Bladder and erectile dysfunctions in the type 2 Diabetic Goto-Kakizaki rat

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OBJECTIVES

- Urological functional complications such as bladder and erectile dysfunctions (ED) significantly impact the quality of life of diabetic patients.
- Most of experimental in vivo studies of ED/bladder dysfunction caused by diabetes have used type 1 diabetes models.
- A robust model for type 2 diabetes urological complications is lacking.

Aim of the study:

- **Evaluate bladder and erectile function in the Goto-Kakizaki (GK) rat model for type 2 diabetes**
- **Evaluate the responses to standard-of-care treatments for overactive bladder and erectile dysfunction in GK rats**

MATERIALS & METHODS

Summary of research design

Experimental groups

Groups	*Strain*	*Cystometry acute iv treatment*	*Erectile function experiments acute iv treatment*
Control	*Wistar*	Vehicle (n=12) or Solifenacin (n=9)	Vehicle (n=9) or sildenafil (n=8)
Diabetic	*Goto-Kakizaki (18 weeks)*	Vehicle (n=9) or Solifenacin (n=10)	Vehicle (n=10) or sildenafil (n=11)

•Male GK rats (n=25, GK/Par colony) and age-matched Wistar rats (n=23) were used between 14 and 19 weeks of age depending on the function evaluated. Metabolic parameters of these non obese GK rat model of type 2 diabetes compared to Wistar rats have been characterized at 14 weeks of age.

All procedures are performed in compliance with the legislation on the use of laboratory animals (NIH publication N°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).

Cystometry experiments

Cystometric investigation was performed in conscious rats. A bladder catheter, implanted 48h before experiment, was connected to a pressure transducer for bladder pressure monitoring and to a syringe-pump for bladder perfusion. The bladder was continuously perfused (50 µl/min) with saline. After stabilization, solifenacin (1mg/kg) or its vehicle was administered by i.v route (250-300 µL during 1 min) and intravesical pressure was recorded during another 60 min period.

The following parameters were analyzed: micturition pressure (mmHg), duration (s) and AUC (mmHg x s) of voiding contraction; basal pressure (mmHg); pressure threshold at which voiding is initiated (mmHg); intercontraction interval (s); bladder capacity (µl, infusion rate x intercontraction interval), voided volume (µl) and voiding efficiency (%, as the ratio of voided volume/infused volume x100). The amplitude (mmHg) and the frequency (contraction per minute) of the non-voiding bladder contractions during the filling phase with an amplitude of >3 mm Hg were analyzed as well as the volume threshold to elicit NVC (percent of total bladder-filling volume).

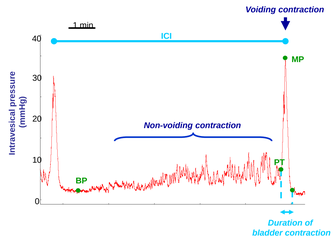


Illustration of the parameters computed for bladder voiding contraction and non-voiding contractions on a cystometrogram.

Erectile function evaluation: electrical stimulation of the cavernous nerve

After 5 minutes of baseline recording of simultaneous computerized measure of mean arterial pressure (MAP) and intracavernous pressure (ICP), the CN was stimulated (6 V, 1 ms for 45 s) at different frequencies (0, 2.5, 5, 7.5, 10, 12.5 and 15Hz) at 3-minute intervals in a randomized manner in order to assess the erectile responses. Erectile responses to ES CN were expressed as a ratio of ICP (mmHg) / MAP (mmHg) x 100, ICP 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 AUCtot / MAP with AUCtot, 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

Statistical analysis

All results were presented as mean ± SEM. Statistical analysis for general features, metabolic and urodynamic parameters were performed using Student's t-test. For OGTT and erectile function evaluation, comparisons of frequency-response curves were performed with a two-way ANOVA statistical analysis test followed by a Bonferroni's post-test. In case of significant interaction between the two factors (i.e. frequency of ES CN and experimental group), the difference between groups of rats will be 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.

