 5-day period until erectile function testing using a well-standardized model of erectile responses to electrical stimulation of the cavernous nerve (ES CN) under urethane anesthesia (1.2 g/kg) (25) and tissue harvesting.

Rats were anesthetized (urethane 1.2 mg/kg) and their body temperature maintained at 37°C using a homeothermic blanket. Rats were tracheotomized to prevent aspiration of saliva and when required to perform artificial ventilation. The carotid artery was catheterized with polyethylene tubings filled with heparinized saline (25 UI/ml) to record blood pressure via a pressure transducer (Elcomatic 750, Glasgow, UK). CN was exposed at the lateral aspect of the prostate, with the aid of a dissecting microscope, and mounted on a bipolar platinum electrode connected to an electrical stimulator (AMS 2100,
Phymer). After 5 min of baseline simultaneous computerized recording of mean arterial pressure (MAP) and intracavernous pressure (ICP), two stimulations of the CN at 6 V, 1 Hz, and 1 ms for 45 s were performed to elicit an increase of ICP to certify the correct implantation of the catheter. Sildenafil (0.3 mg/kg) or vehicle was then intravenously injected. Exactly 4 min thereafter the CN was stimulated (6 V, 1 ms for 45 s) at different frequencies (0, 2.5, 5, 7.5, 10, 12.5, and 15 Hz) at 3-min intervals in a randomized manner to assess the erectile responses in presence or absence of sildenafil, as previously described (8). Each ES CN was repeated twice in view of establishing a frequency-response curve for each animal. Erectile responses to ES CN were expressed as a ratio of ∆ICP (mmHg)/MAP (mmHg) × 100, IACP being the difference between ICP in the flaccid state; i.e., before stimulation and ICP during the plateau phase of the erectile response, and MAP, the mean arterial pressure during the plateau phase, and as the ratio of AUCcotl/MAP with AUCcotl, with the area under the curve during the entire erectile response, measured from the beginning of the electrical stimulation until the end of the erectile response and determined using the ICP level in the flaccid state before the onset of the stimulation, as previously described (39).

At the end of the ES CN experiments, the rats were killed with an overdose of pentobarbital and the bladder, the prostate lobes, corpora cavernosa, and the kidneys were immediately harvested and weighed. Statistical analysis. All results were presented as means ± SE. Statistical analysis for general features, metabolic and urodynamic parameters were performed using Student’s t-test. For oral glucose tolerance test and erectile responses comparisons were performed with a two-way ANOVA statistical analysis test followed by a Bonferroni’s posttest. In case of significant interaction between the two factors (i.e., frequency of ES CN and experimental group), the difference between groups of rats have been examined by the modified Student’s t-test with the Bonferroni’s adjustment for multiple comparisons. Statistical analysis was performed with GraphPad Prism 5.02 software. P values <0.05 were considered significant.

Drugs and chemicals. Sildenafil citrate and solifenacin succinate were purchased from Alsachim SAS (Strasbourg, France). All other drugs and chemicals were purchased from Sigma-Aldrich (Saint Quentin Fallavier, France).

RESULTS
Metabolic parameters and general features of the animals. Metabolic parameters for control Wistar and diabetic GK rats are summarized in Table 1. The body weight of GK rats was significantly lower than Wistar rats (P < 0.001). Proteinuria was evidenced in 24-h urines from GK rats compared with Wistar rats (P < 0.05). When compared with Wistar rats, GK rats displayed significant elevated plasma glucose levels (P < 0.001) and mildly but not significantly elevated insulin levels. GK rats also showed increased circulating levels of total cholesterol (P < 0.001) and HDL cholesterol (P < 0.001) and decreased circulating levels of triglycerides (P < 0.01). FFA levels were not significantly different between both groups. Testosterone levels were significantly decreased by 1.7-fold in GK rats compared with Wistar rats (P < 0.05).

Figure 1 illustrates changes in plasma glucose levels during OGTT in GK and Wistar rats. Glycemic responses to OGTT were significantly greater in GK rats than those in Wistar rats evidencing the impaired glucose tolerance of GK rats (P < 0.001).

