08. Respiratory, Urinary and Reproductive Pharmacology

08.001 Serotonin contracts the rat corpus cavernosum, but how?

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Introduction: Erectile dysfunction is characterized by the inability to obtain and/or maintain an erection for satisfactory sexual performance[1]. The role of 5-hydroxytryptamine (5-HT, serotonin) for penile erection is not well established despite the increasing use of drugs that alter the homeostasis of 5-HT, such as fluoxetine. Preliminary data from our laboratory show that the contractile response induced by 5-HT is not reproducible in the erectile tissue from the penis, the corpus cavernosum (CC), suggesting tachyphylaxis. To study this tachyphylactic event, the mechanisms by which 5-HT elicits contraction of the CC must be firstly determined. In this study, the characterization of the receptor activated by 5-HT to induce contraction was performed using agonists for different 5-HT receptor subtypes; the involvement of the 5-HT metabolite (5-HIAA) in the contractile response of CC was also evaluated. **Methods:** Corpus cavernosum segments obtained from male Wistar rats (250-330g; CEUA PP00706) were mounted in isolated organ chambers for isometric tension recording. The segments were stimulated once by one different serotonergic agonist (100 μM) for multiple 5-HT receptor subtypes as follows: Buspirone (partial 5-HT_{1A}), 8-OH-DPAT(5-$HT_{1A/7}$) ,CGS 12066B (5-HT_{1B}) DOI (5-$HT_{2A/2C}$), MK-212 (5-HT_{2C}), BW 723C86 (5-HT_{2B}), mCPP (5-$HT_{2B)}$, WAY 161503 (5-HT_{2C}).To evaluate the participation of 5-HIAA in the contraction, segments were incubated with pargyline (100 μM), an inhibitor of the enzyme monoamine-oxidase responsible for 5-HT metabolization into 5-HIAA 30 min before the stimulation with 5-HT (100 μM). One set of segments was directly stimulated with 5-HIAA (100 μM). Student's t-test was used when appropriate. **Results and discussion:** No changes in the magnitude of 5-HT-induced contraction (60 mg ± 5; n = 8) were observed when CC segments were preincubated with pargyline (54 ± 6; n = 8). 5-HIAA (n = 4) failed to induce contraction of CC segments. From all 5-HT agonists tested (n = 4), only 8-OH-DPAT was able to contract the CC segments (30 mg ± 11; n = 6). Our results indicate that inhibition of 5-HT metabolism does not affect 5-HT-induced contraction of CC segments. Our results also suggest that the contraction induced by 5-HT in the rat CC is mediated by 5-HT_{1A} and/or 5-HT_7 receptor activation and these receptors may be responsible for the tachyphylactic response induced by 5-HT in this tissue. Since 5-HT is found in the blood, especially in platelets, this may be an easy way for 5-HT to reach its targets. However, further investigation is needed to evaluate the mechanisms involved in 5-HT-induced tachyphylactic contractions in the rat CC. Supported by FAPESC/CNPq and PPG-FMC. **References**: [1]REIS, J. M. et al. Impotência sexual: abordagem multidisciplinar. Instituto H. Ellis, 1993.

08.002 Effects of clonidine in the isolated rat testicular capsule. Silva-Júnior ED[1], Rodrigues JQD, Souza BP, Jurkiewicz A, Jurkiewicz NH Unifesp – Farmacologia