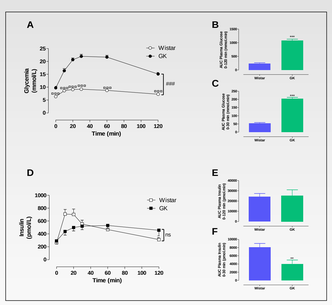
RESULTS

Metabolic parameters and general features of the animals

General features and metabolic parameters of GK and age-matched Wistar rats

	Wistar	GK
Metabolic parameters		
Body weight (g)	415.1±5.8	356.8±4.1***
Glycemia (mmol/L)	6.13±0.17	9.71±0.63***
Insulinemia (pmol/L)	264.12±25.20	299.40±46.15
Triglycerides (mmol/L)	2.05±0.14	1.46±0.09**
HDL cholesterol (mmol/L)	1.25±0.03	1.74±0.03***
Total cholesterol (mmol/L)	1.66±0.05	2.14±0.04***
FFA (mmol/L)	0.55±0.02	0.47±0.03
Diuresis (ml/24h)	10.6± 0.6	10.4±0.9
Testosterone (ng/mL)	5.15±0.85	3.10±0.25*

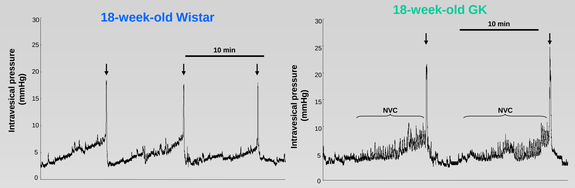
HDL: high density lipoproteins; FFA: free fatty acids.
*Data are the mean ± SEM of n= 25 Wistar rats and n= 23 GK rats. *p< 0.05, **p<0.01, *** p< 0.001, versus age-matched Wistar rats, Student's t-test*



*Blood glucose (A) and blood insulin levels (D) in 18 weeks GK rats and in age-matched Wistar rats 0, 10, 20, 30, 60 and 120 min after oral glucose challenge (2g/kg body weight). Area under the curve (AUC) of glucose or insulin time course curves from 0 to 120 minutes (B and E, respectively) or 0 to 30 minutes (C and F, respectively). Data are mean ± SEM of experiments performed in Wistar rats (n= 25 animals) and GK rats (n= 23 animals). ###p<0.001 two-way ANOVA with interaction; followed by post hoc modified Student's test ===p< 0.001, Wistar versus GK. ns: non significant, two-way ANOVA analysis. **p<0.01, ***p<0.001, Student's t-test.*

➡**As expected, diabetic GK rats showed hyperglycemia, hyperinsulemia, hypercholesterolemia, and impaired glucose tolerance. In accordance with previous studies describing this model, GK rats used in the current study represent a suitable model of type 2 diabetes to investigate urological complications.**

Urodynamic evaluation of GK rats



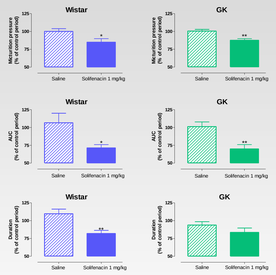
Representative tracing of cystometrogram of conscious 18 weeks GK rat and age-matched Wistar rat. Arrows represent micturition contractions. NVC: non- voiding contractions characteristic of detrusor overactivity.

Comparison of urodynamic parameters between Wistar and GK rats

	Wistar (n=21)	GK (n=20)
Micturition pressure (mmHg)	20.8 ± 0.8	23.7 ± 1.0 *
AUC micturition (mmHg x s)	114.6 ± 9.1	191.5 ± 13.4 ***
Duration micturition (s)	22.3 ± 0.9	27.4 ± 1.5 **
Threshold pressure (mmHg)	9.6 ±0.6	8.8 ± 0.6
Basal pressure (mmHg)	5.9 ± 0.6	4.5 ± 0.4
Intercontraction interval (s)	443.7 ± 36.5	743.3 ± 58.2 ***
Bladder capacity (µl)	369.8 ± 30.5	619.4 ± 48.5 ***
Micturition volume (µl)	371.0 ± 44.1	636.9 ±65.1 **
Voiding efficiency (%)	97.6 ± 7.1	101.6±3.6
Amplitude of non-voiding contractions (mmHg)	3.1 ± 0.3	5.0 ± 0.4 ***
Frequency of non-voiding contractions (contractions per min)	0.3 ± 0.1	1.5 ± 0.2 ***
Volume threshold to elicit non-voiding contractions (%)	92.2 ± 1.6	65.2 ± 4.6 ***