Plasma insulin levels significantly decreased (P < 0.01) during the first 30 min after glucose load showing the defective first phase of insulin response to glucose in GK rats (Fig. 1, D and F).

Thus, as expected, GK rats showed hyperglycemia, impaired glucose tolerance, defective insulin response to glucose, hypercholesterolemia, and proteinuria. In accordance with previous studies describing this model (10, 38, 41, 46, 47), these results provide evidence that GK rats used in the current study represent a suitable model for Type 2 diabetes.

Bladder weights as well lateral prostate weights normalized to animal body weights did not significantly differ between Wistar and GK rats (Table 1). In contrast, ventral prostate and corpus cavernosum were significantly atrophied in GK rats compared with Wistar rats (P < 0.05 and P < 0.001). Kidney weights normalized to animal body weights were significantly increased in GK rats, which could indicate the development of a diabetic nephropathy (P < 0.001).

Cystometry experiments. Cystometrograms between GK and Wistar rats displayed marked differences as illustrated in Fig. 2. The bladder capacity and intercontraction interval were significantly increased by 67% in GK compared with Wistar rats (P < 0.001, Table 2). The micturition pressure in GK rats was also significantly increased by 14% compared with Wistar rats (P < 0.05). This increase was associated to an increase in the duration of the micturition contractions by 23% (P < 0.01) and consequently to an increase in the AUC of the micturition contraction by 67% (P < 0.001). Such increases were consistent with the significant larger micturition volume in GK rats compared with Wistar rats (P < 0.01). Basal pressure, threshold pressure necessary to elicit voiding contraction and voiding efficiency, were not significantly different between both groups.

During the filling phase, intravesical pressure oscillations before voiding were identified in both GK and Wistar rats. These oscillations were not accompanied by release of fluid from the urethra and were termed NVC. These NVCs were rhythmic and increased as the bladder filled both in terms of weight and capacity.

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<th>Table 1. General features, metabolic parameters, and organ weights of GK and age-matched Wistar rats</th>
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Data are means ± SE of n = 25 Wistar rats and n = 23 Goto-Kakizaki (GK) rats. HDL, high-density lipoproteins; FFA, free fatty acids. *P < 0.05, †P < 0.01, ‡P < 0.001, vs. age-matched Wistar rats, Student’s t-test.
frequency (+400%, \( P < 0.001 \)) and amplitude (+62%, \( P < 0.001 \)) in GK rats in contrast to Wistar rats. The volume threshold necessary to elicit the occurrence of these NVCs was significantly decreased by 29.3% (\( P < 0.001 \)) in GK compared with Wistar rats meaning that nonvoiding contractions appeared more rapidly during bladder filling in GK than in Wistar rats.

**Effect of solifenacin.** Solifenacin (1 mg/kg iv) significantly reduced the micturition pressure by 15% (\( P < 0.05 \)) and 13% (\( P < 0.01 \)), the AUC of micturition contractions by 33% (\( P < 0.05 \)) and 31% (\( P < 0.01 \)), and the duration of micturition contraction by 25% (\( P < 0.01 \)) and 11% in Wistar and GK rats, respectively (Figs. 2 and 3).

Solifenacin (1 mg/kg iv) did not elicit significant changes compared with saline on basal pressure, threshold pressure, bladder capacity, intercontraction interval, micturition volumes, or NVCs neither in GK nor in Wistar rats (\( P = \) nonsignificant, Student’s t-test, data not shown).

**Erectile function.** The erectile responses elicited by ES CN (6V, 1 ms for 45 s) were markedly decreased in GK compared with Wistar rats at frequencies above 5 Hz (Figs. 4 and 5). Nevertheless, erectile responses displayed a similar profile: a rapid increase in ICP following CN stimulation, maintained as long as the CN stimulation lasted, and at the end of the 45-s period of stimulation, ICP returned to its prestimulation level. The increases in ICP induced by ES CN were frequency dependent in both GK and Wistar rats.