Introduction: The testicular capsule contracts in response to noradrenaline and adrenaline by activation of α_1-adrenergic receptors (α_1-AR). However, the effects of clonidine, α_2-adrenergic receptor (α_2-AR) agonist, were still not evaluated. Therefore, this study was carried out to evaluate the effects of clonidine in the isolated testicular capsule. **Methods:**The testicular capsules from adult male Wistar rats were isolated and mounted in organ bath. Cumulative concentration curves were performed for clonidine (10^{-9} - 3.10^{-5}M) and compared with other α-AR agonists. The effects of pretreatment with clonidine for 10 min were tested against curves for noradrenaline (10^{-8} - 10^{-4}M) or phenylephrine (10^{-8} - 10^{-4}M) in the absence or presence of α-AR antagonists. The results were expressed as Mean±SEM from 4 to 6 experiments. All procedures were approved by UNIFESP Animal Ethics Committee (protocol number: 0016/13). **Results:** The order of potency for agonists (pD_2) was clonidine (6.9±0.12) = adrenaline (6.9±0.10) > noradrenaline (5.9±0.07) > phenylephrine (5.0±0.1) > methoxamine (3.5±0.05). The clonidine behaved as partial agonist in relation to noradrenaline. Moreover, the consecutive curves for clonidine showed tachyphylaxis with 3-fold rightward shift and E_{max} reduction of 40%. In contrast, the curves for the other agonists presented reproducible responses. The pretreatment with clonidine at 10^{-5}, 10^{-4} or 10^{-3}M for 10 min was able to rightward shift the noradrenaline curves by about 4.5, 19 and 190-fold, respectively, and decreased the E_{max} by about 20%. In addition, clonidine (10^{-5}M for 10min) did not change the pD_2 for phenylephrine (α_1-AR agonist), but the E_{max} was depressed by about 50%. Clonidine (10^{-5}M for 10min) was unable to shift the noradrenaline curves if the treatment was performed in the presence of idazoxan 3.10^{-7}M (α_2-AR antagonist) whereas prazosin 3.10^{-8}M (α_1-AR antagonist) was unable to prevent the clonidine effects. The effect of idazoxan or prazosin on noradrenaline curves were also evaluated after clonidine treatment. The rightward shift promoted by idazoxan 3.10^{-7}M in the noradrenaline curves was decreased in 50% after clonidine pretreatment, as reflected by the concentration ratio (CR) of 5.2±1.2 (treated tissue) and 10.1±1.0 (untreated tissue). However, the CR values for prazosin 3.10^{-8}M were unchanged. So far, the results indicate the presence of functional α_2-AR which could participate in the noradrenaline-induced contraction and to undergo a desensitization induced by clonidine. The involvement of α_2-AR in the contractions induced by noradrenaline was studied by the pretreatment with phenoxybenzamine 3.10^{-6}M plus idazoxan 10^{-6}M (P/I) for 30 min. After carefully wash out, a subsequent noradrenaline curve was constructed. The P/I treatment was able to rightward shift the noradrenaline curves by about 5-fold and diminish the E_{max} in 68%. Moreover, the residual contraction was competitively antagonized by idazoxan 3.10^{-7}M, but not prazosin 3.10^{-8}M. **Discussion:** The results indicate the presence of functional α_2-AR in rat testicular capsule which could be activated by clonidine or noradrenaline. Furthermore, this receptor may be desensitized by clonidine, promoting a decreased potency of the endogenous agonist noradrenaline. **Financial agencies:** CAPES, CNPq and FAPESP.

08.003 Soluble guanylate cyclase (sGC) degradation and impairment of nitric oxide-mediated responses in urethra from obese mice: **Reversal by the sGC activator Bay 60-2770**. Alexandre EC[1], Leiria LO[1], Silva FH[1], Davel APC[2], Mónica FZ[1], Antunes E[1] [1]Unicamp – Farmacologia, [2]IB-Unicamp

