07. Endocrine, Reproductive and Urogenital Pharmacology

07.001 Mirabegron relaxes urethral smooth muscle by a dual mechanism involving β3-Adrenoceptor activation and α1-adrenoceptor blockade. Alexandre EC[1], Kiguti LR[2], Calmasini FB[1], Ferreira R[3], Silva FH[1], Silva KP[2], Ribeiro CA[2], Mónica FZ[1], Pupo AS[2], Antunes E[1] [1]FCM-Unicamp – Farmacologia, [2]IBB-Unesp, [3]FCM-Unicamp – Hematologia e Hemoterapia

Introduction: Overactive bladder syndrome (OAB) is a subset of storage LUTS (lower urinary tract symptoms) highly prevalent in diabetes, obesity and hypertension. Benign prostatic hyperplasia (BPH) in aging men is another pathological condition highly associated with OAB secondary to bladder outlet obstruction (BOO). The β3-adrenoceptor apparently is the major receptor to induce bladder relaxations. Mirabegron is the first β3-adrenoceptor (β3-AR) agonist approved for OAB treatment (Chapple et al., 2014). Urethral smooth muscle plays a critical role to urinary continence, but no studies have examined the mirabegron-induced urethral relaxations. **Aims**: This study was designed to investigate the mirabegron-induced mouse urethral relaxations. In preliminary assays, mirabegron showed an unexpected action by competitively antagonizing the urethral contractions induced by the α1-AR agonist phenylephrine. Therefore, this study also aimed to characterize the α1-AR blockade by mirabegron, focusing on the α1-AR subtypes in rat vas deferens and prostate (α1A-AR), spleen (α1B-AR) and aorta (α1D-AR) preparations. **Methods**: Functional assays were carried out in mouse urethra rings, and rat vas deferens, prostate, aorta and spleen. β3-AR expression (mRNA and immunohistochemistry) and cyclic AMP levels were determined in mouse urethra. Competition assays for the specific binding of [3H]Prazosin to membrane preparations of HEK 293 cells expressing each of the human α1-ARs subtypes were performed. **Results**: Mirabegron (1 nM-100 μM) produced concentration-dependent urethral relaxations that were right shifted by the selective β3-AR antagonist L 748,337, but unaffected by β1- and β2-AR antagonists (atenolol and ICI 118,551, respectively). The non-selective β-AR agonist isoprenaline produced biphasic relaxant responses that were turned into a monophasic concentration-response curve in presence of L 748,337. Mirabegron-induced relaxations were enhanced by the phosphodiesterase-4 inhibitor rolipram, and associated with β3-AR-stimulated cAMP synthesis. Mirabegron (1 to 100 μM) also produced rightward displacements in urethral contractions induced by phenylephrine. Schild regression analysis revealed that mirabegron behaves as a competitive antagonist of α1-AR in urethra, vas deferens and prostate (α1A-AR, pA2 ≈ 5.6) and aorta (α1D-AR, pA2 ≈ 5.6), but not in spleen (α1B-AR). The affinities estimated for mirabegron in functional assays were consistent with those estimated in radioligand binding with human recombinant α1A- and α1D-ARs (pKi ≈ 5.6). RT-PCR and immunohistochemistry confirmed the presence of β3-AR in mouse urethra. **Conclusion**: The effects of mirabegron in mouse urethra smooth muscle are the result of β3-AR agonism together with α1A / α1D-AR antagonism. The combination of both of these pharmacological actions may contribute to the efficacy of mirabegron in treating OAB-associated with BOO. **Financial Support**: FAPESP (2014/02196-2) CEUA 3514-1. **References**: Chapple et al., (2014) BJU int 113.6: 847-848.

07.002 Effects of testosterone replacement at physiological levels in the lower urinary tract of ovariectomized (OVX) rat. Becerra SB, Oliveira MG, Moscoso JR, Calmasini FB, Campos RM, Iwamoto RD, Antunes E FCM-Unicamp – Pharmacology