ICP/MAP was significantly decreased in GK compared with age-matched Wistar rats (at 15 Hz: \(-46\%\), \( P < 0.0001 \), Fig. 5A).

**Effect of sildenafil.** Sildenafil (0.3 mg/kg iv) significantly increased the erectile response to ES CN in both Wistar and GK rats (\( P < 0.01 \)). The magnitude of this improvement was similar in both groups (Fig. 5, B and C). However, sildenafil failed to restore erectile responses in GK rats to the level of those from Wistar rats (Fig. 5A).

**DISCUSSION**

In the present study, we have evidenced that the Type 2 diabetes GK rat model displays DBD characterized by OAB and ED. GK rats were responsive to the mainstay of oral pharmacotherapy currently used to treat OAB since solifenacin, the antagonist of muscarinic receptor, reduced the enhanced micturition detrusor contractions in GK rats. Conversely, the PDE5 inhibitor sildenafil failed to restore normal erectile function in GK rats despite enhancing somewhat their erectile responses elicited by ES CN.

DBD encompasses a spectrum of clinical symptoms due to a wide array of disorders ranging from bladder overactivity to impaired bladder contractility. A review of urodynamic profiles in diabetic patients (\( n = 182 \)) revealed that 55% had detrusor overactivity, 23% had impaired detrusor contractility, 11% had indeterminate findings, 10% had detrusor areflexia, and 1% was normal (30). Other urodynamic studies also reported that detrusor overactivity was the predominant profile observed in diabetic patients (7). Cystometrograms performed in 18-wk-old GK rats revealed involuntarily and spontaneous nonvoiding contractions during the filling phase of the micturition cycle, which are characteristic of detrusor overactivity as defined by the International Continence Society (1). The GK
DBD at 18 wk of age thus mimics the most common bladder dysfunction pattern described in the diabetic population; i.e., bladder overactivity associated with detrusor overactivity. In addition, GK rats displayed increased micturition contractions associated with increased micturition volumes as observed in patients with OAB, as well as higher intercontraction interval between two micturition events than in control rats reflecting an increase in bladder capacity as previously observed in diabetic patients (7).

Given the mixed clinical picture of DBD in diabetic patients and the fact that bladder function impairment in streptozotocin (STZ) rats is time dependent, Daneshgari et al. (17, 18) proposed a temporal theory for DBD pathophysiology. In an early phase, hyperglycemia could induce an increased compensatory bladder function with increased detrusor contractility and overactive bladder followed by a later phase, in which bladder tissue and function decompensate yielding a hypococontractile bladder. According to this theory, 18-wk-old GK rats might be in the early phase of DBD pathophysiology with an overactive bladder profile. Interestingly, a very recent longitudinal study in GK rats has reported a normal urodynamic profile in 10-wk-old GK rats, thereby indicating that DBD requires some time to set in, and evidences of bladder underactivity in 46-wk-old GK rats, thereby supporting this temporal theory for DBD pathophysiology (3). To establish the mechanisms responsible for the onset, the development, and the progression of DBD, the GK rat model represents a useful experimental model mimicking the various clinical symptoms reported in diabetic men.

In nondiabetic male patients, the presence of bladder outlet obstruction (BOO) mostly due to benign prostatic enlargement can be responsible for overactive bladder, resulting in both irritative (or storage) and obstructive (voiding) lower urinary tract symptoms (LUTS). Such is not the case in diabetic patients, since BOO has also been reported but with a lower...
incidence than in the nondiabetic patient population (28% vs. 70%) (2, 7). In line with this lower incidence, GK rats in the present study have a ventral prostate weight that is inferior to the Wistar. Despite this lower incidence of BOO, however, diabetes is associated with an increased incidence of LUTS. This likely indicates that the main culprit in LUTS in male diabetic patients may be bladder dysfunction due to the alteration in the detrusor smooth muscle, neuronal dysfunction, and/or urothelial dysfunction associated to diabetes (31, 37, 44, 45).