Introduction: Novel agonists of soluble guanylate cyclase (sGC) have emerged as valuable tools to elucidate the physiopathology of the NO–cGMP signaling pathway. BAY 60-2770 is a novel described sGC activator that acts by NO- and haem-independent mechanisms (Stasch and Hobbs; Handb Exp Pharmacol 191: 277-308, 2009). Activators of sGC are of potential interest for the treatment of cardiovascular diseases associated with thrombotic complications (Mendes-Silverio et al; PLoS One 7(11): e47223, 2012). Recent studies have implicated obesity as a major contributing factor for voiding dysfunction and overactive bladder (Leiria et al; PLoS One 7(11): e48507, 2012). We have used here a murine model of obesity-associated voiding dysfunction to evaluate the effects of BAY 60-2270 in the urethral dysfunction *in vitro*. **Methods**: The experimental protocols were approved by the Animal Ethical Committee of UNICAMP (CEEA-IB/UNICAMP, 2582-1). C57BL/6 male mice fed for 12 weeks with standard chow or high-fat diet were used. Control and obese mice were treated with vehicle (transcutol:cremophor:water) or BAY 60-2770 (1 mg/kg/day for 2 weeks). Concentration-response curves to NO donors in isolated urethra, as well as determination of cGMP and reactive-oxygen species (ROS) levels, and Western blotting for sGC in the urethral tissues were performed. **Results**: The NO-donors S-Nitrosoglutathione (SNOG; 0.001-100 μM; n=5) and glyceryl trinitrate (GTN; 0.001-100 μM; n=5) produced concentration-dependent urethral relaxations that were significantly lower ($p<0.05$) in obese (Emax: 36.3±4.2 and 29.3±1.4%, respectively) compared with control group (Emax: 66.3±7.3 and 43.7±3.3%, respectively). Urethral relaxations to acidified sodium nitrite (0.001-300 μM; n=6) were also 27% lower in obese compared with control group ($p<0.05$). Two-week treatment with BAY 60-2770 fully restored the impaired urethral relaxations to acidified sodium nitrite in obese group (n=6). In urethral tissue from obese mice, BAY 60-2770 increased by 2.5-fold ($p<0.01$; n=3) the cGMP above baseline, whereas no changes in cGMP levels by BAY 60-2770 were observed in control group. Protein expression of β1 subunit of sGC was decreased by 37% ($p<0.05$) in urethra tissues of obese animals, which was restored by BAY 60-2770. A 118% increase in ROS generation in urethra tissues of obese mice was observed, and that was also normalized by BAY 60-2770. **Discussion**: Urethral smooth muscle from obese mice present sGC degradation and impairment of NO-mediated urethral relaxations, which may be secondary to increased ROS generation. It is likely that such mechanism underlies the overactive bladder in adiposity. Moreover, therapy with the BAY 60-2770 attenuates ROS formation and restores the protein levels of sGC in urethral tissues that results in amelioration of the urethral dysfunction in obese mice. **Financial Support**: CNPq – National Counsel of Technological and Scientific Development.

08.004 Increased prostate smooth muscle reactivity in middle-aged rats. Calmasini FB, Silva FH, Rodrigues RL, Báu FR, Antunes E FCM-Unicamp – Pharmacology

Introduction: Benign prostate hyperplasia (BPH) is one of the most common disorders affecting older man. It is characterized by prostate enlargement and increased smooth muscle tone, thus contributing to overactive bladder and lower urinary tract symptoms (LUTS). Previous studies showed aging-dependent reduced in prostate nitrergic innervation and distribution of autonomic receptors (Aikawa K, Prostate, 48, 40, 2001; Kondo S, Urol Int, 49, 201, 1992). In the present study we explored the pathophysiological alterations in prostate from middle-aged rats, looking at both relaxant and contractile machinery in prostate smooth muscle (PSM). **Methods:** The experimental protocols were approved by the Animal Ethical Committee of UNICAMP (n° 2110-1). Wistar rats were divided into two groups: (a) young (14-15 weeks) and (b) middle-aged rats (37-38 weeks). Concentration-response curves to the contractile agents phenylephrine (α1-adrenoceptor agonist; 1 nM-100 μM) and α,β-methylene ATP (P2X1 agonist; 1-10 μM), 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) were also performed. The levels of cAMP in prostate homogenate were determined by ELISA assays. **Result:** A significant increase in phenylephrine- and α,β-methylene ATP-induced PSM contractions were observed in middle-aged rats (Emax: 4.60±0.33 and 2.69±0.13 mN, respectively; $P<0.05$) compared with young rats (Emax: 3.52±0.15 and 2.03±0.2 mN, respectively). EFS-induced PSM contractions were also higher in middle-aged group (32Hz: 3.98±0.39 mN, $P<0.05$) compared with control group (2.52±0.25 mN). 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 young 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. **Discussion:** Our findings show that PSM from middle-aged rats exhibit hypercontractility in response to a1-adrenergic and purinergic P2X1 receptor activation, which is associated with impaired cAMP- and cGMP-mediated relaxations. Whether such alterations contribute to BPH development is under current investigation. **Financial support:** CNPq.