➡**The type 2 diabetes GK rat model displays severe diabetic bladder dysfunction characterized by bladder overactivity. They display increased micturition pressures, increased bladder capacity and detrusor overactivity**

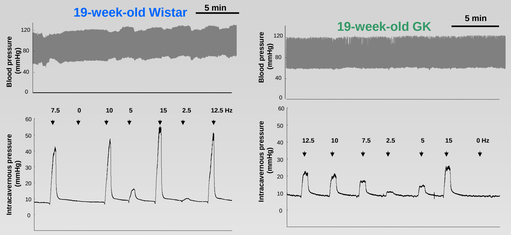
Effect of i.v solifenacin (1 mg/kg) or saline on urodynamic parameters characterizing micturition contraction in Wistar and GK rats.



*Data are mean ± SEM of experiments performed in Wistar rats (n= 12 and 9 animals injected with vehicle and solifenacin respectively) and GK rats (n=9 and 10 animals injected with vehicle and solifenacin respectively). *p<0.05; **p<0.01 versus saline group, Student's t-test.*

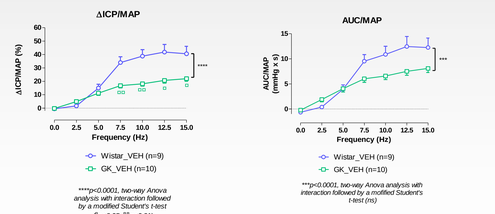
➡**Solifenacin (1 mg/kg) inhibited the parameters characterizing the micturition contraction in either GK or Wistar rats compared to saline injection without impacting voiding efficiency**

Erectile function evaluation of GK rats



Representative tracing of blood and intracavernous pressures recording in anaesthetized 19 weeks GK rat and age-matched Wistar rat

Erectile responses elicited by cavernous nerve stimulation at increasing stimulation frequencies in anaesthetized Wistar and GK rats.

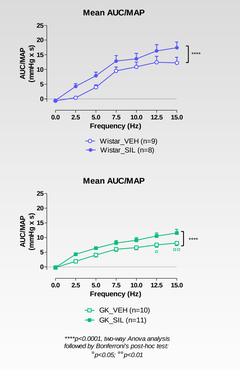


*****p<0.0001, two-way Anova analysis with interaction followed by a modified Student's t-test (**p<0.05, ***p<0.01)*

****p<0.0001, two-way Anova analysis with interaction followed by a modified Student's t-test (ns)*

➡ **The erectile responses elicited by electrical stimulation of the cavernous nerve (6V, 1 ms for 45s) were considerably decreased in GK compared to Wistar rats at frequencies above 5 Hz**

Effect of i.v sildenafil (0.3 mg/kg) or saline on erectile responses elicited by cavernous nerve stimulation at increasing stimulation frequencies in anaesthetized Wistar and GK rats



*****p<0.0001, two-way Anova analysis followed by Bonferroni's post-hoc test*
**p<0.05, **p<0.01*

➡**Sildenafil (0.3 mg/kg) significantly increased the erectile response to ES CN in either Wistar or GK rats. The magnitude of this improvement was similar in both rats: at 15 Hz, AUC/MAP was increased by approximately 30% in both strains.**

CONCLUSIONS

- The present study demonstrates that GK rats have many pathophysiological features in term of urological complications which are common to diabetic patients. They display diabetic bladder dysfunction characterized by detrusor overactivity, an increase in bladder capacity and micturition pressures. These rats also have an associated erectile dysfunction.
- Furthermore, standard of care treatments for both disorders are effective in GK rats.
- Thus, GK rats represent a suitable and validated research model to better understand the pathophysiology of type 2 diabetes-associated bladder and erectile complications and to assess efficacy of new therapeutic agents targeting diabetic bladder and/or erectile dysfunctions.