

# Targeting both the dynamic and static components of LUTS/BPH using a soluble guanylate cyclase stimulator compared to vardenafil: preclinical evidences

D.Behr-Roussel<sup>1,2</sup>, A. Oudot<sup>1,2</sup>, S. Oger-Roussel<sup>1,2</sup>, D. Gorny<sup>1,2</sup>, P. Sandner<sup>3</sup>, J. Bernabé<sup>1,2</sup>, L. Alexandre<sup>1</sup>, F. Giuliano<sup>2,4</sup>

<sup>1</sup> Pelvipharm, Orsay, France; <sup>2</sup> EA 4501 Université Versailles Saint Quentin en Yvelines, Garches, France; <sup>3</sup> Bayer Pharma AG, Wuppertal, Germany; <sup>4</sup> AP-HP, Neuro-Uro-Andrology, Dept. of Physical Medicine and Rehabilitation Raymond Poincaré Hospital, Garches, France



## BACKGROUND

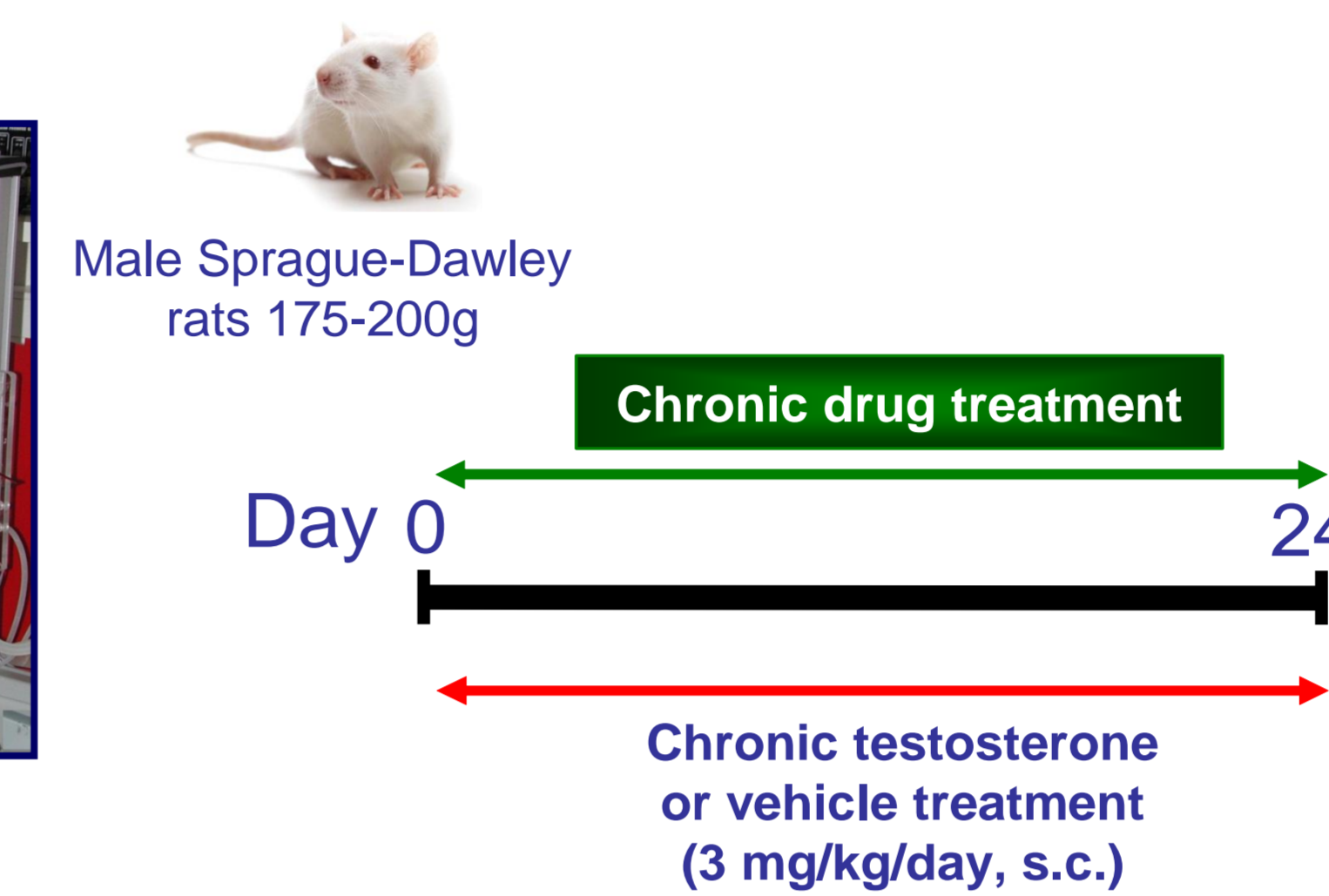
In randomized clinical trials, PDE5 inhibitors improve LUTS related to BPH.