Most previous studies in diabetic rats with in vivo cystometry to assess DBD have been performed using the model of STZ-induced Type 1 diabetes. Consistent with the present results in GK rats, an increase in micturition pressure, bladder capacity, micturition volume, spontaneous activity during filling, and no change in basal and threshold pressure were reported in STZ rats (15, 19). However, very few studies investigating bladder function in Type 2 diabetes models have been performed, and none to date have studied the efficacy of antimuscarinics. In the spontaneously diabetic Torri model, the intercontractation interval between micturitions as well as the micturition pressures were increased in 22- and 36-wk-old rats (36). A previous cystometric study performed in GK rats did not find any differences in micturition pressure and voided volume at 12- or 70-wk-old (43), whereas, consistent with the present study, 16- or 32-wk-old GK rats had increased detrusor contractile responses to carbachol or ATP (55). Very recently, another study using GK at 10 and 46 wk of age reported sensory bladder dysfunction associated with diabetes Type 2, along with urodynamic features characterisics of bladder underactivity at 46 wk of age (decreased micturition pressures and detrusor contractile responses to carbachol, but without significant increases in mean postvoid residual volumes or decrease in mean urinary flow rate). At 46 wk of age, these GK rats did not display any nonvoiding contractions. Conversely, at 10 wk of age, there was no notable urodynamic modification in these rats (3). Such discrepancies may be due to differences in rat strain since the colonies used differed between these studies. Alternatively, they may result from the natural history of lower urinary tract dysfunction in this experimental model, as is also described in diabetic patients, thereby again highlighting the temporal theory of DBD pathophysiology. Finally, voiding function has previously been studied by cystometry in obese and diabetic female ZDF rats after 10 wk on a high-fat diet, highlighting the decrease of both nonvoiding contractions, voiding frequency, and residual volume, although early onset of diabetes in this model may not have been responsible for the deleterious effects on lower urinary tract function but additional to those attributed to obesity (23). The various phenotypes reported for each considered experimental model highlights this importance of a careful characterization of the model.
before use to best exploit its features. However, this variety of bladder disorder profile is not surprising because DBD in diabetic patients is also multifaceted.

Antimuscarinics are standard of care for OAB but their tolerability profile is not optimal (5). Blockade of the postganglionic muscarinic receptors of the detrusor smooth muscle, thereby preventing acetylcholine-induced bladder contraction, is likely the primary mechanism of action for antimuscarinics in OAB (5). Thus the common urodynamic effect of antimuscarinics; i.e., solifenacin is to decrease intravesical pressure in patients with OAB (49). Results of the present experiments are coherent with the clinical situation, since solifenacin significantly decreased the parameters characterizing bladder contractions; i.e., micturition pressure, duration, and AUC of bladder contractions in both normal and diabetic rats without impacting voiding efficiency, suggesting the absence of urine retention side effect. Interestingly, the expression of the muscarinic receptors M2 and M3 was shown upregulated in 70-wk-old but not in 12-wk-old GK rats (43). Thus antimuscarinic effects on urodynamic parameters could even be greater in GK rats older than 18 wk old, deserving further investigations. All in all, the response to solifenacin in GK rats reported in the present study is in accordance with the reported efficacy and current guidelines proning antimuscarinics as the cornerstone treatment for diabetic patients presenting overactive bladder (26).