Introduction and Aim: Post-menopause is the end of menstrual cycles that is accompanied by sexual hormones reduction, vasomotor symptoms and sexual dysfunction. Menopause also causes overactive bladder syndrome (OAB), characterized by frequency, nocturia, urgency and urge incontinence. Testosterone replacement therapy has been used to relieve hormone deficiency symptoms (1). However, the efficacy of testosterone replacement on OAB of post-menopause women is poorly investigated, although recent studies have reported the use of supra-physiological plasma concentrations (2, 3). The effect of testosterone replacement at physiological levels on the lower urinary tract (bladder and urethra) were investigated in the present study. **Materials and Methods**: Two-month old female Sprague–Dawley rats (250-280g) were anesthetized (ketamine/xylazine, 60: 6 mg/kg, IP). Bilateral incisions were made, the vascular supply ligated and the ovaries removed. SHAM-operated rats were subjected to surgery and the ovaries were manipulated but left intact. Three months later, animals were divided into groups and treated with a single dose, intramuscular, as follows: SHAM (castor oil); OVX (castor oil); OVX-T_1–testosterone undecanoate (1 mg/kg), OVX-T_{10}–testosterone undecanoate (10 mg/kg); and OVX-T_{100}–testosterone undecanoate (100 mg/kg). After 30 days, blood samples were collected for testosterone serum quantification by chemiluminescence. Uterus, bladder and urethra weights were evaluated. **Results**: OVX markedly reduced the serum testosterone levels ($p < 0.001$) compared with SHAM (5.9 ± 0.3 and 22.6 ± 2.3 ng/dL, respectively), which was increased by 4- and 8-times in animals treated with testosterone at 10 and 100 mg/kg. Testosterone levels in OVX-T_1 did not differ from OXV group. Body weight of all OVX rats treated with vehicle or testosterone at 1, 10 and 100 mg/kg was significantly higher compared with SHAM (11%, 6%, 20% and 9% increase, respectively; $p < 0.05$). OVX reduced by 79% the uterine weight ($p < 0.001$), but testosterone replacement had no significant effect on this parameter. The bladder and urethra weights did not differ among groups. **Conclusion**: Testosterone replacement does not cause structural changes in the uterus and the lower urinary tract. Functional studies should be conducted at physiological levels of testosterone to investigate its effect on OAB. **References**: 1. Jong Kyu Kwon. Maturitas 79 (3): 311-315, 2014. 2. Tiago J. Costa. Am J Physiol Heart Circ Physiol 308: H723–H732, 2015. 3. Yanlan Yu. Urology 73 (6), 1210-7, 2009. **Financial support**: CAPES. All animal procedures were approved by the Ethical Committee on Animals Use CEUA/UNICAMP (CEUA; No. 3500-1).

07.003 Androgen-induced changes in the expression of the β-defensin Spag11c during rat Wolffian duct morphogenesis. Ribeiro CM[1], Silva EJR[2], Thimoteo DS[1], Hinton BT[3], Avellar MCW[1] – [1]Unifesp-EPM – Farmacologia, [2]Unesp – Farmacologia, [3]University of Virginia – Cell Biology

Introduction: Androgens are essential for the differentiation of Wolffian duct (WD), the anlagen of the epididymis. The early effects of androgens on the epithelium of WD are mediated indirectly via unknown paracrine signals from the mesenchyme. Our group has recently showed that androgens drive the temporal, cell-type and region-specific expression of the β-defensin SPAG11C in the epididymis during rat ontogeny. We showed SPAG11C predominantly immunolocalized in the WD mesenchymal cells. **Aim**: to investigate whether SPAG11C is a downstream androgen target in the morphogenesis of the rat WD. **Methods**: WDs were collected from male Wistar rats at embryonic days (e) e12.5-e20.5 and the whole epididymides at postnatal day (pnd) 1. Organotypic cultures were performed using WDs collected from male embryos at e17.5 for 24-72h on Millicell inserts floating on serum-free medium (DMEM/F12, 1% ITS, 50 µg/mL ampicillin) in the absence or presence of testosterone (10 nM), flutamide [a competitive androgen receptor (AR) antagonist; 10 µM], and PD98059 [a specific inhibitor of the activation of mitogen-activated protein kinase kinase (MAPKK); 50 µM]. WD morphological differentiation was assessed by gross morphology and RT-qPCR studies evaluated Spag11c mRNA levels. **Results**: Spag11c mRNA was observed in the male urogenital rudiment collected as early as e12.5, thus before the onset of AR expression and fetal testosterone synthesis. Between e12.5 and e17.5, when testosterone stabilizes WD in males, Spag11c mRNA levels increased. Its expression then decreased between e17.5 and pnd1, a period when the WD differentiates into epididymis. Organotypic WD cultures were used to further investigate the regulation of Spag11c by androgens. WDs cultured in the presence of testosterone elongated and coiled, an effect abrogated in the absence of androgens or co-incubation of testosterone and flutamide. Testosterone induced a down-regulation of Spag11c mRNA levels in cultured WDs, which reproduced the in vivo modulation of this transcript observed in WDs at ages e17.5 and e20.5. This effect was prevented by flutamide, confirming the involvement of androgens/AR signaling in the regulation of Spag11c expression in the WD. Furthermore, treatment with PD98059 prevented the morphological differentiation of the WD and the down-regulation of Spag11c mRNA induced by testosterone, indicating that ERK/MAPK signaling pathway participates in the androgen action in this tissue**. Conclusions**: Our data revealed that androgens induce both WD differentiation *in vitro* and regulation of the expression of Spag11c mRNA, which is a mesenchyme-derived factor, through AR and ERK/MAPK signaling. These results suggest that SPAG11C can act collectively with testosterone in the epididymal morphogenesis, which can broaden the putative functional repertoire of β-defensins. **Financial Support**: CNPq/Ciência Sem Fronteiras (PVE 401932/2013-3; PDJ 150040/2015-6), CNPq, NIH−NICHD #069654. This study was approved by the Research Ethics Committee from Unifesp/EPM (CEUA N. 1776201213).