Soluble guanylate-cyclase (sGC) stimulators bypass the need for an NO drive while activating the same signaling pathway. We have assessed and compared BAY 41-2272, a sGC stimulator, to vardenafil with respect to their relaxing effects on human prostate and their antiproliferative / pro-apoptotic effects on prostate growth induced by testosterone supplementation in the rat.

## OBJECTIVES

- To assess and compare BAY 41-2272, a sGC stimulator, to vardenafil with respect to their relaxing effects on human prostate
- To evaluate quantitatively the chronic effects of the sGC stimulator, BAY 41-2272, and vardenafil on the prostatic expression of proliferation and apoptosis markers in the testosterone-induced rat model of BPH

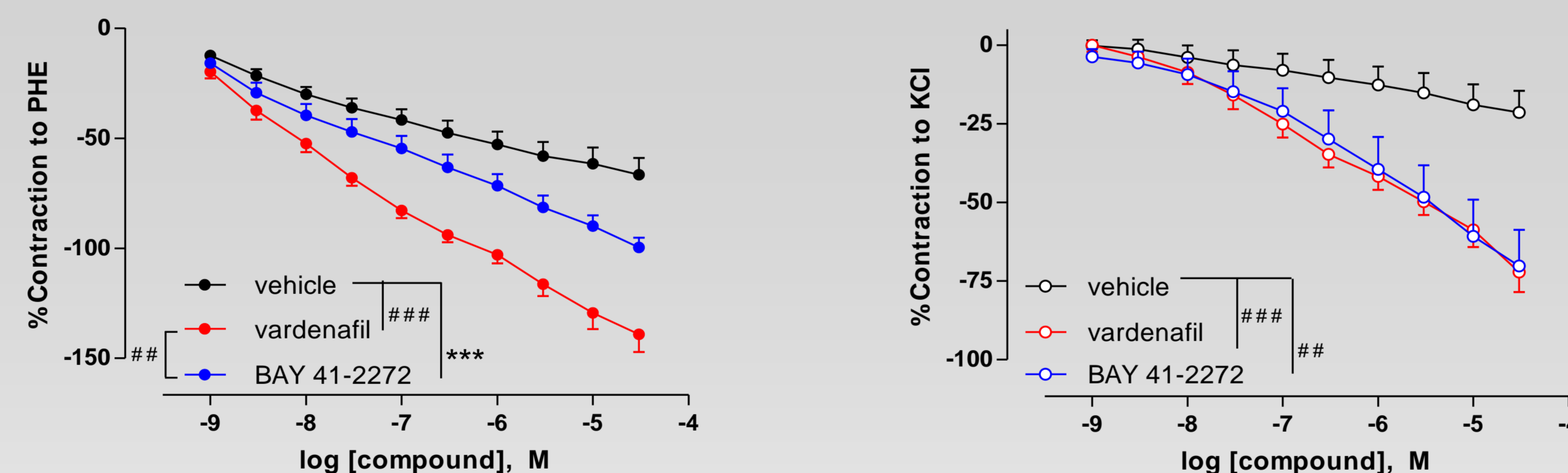
## MATERIALS & METHODS



Adult male Sprague-Dawley rats (n=40) were treated for 24 days by daily subcutaneous injection with testosterone (3 mg/kg) or sesame oil together with an oral gavage twice a day of BAY 41-2272 (0.3mg/kg), vardenafil (3mg/kg), or vehicle (10 % Transcutol / 20 % Cremophor / 80 % tap water). At the end of the treatment period, lateral lobes of the prostates were harvested, homogenized and evaluated for the expression of cleaved caspase-3 and -9, markers of apoptosis and PCNA and cyclin D1, markers of proliferation by western blot on a denaturing SDS-polyacrylamide gel. After electroblotting onto PVDF membrane (Millipore, France) (Trans-blot® SD Semi-Dry Transfer Cell Bio-Rad) and immunodetection, proteins of interest were detected by enhanced chemiluminescence and exposed to autoradiographic films. The films were then scanned and relative density against an internal control determined (NIS-Elements, Nikon, France) to allow inter-membrane comparisons. Results are expressed as mean ± SEM. Comparisons were performed with a Student's t-test and P values < 0.05 were considered significant.