Diabetes is a major risk factor for ED in men (34). In diabetic patients, the prevalence of ED is increased with age, poor glycemic control, and duration of diabetes (22). In the present study, erectile responses following electrical stimulation of the cavernous nerve in GK rats were markedly impaired. Previous studies using models of obesity or Type 2 diabetes have been previously published, although methodologies employed differed somewhat from the one used in the present paper, thereby yielding different claims. In BBZ/WOR rat, a congenital model of Type 1 and 2 diabetes, penile reflexes were lost, but with no impairment of the response to electrical stimulation, hence casting some doubts on the usefulness of this model (52). On the other hand, Kovanez et al. (33) failed to report a significant reduction in the erectile responses to papaverine after 9 wk of diabetes compared with age-matched lean Zucker rats. However, after 4.5 mo of diabetes, a corporal venoocclusive dysfunction was detected in these Zucker fa/fa rat, by evaluating the fall in intracavernosal pressure from 100 mmHg within 1 min after corporal saline infusion compared with rats at 9 wk of age. It is to be noted that in this study, age-matched lean Zucker rats were not provided for this comparison. In accordance with our study, Carneiro et al. (12) have reported reduced erectile responses in the GK rat following electrical stimulation. However, caution must be exercised in interpreting their data since frequency-response curves of erectile responses were obtained by cumulative electrical stimulations of the cavernous nerve every 45 s, thus not allowing detumescence of the penis and a return to flaccid ICP between two stimulations. This major difference in the experimental setting, apart from the anesthetic applied, impairs the comparison interstudy. Nonetheless, they show a significant reduction of the AUC at 12 Hz of ~55% whatever the age of the GK rat considered, which is in accordance with our paper (~46% at 15 Hz). Interestingly, Wingard et al. (54) have evaluated the erectile responses of Zucker Diabetic Fatty fa/fa rats at 16–20 wk of age compared with male lean counterparts and have also reported a reduction of 40% of the electrically elicited erectile responses at stimulation voltages greater than 2 V. It is to be noted, however, that we have never been able to reproduce these results in house (Behr-Roussel D, Mevel K, Bernabe J, Giuliano F; unpublished results). Because these ZDF rats were not obtained from the same provider, this discrepancy may better reflect a genetically driven phenomenon rather than an effect of induced diabetes. Finally, the db/db (obesity and Type 2 diabetes caused by a leptin receptor mutation) mouse strain was also described to exhibit signifi-

Fig. 4. Representative tracings of blood and intracavernous pressure recordings in anaesthetized 19-wk-old GK rat (A) and age-matched Wistar rat (B). Arrows correspond to each electrical stimulation of the cavernous nerve (6 V, 1 ms for 45 s) at different frequencies (0, 2.5, 5, 7.5, 10, 12.5, and 15 Hz) at 3-min intervals in a randomized manner. Mean arterial pressure for GK and Wistar were 79.5 ± 2.1 vs 87.1 ± 3.6 mmHg; P = nonsignificant, Student’s t-test.
Fig. 5. Erectile responses elicited by cavernous nerve stimulation at increasing stimulation frequencies in anaesthetized Wistar rats (n = 9 treated with saline and n = 8 treated with sildenafil 0.3 mg/kg iv) and GK rats (n = 10 treated with saline and n = 11 treated with sildenafil 0.3 mg/kg iv) reported as intracavernosal pressure/mean arterial pressure (∆ICP/MAP) rise. Basal flaccid ICP values were 7.35 ± 0.82 mmHg in Wistar rats compared with 7.96 ± 0.83 mmHg in GK rats (ns, Student’s t-test). Data are means ± SE of experiments performed. ###P < 0.0001, Two-way ANOVA vs. Wistar-saline followed respectively by post hoc modified Student’s test if interaction or Bonferroni’s: $tP < 0.05$; ###$P < 0.001$, ##$P < 0.0001$. ***P < 0.0001, two-way ANOVA vs. GK-saline followed by post hoc Bonferroni’s: $xP < 0.05$.