07.004 Characterization of increased prostate smooth muscle reactivity in middle-aged rats: Lack of effect of testosterone replacement. Calmasini FB, Silva FH, Alexandre EC, Rodrigues RL, Báu FR, Barbosa APL, Anhê GF, Antunes E FCM-Unicamp – Farmacologia

Introduction: Benign prostate hyperplasia is one of the most common disorders affecting older men. Previous studies have been shown aging-dependent prostate dysfunctions.[1] It is know that aging leads to decreased serum testosterone, which can contribute to prostate alterations.[2] In the present study we explored the pathophysiological alterations in prostate smooth muscle (PSM) from middle-aged rats, and evaluated the functional and molecular effects of testosterone replacement. **Methods**: Control (3.5-month old) and middle-aged (10-month old) male Wistar rats were used. Rats were divided into three groups: 1) control and 2) middle-aged rats that received peanut oil subcutaneously (s.c.) and 3) middle-aged rats that received daily 0.5 mg/Kg of testosterone cipionate diluted in peanut oil s.c. Concentration-response curves to the contractile agents phenylephrine (□1-adrenoceptor agonist) and α,β-methylene ATP, as well as to the relaxing agents isoproterenol (ISO), sodium nitroprusside (SNP) and Y27632 (Rho kinase inhibitor) were obtained in PSM. Neurogenic contractions produced by electrical-field stimulation (1-32 Hz, 50V, 10 sec), along with measurement of [^{3}H]-noradrenaline release were performed. The levels of cAMP in prostate homogenate and soluble guanylyl cyclase (sGC) protein expression were also determined. **Result**: A significant increase ($P<0.05$) in phenylephrine- and α,β-methylene ATP-induced PSM contractions were observed in middle-aged compared with control rats (30% and 32% increase). EFS-induced PSM contractions were 60% higher in middle-aged compared with control group ($P<0.05$). PSM contractions in middle-aged group were accompanied by greater [^{3}H]-noradrenaline release. The PSM-induced relaxations in response to SNP, isoproterenol and Y27632 were lower in middle-aged rats (Emax: 59.4±4%, 48.6±4% and 76.1±3%, respectively; $P<0.05$) in comparison with control rats (76.37±1%, 63.5±3% and 92.3±4%, respectively). The cAMP levels in prostate homogenate were 25% lower ($P<0.05$) in middle-aged compared with control group. The sGC protein expression was reduced in prostate from middle-aged rats compared with control group (4.9- and 2.8-fold reductions to α1 and β1 subunit, respectively). Testosterone replacement restored neither the enhanced PE- and EFS-induced contractions nor the sGC expression in prostate from middle-aged rats. **Conclusion**: PSM from middle-aged rats exhibit hypercontractility in response to α1-adrenergic and purinergic P2X1 receptor activation, which is associated with impaired cAMP- and cGMP-mediated relaxations. Furthermore, the failure of testosterone therapy to restore the normal contractile pattern and sGC expression in prostate from middle-aged rats suggests that reduced levels of serum testosterone do not play a key role in the PSM hypercontractility associated with aging. **Financial support**: FAPESP / CNPq **CEUA**: n° 3171-1

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07.005 Local cytokine responses to LPS or LTA in a rat model of acute epididymitis. Silva EJR[1,2], Ribeiro CM[2], Avellar MCW[2] [1]Unesp – Farmacologia, [2]Unifesp-EPM – Farmacologia