## RESULTS

### Ex vivo experiments on human prostate samples

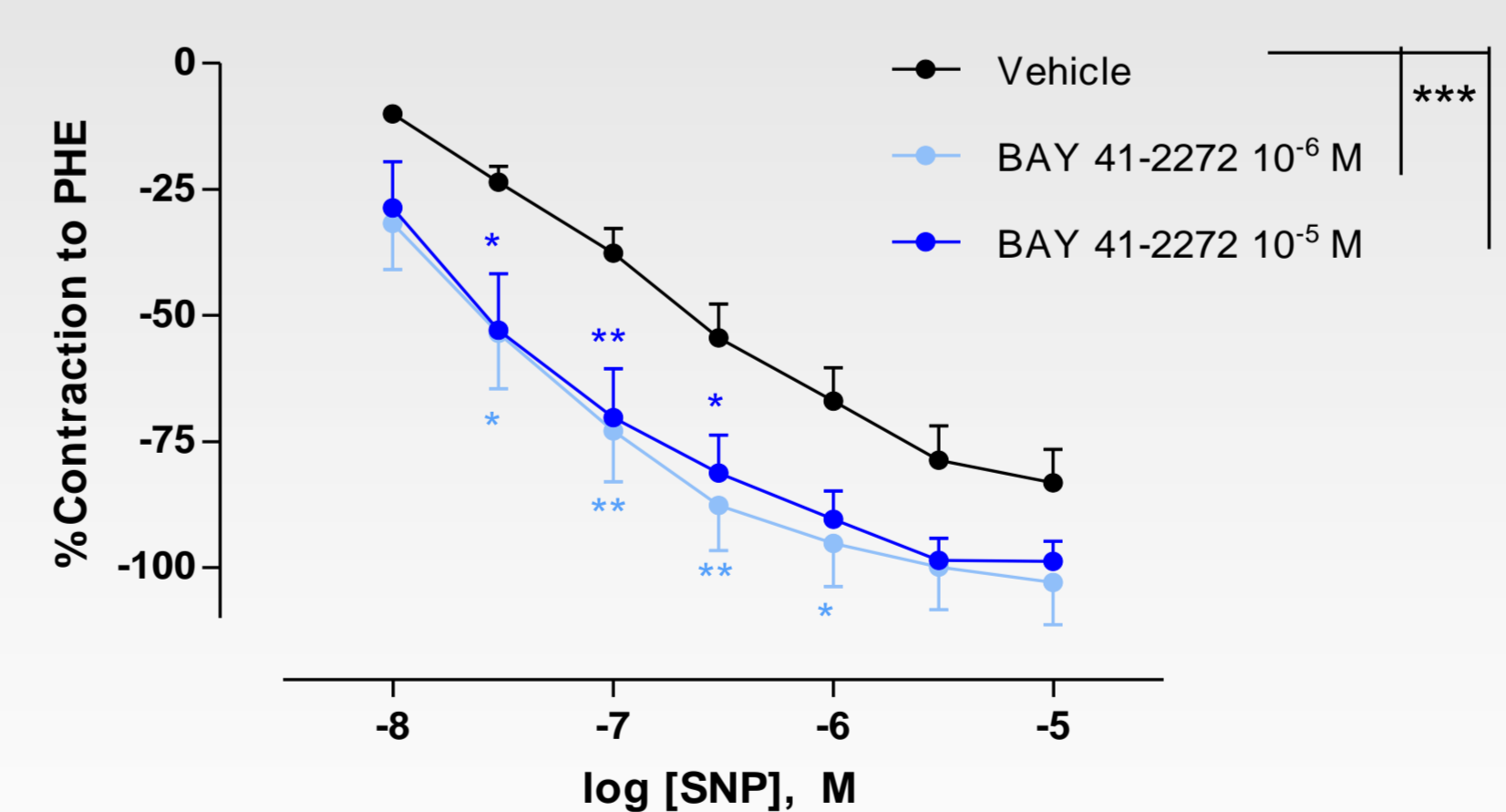


Phenylephrine-induced precontractions				
	pD2**	E <sub>max</sub> <sup>ns</sup> (%)	PHE <sup>ns</sup> (mg)	N
CRC to vehicle	-	-	479 ± 204	8
CRC to vardenafil	7.6 ± 0.1 †	> 100 † (¶)	262 ± 83	6
CRC to BAY 41-2272	6.9 ± 0.2	-90.0 ± 4.3	317 ± 38	6