The first-line treatment for ED in diabetic men is PDE5 inhibitors (27) even though the overall response rate is less than 50% (22, 40). In fact, efficacy of the PDE5 inhibitor sildenafil in preclinical models has not been reported in previous studies. In our study, sildenafil improved erectile responses of GK rats but without achieving a similar level of erectile responses observed in Wistar rats. To be noted, the pro-erectile facilitator effect of sildenafil in Wistar rats was of comparable amplitude to the one reported in GK rats, possibly because Wistar rats already fully responded to ES CN and thus could not respond much more to sildenafil (50). Of importance, it might be argued that a higher dosing than the one tested (0.3 mg/kg) could have had greater potentiating effects on erectile function. However, increasing the dose of sildenafil will notably decrease the arterial pressure, as this agent provokes clear drops in blood pressure. In our set of experiments, it was already determined that MAP in GK rats dropped from 82 to 67 mmHg within the first minutes of sildenafil intravenous (slow) injection. Increasing the dosage of sildenafil might thus introduce a confounding blood-lowering effect, thus masking the pro-erectile effect of this drug given the relationship between MAP and erectile responses (24). Alternatively, Carneiro et al. (12) did not observe changes in neuronal nitric oxide synthase (nNOS) protein expression but reported a decreased expression of endothelial NOS (eNOS) protein in cavernosal tissue from 1-wk-old GK rats. Given the fact that penile erection relies mainly on coordinated activation of both nNOS and eNOS (28), this defect may well explain the reduced erectile responses to ES CN in GK rats. Moreover, Cho et al. (14) demonstrated that the response to PDE5 inhibitors could depend on the stage of development of ED. In 8-wk-old STZ-induced diabetic rats, impaired erectile function was restored by PDE5 inhibitors but not in 10-wk-old or older diabetic rats. In the present study, the decrease in erectile responses of GK rats might not have been severe enough to fully abolish the proerectile effect of sildenafil. Again, evaluation of erectile function and the effect of PDE5 inhibitors in older GK rats should be interesting to evidence a potential greater failure to respond to PDE5 inhibitors. However, by no means did sildenafil (0.3 mg/kg) restore the erectile responses to ES CN in these GK rats, thereby reflecting the clinical overall poor response rate to PDE5 inhibitors in diabetic patients (40).

The effects of androgens on male desire/interest and sexual behavior are well established. One report has shown direct cavernosal dependency on androgens (34). Hypogonadism has been proposed as a pathological factor contributing to ED in diabetic patients (16). It has been found that diabetic patients with ED have lower testosterone levels (20). Interestingly and in line with these data, we found that ED in GK rats is associated with lower total testosterone levels than Wistar rats suggesting that this deficiency could be involved in the impaired corporal smooth muscle relaxation as previously described in STZ rats (56). Moreover, this decrease in total testosterone levels in GK rats was associated with an overt biological condition of hypogonadism since the weight of androgen target tissues, i.e., corpus cavernosum and prostate, were decreased. Although it would be of interest to report free testosterone levels in this model in future experiments to confirm these data, the observed hypogonadism in the GK rat may well be involved in the pathophysiology of ED, such as is seen in Type 2 diabetic patients. Furthermore, this corroborates the fact that androgens are involved in every aspect of prostate development, growth, and function from early in male embryogenesis to prostatic hyperplasia in aging men and dogs. Indeed, androgen deprivation at any phase of life causes a decrease in prostate cell number and DNA content (53). Moreover, only the ventral lobe of the prostate was decreased in the GK rat, which is in line with several papers describing the fact that there is a differential regulation of androgen receptors depend-
ing on the lobe considered (42). In fact, a lower rate of cell death consecutive to aging was observed in the lateral lobes of 24-mo-old castrated Brown Norway rats compared with the ventral lobe, thereby highlighting that the regression of the prostate is lobe specific (6, 48).

In conclusion, the present study demonstrates that 18-wk-old male GK rats have urogenito-sexual dysfunctions or alterations which are similar to male diabetic patients. They display DBD characterized by detrusor overactivity, an increase in bladder capacity and micturition pressures, and ED associated to hypogonadism. Furthermore, standard-of-care treatment for bladder overactivity is effective in GK rats, while standard-of-care treatment for ED does not restore normal erectile function.

Perspectives and Significance

Because of the many similarities found in the GK rats with respect to diabetes-related urogenital complications, it represents a promising research model to better understand the pathophysiology of Type 2 diabetes-associated bladder and erectile dysfunctions. A longitudinal study of these dysfunctions in GK rats is now warranted to continue the exploration of the pathophysiological mechanisms responsible for the onset, the development and the progression of DBD and ED. Furthermore, the response of GK rats to standard-of-care treatments allows its future use to assess efficacy of new therapeutic agents targeting one or both complications.

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