Introduction: Epididymitis is a common inflammatory condition usually caused by pathogens ascending from the urethra into the male excurrent ducts. Bacterial infections represent a major etiological factor of acute epididymitis, which may result in transient or permanent infertility. **Aim**: to investigate the local expression of cytokines in the rat epididymis after the retrograde injection of lipopolysaccharide (LPS) from *Escherichia coli* or lipoteichoic acid (LTA) from *Enterococcus faecalis* into the vas deferens. **Methods**: Wistar rats (90 days, n=6 rats/group) were anesthetized with ketamine/xylazin (100 and 10 mg/kg, i.p.). The epididymal portion of the vas deferens was exposed by a scrotal incision and twenty five microliters of sterile saline (0.9% NaCl) containing or not ultrapure LPS (25 µg/epididymis) or LTA (125 µg/epididymis) were injected into the lumen of the vas deferens near to the cauda epididymis using a 30-G needle and a 0.1-ml Hamilton syringe. Rats were sacrificed at 6 and 24 h after the injection and their epididymides were harvested and processed either for histopathological examination or for the determination of the concentration of different cytokines (IL-1A, IL1-B, IL6, IL17-A, IL-10, IL-18, GM-CSF, MIP-2, MCP-1 and RANTES) using a magnetic bead multiplex assay. **Results**: Epididymides from both LPS- and LTA-treated rats showed evidences of ongoing inflammatory reactions as demonstrated by the presence of intense interstitial granulocyte infiltrates and immune cells into the lumen of the cauda epididymis. The basal concentrations (mean ± SEM, pg/mg of protein) of cytokines in the cauda epididymis of control rats were: IL-1A (22.3 ± 2.1), IL-1B (79.2 ± 5.5), IL-6 (500.3 ± 44.8), IL-10 (21.5 ± 2.3), IL-17A (10.9 ± 1.1), IL-18 (284.9 ± 17.2), GM-CSF (17.5 ± 1.6), MIP-2 (30.0 ± 2.7), MCP-1 (60.2 ± 4.8) and RANTES (331.4 ± 26.7). After 6 h, tissue concentrations of IL-1B, IL-10 and MCP-1 in the cauda epididymis were significantly increased in both LPS- and LTA-treated groups in comparison to control groups ($p < 0.05$, ANOVA followed by Tukey test). At this time point, the concentrations of IL-1A, IL-6 and MIP-2 were increased in the LPS-treated group, whereas the concentration of RANTES was increased in the LTA-treated group only. After 24 h, tissue concentrations of all cytokines tested returned to the same levels observed in the control groups. **Conclusions**: Our study demonstrates that the luminal exposure of the epididymis to bacterial-derived products induces an acute up regulation of a specific set of cytokines depending on the type of inflammatory stimulus. Overall, our results provide new insights on how innate immune responses are regulated by pathogen-associated molecular patterns in the epididymis, which may influence the clinical outcomes of infectious epididymitis. **Financial Support**: This study was supported by Science without Borders Program (CSF/CNPq), CNPq, Fapesp, and Capes. Approved by the Research Ethics Committee from Unifesp/EPM (process #0310/12). **Acknowledgments**: the authors thank Dr. Marlon Vilela and Bruno Salu for their assistance with the multiplex assays.

07.006 Effects of creatine supplementation in diabetic rats induced by streptozotocin. Medeiros MA[1], Lemos LIC[1], Silva FS[1], Abreu BA[1], Sobral MV[2], Santos LRSO[1], Medeiros KCP[1] [1]UFRN, [2]UFPB

Introduction and Aims: Diabetes Mellitus (DM) is a chronic metabolic disease characterized by hyperglycemia secondary to a reduction in circulating insulin levels. Many studies seek alternative or complementary therapies to reduce side effects of insulin, used as standard treatment in DM. The creatine is a dietary supplement that has been the subject of research in many diseases, with wide possibility of clinical applications. Studies show the effect of creatine in increasing glucose uptake by elevating the expression of GLUT-4 receptors in metabolic disorders like diabetes mellitus type II, however, there are no studies reporting the effects of creatine in diabetes mellitus type I. As thus, this study aims to evaluate the effect of creatine supplementation in diabetic rats induced by streptozotocin. **Methods**: 32 female Wistar rats were separated into 3 groups: (CON) non-diabetic (n = 8), (DB) diabetic (n = 12), and (DBCr) with creatine treated diabetic (n = 12). The DB and DBCr groups received single dose of streptozotocin (40 mg / kg ip). Clinical, biochemical and morphological pancreas were held. **Results**: The use of creatine was able to enhance weight loss percentage (15 ± 6) and polydipsia (481 ± 98,) group when compared to the DB (25 ± 8; 631 ± 101 respectively). In the biochemical analysis, creatine supplementation was effective in reducing hyperglycemia (493 ± 51) and uremia (171,87 ± 29) caused the DB animals (551 ± 51; 223 ± 28 respectively) and when left for morphological evaluation of the pancreas, target organ in DM, an atrophy of the pancreatic islets was observed in animals and DB creatine was able to restore pancreatic islet atrophy. **Conclusion**: Creatine supplementation has been shown to reduce hyperglycemia classical DM, the polidispsia and urea as well as the ability to regenerate pancreatic beta cells and thus can be considered a promising product for future clinical applications associated with other therapies. **Financial Support**: CAPES/CNPq Research approved by the Animal Research Ethical Committee of Federal University of Rio Grande do Norte (process number: 020/2015)