KCl-induced precontractions				
	pD2**	E <sub>max</sub> <sup>ns</sup> (%)	KCl <sup>ns</sup> (mg)	N
CRC to vehicle	-	-	498 ± 84	11
CRC to vardenafil	6.6 ± 0.2 §	-62.9 ± 6.0	498 ± 60	6
CRC to BAY 41-2272	6.1 ± 0.2 §§	-70.3 ± 10.7	555 ± 211	8

Potency (pD2) and maximum response (E<sub>max</sub>) obtained using four-parameters logistic regression for vardenafil and BAY 41-2272-induced relaxations on PHE or KCl-induced contractions of human prostatic strips. Data are mean ± SEM of N experiments using prostate samples from N different patients. p = ns, † p < 0.01, one-way ANOVA followed by Newman-Keuls post-hoc test, ‡ p < 0.05, §§ p < 0.001 versus BAY 41-2272.



➔ Vardenafil and BAY 41-2272 induce significant concentration-dependent relaxations of both PHE- or KCl-precontracted human prostatic strips with a greater effect of vardenafil on PHE-precontracted prostatic strips. BAY 41-2272 enhances the relaxant effect of SNP on PHE pre-contracted human prostatic strips.

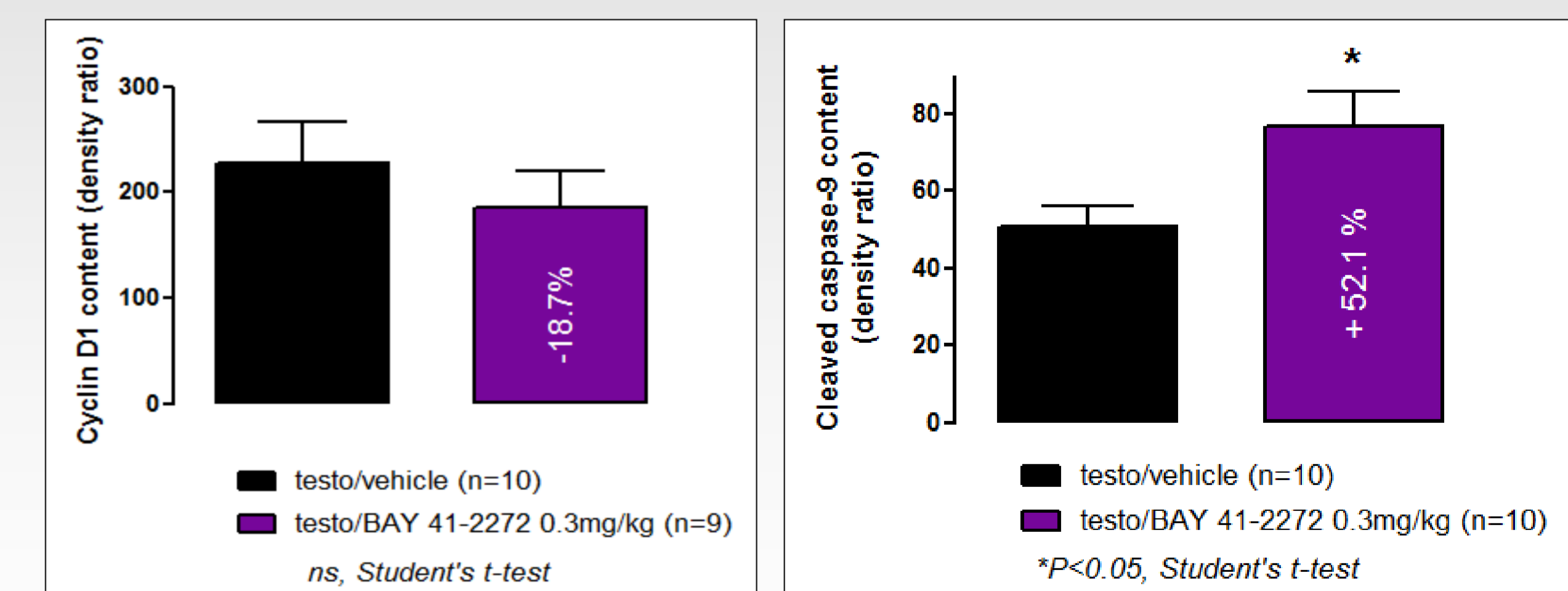
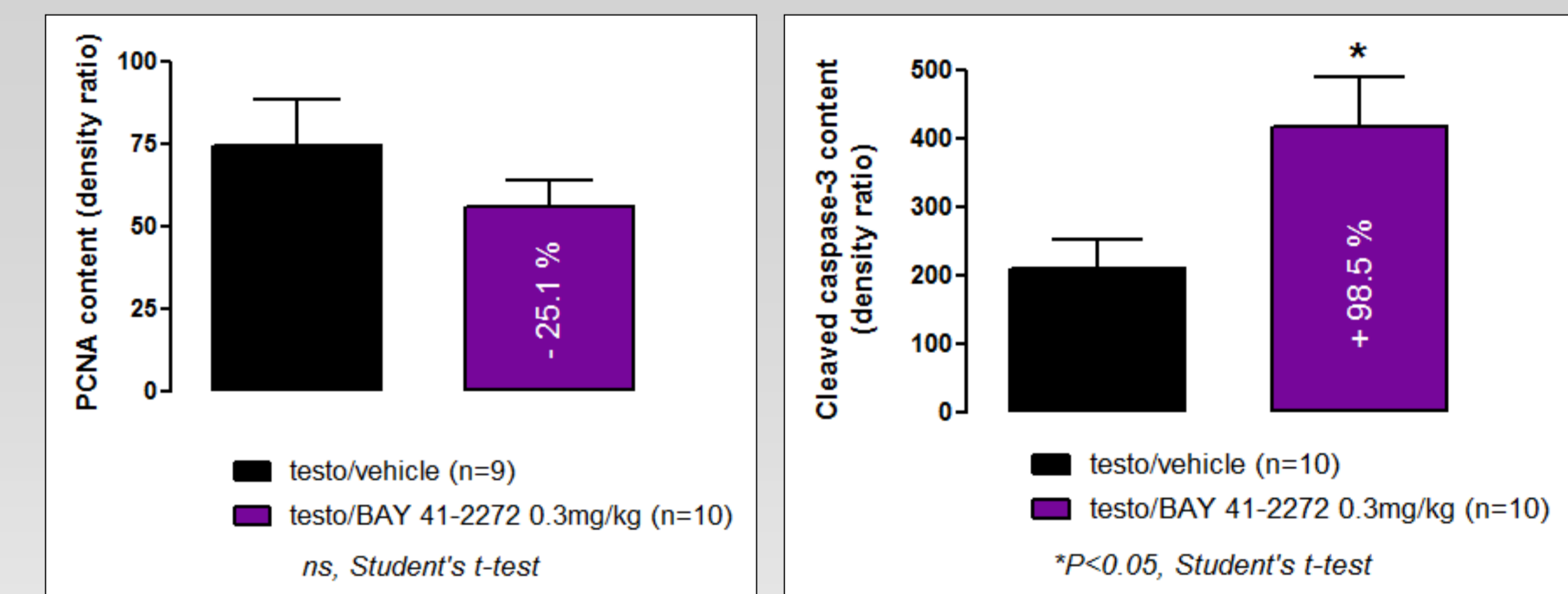
### In vivo experiments in testosterone-supplemented rats