07.007 Corticosterone control of pineal gland nuclear factor kappa B-related genes couples rest/activity to light/dark rhythm. da Silveira Cruz-Machado S[1,2], Tamura EK[1], Carvalho-Sousa CE[1], Cecon E[1], Fernandes PA[1], Markus RP[1] [1]IB-USP – Cronofarmacologia

The daily temporal organization in mammals involves the activity of two major endocrine glands, the pineal and adrenal glands. Their hormones, melatonin and corticosterone regulate the pivotal signaling of inflammatory response, the NF-κB mediated-genes. Melatonin signalizes the dark phase of the day, whilst corticosterone prepares the organisms for activity. Therefore, corticosterone peak occurs at the light/dark transition in nocturnal rodents. Considering the relevance of the proper timing for drug administration, it is important to disclose how these two hormones interacts in healthy conditions. Here we evaluated the daily rhythm of transcription of 84 genes involved in NF-κB activation and deactivation in the rat pineal gland. The data obtained clearly disclosed a new and important pattern of interaction of both hormones at the pineal gland level. Pineal glands from adult male Wistar rats (Animal Facility/Dep of Physiol., IB-USP) kept under 12/12 h L/D cycle (lights ON at 06h00 = ZT0) were obtained at ZT0, ZT6, ZT12L (light), ZT12D (dark) and ZT18. The expression of 84 genes related to NF-κB signaling (qPCR array profiler, PARN-018A, SABioscience, USA), and the nuclear content of NF-κB (EMSA) were determined. Corticosterone and melatonin levels were determined by radioimmunoassay (ImmuChem, USA) or ELISA (IBL, Germany), respectively. Data presented as mean ± S.E.M. were compared by unpaired Student's t test or ANOVA followed by Newman–Keuls post-test. Seven out of 84 genes were not expressed at any time (Il1a, Il6, Ifna1, Ifnb, Ifng, Tnf and Tlr5) while seven genes were expressed in a non-rhythmic manner (Cd80, Csf2, Csf3, Il10, Lta, Rela, and Tlr9). The other 70 genes showed a robust daily rhythm: highest expression at the end of the light and a sharp decrease at the beginning of the dark phase. Given the well-documented inhibitory effects of glucocorticoids on NF-κB activity, we tested whether the rhythmic corticosterone peak triggers the reduction of pineal NF-κB transcriptional program. Notably, we detected an inverse correlation between plasma corticosterone and nuclear pineal NF-κB content in animals kept in constant darkness (CT12 and CT21, $p < 0.05$, $r2 = -0.82$, $N=8$). In addition, 13 out of the 84 genes (Tlr1, Tlr2, Tlr4, Myd88, Mal, Il1r1, Il6r, Il1b, Cd80, Casp8, Cxcl10, Eif2ak2 and Mapk8ip3) are regulated by GR activation, as the competitive antagonist, mifepristone (10 mg/kg) reverted the decreased gene expression at beginning of the night. Overall, GR activation controls a group of gene that provides a genomic signature, under the control of NF-κB, which signals activity initiation to pineal glands. When this information is blocked, the pineal production of melatonin is impaired. Indeed, the plasma level of melatonin at ZT18 was significantly lower in mifepristone-treated animals (175.4 ± 24.9 pg/mL; n=5), compared to vehicle (252.1 ± 12.1 pg/mL, n=5). The present study provides new molecular basis for better understanding the synchronization of the endocrine system. Because corticosterone and melatonin plays pivotal roles in the regulation of immune surveillance, the cooperation between adrenal and pineal glands may be relevant for regulating homeostasis and opens a new approach for therapeutics. Financial Support: FAPESP 2013/01961-3. CEUA license 115/2010.