Total prostate weight (mg/100 g BW)		
Treatment	Mean	N
Sesame oil/veh	203 ± 6	10
Testo/veh	302 ± 11	10
Testo/vardenafil	308 ± 13	10
Testo/BAY 41-2272	329 ± 10	10

Lateral lobes weight (mg/100 g BW)		
Treatment	Mean	N
Sesame oil/veh	133 ± 6	10
Testo/veh	189 ± 9	10
Testo/vardenafil	196 ± 10	10
Testo/BAY 41-2272	207 ± 7	10

Evaluation of prostate enlargement by weighing of the gland. Data are mean ± SEM. p=ns, one-way ANOVA.

➔ Neither vardenafil nor BAY 41-2272 modified prostate weight in testosterone-treated rats.



Densitometric analysis of immunoblots for PCNA, cyclin D1, cleaved caspase-3 and -9 in the lateral prostate homogenates from testosterone/vehicle and testosterone/BAY 41-2272 (0.3mg/kg b.i.d) treated rats. Data are mean ± SEM. \*p<0.05, Student's t-test.

➔ Chronic administration of BAY 41-2272 (0.3 mg/kg b.i.d) significantly increased both cleaved caspase-3 and -9 expression, markers of apoptosis, and decreased, albeit not significantly, PCNA and cyclin D1 expression, markers of proliferation, thus favoring prostate regression.

## CONCLUSIONS

- In addition to a potential relaxant effect on prostate smooth muscle fibers (dynamic component), sGC stimulation might favor prostate regression (static component).
- Of note, the effects of sGC stimulation on urethral pressure and voiding efficiency are still unknown.
- sGC stimulation could thus represent a novel promising pharmacological mechanism of action for the treatment of LUTS related to BPH.