08.005 Bay 60-2770, **a soluble guanylate cyclase activator relaxes corpus cavernosum from rabbit**. Estancial CS, de Nucci G, Antunes E, Mónica FZ Unicamp – Farmacologia

Introduction: Activation of NO-cGMP signaling pathway results in corpus cavernosum (CC) relaxation. BAY 60-2770 is known as activator of soluble guanylate cyclase (sGC) that acts by NO- and haem-independent mechanisms (Pankey, E.A., Am J Physiol Heart Circ Physiol, 300, 792, 2011.). This study aimed to characterize by *in vitro* assays the effect of BAY 60-2770 in CC cavernosum from rabbit. **Methods**: Male New Zealand rabbits (3-4 Kg) were used. Corpus cavernosum was retrieved, dissected out and mounted in a 10 mL organ bath containing Krebs solution keep at 37^{o}C, continuously aerated by a mixture of 95%O_2 and 5%CO_2. Then, the tissues were connected to isometric force transducers. First, concentration response curve to BAY 60-2770 (0.01-10 μM) was carried out in the absence (control) and in the presence of inhibitors of sGC (ODQ, 10 μM), nitric oxide synthase (L-NAME) and phosphodiesterase type 5 (tadalafil, 100 nM). The potency (pEC_{50}) and maximal response (E_{max}) values were determined. Second, contraction induced by electrical field stimulation (EFS, 50 V, 4-16 Hz, 10 seconds of stimulation) was carried out in the absence (control) and in the presence of BAY 60-2770 (1 μM). In some protocols ODQ (10 μM) was co-incubated with BAY 60-2770 (1 μM). The experimental protocols were approved by the Animal Ethical Committee of UNICAMP (2720-1). **Results**: BAY 60-2770 relaxed isolated CC from rabbit with pEC_{50} and E_{max} values of 7.53 ± 0.27 and 81 ± 4 % (n= 7), respectively. Neither ODQ or L-NAME affected significantly the pEC_{50} (7.73 ± 0.28 and 7.43 ± 0.22, respectively, $P>0.05$) and E_{max} (84 ± 5 and 67 ± 5 %, respectively, $P>0.05$, n=4) values in comparison to the control curve. On the other hand, the addition of phosphodiesterase type 5 inhibitor (tadalafil, 100 nM) produced a 10 fold ($P<0.05$, n=5) leftward shift in the BAY 60-2770-relaxing response. The addition of BAY 60-2770 (1 μM) alone did not affect the contraction induced by EFS. However, co-incubation of ODQ (10 μM) reduced by, approximately, 73 and 52% ($P<0.05$, n=3) the EFS-induced contraction in lower frequencies (4 and 8 Hz), respectively. **Discussion:** Our results clearly demonstrated that BAY 60-2770 would be of great value to treat erectile dysfunction caused by sGC oxidation and/or lower levels of NO. **Financial support**: CAPES

08.006 Mirabegron, **a beta-3 adrenergic agonist relaxes rat corpus cavernosum and rabbit prostate**. Candido TZ, Antunes E, de Nucci G, Mónica FZ Unicamp – Farmacologia

Introduction: In cardiomyocytes and blood vessels, beta 3-adrenoceptor (ß-3AR) is coupled to Gs and/or Gi, leading to cAMP accumulation and nitric oxide synthase activation (Ursino et al., Pharmacological Research, (59):221-234, 2009.), respectively. Mirabegron (trade name Myrbetriq) is a new ß-3AR agonist approved by US Food and Drug Administration (FDA) in 2012 for the treatment of overactive bladder (OAB). Benign prostatic hyperplasia is an underlying cause of OAB and with less evidence erectile dysfunction. Thus, the aim of the present study was to verify the effect of mirabegron in isolated prostate from rabbit and corpus cavernosum (CC) from rat. **Methods**: Male wistar rats (300-350 g) or New Zealand rabbits (3-4 Kg) were used for experimental protocols. The CC or prostate were removed, cut into strips, suspended in a 5 or 10ml organ bath containing Krebs solution at 37ºC and bubbled with a mixture of 95% O_2 and 5% CO_2. Following 60 min of equilibration period, the resting tension was adjusted to 5 mN (CC) and 10 mN (prostate). In rat CC, concentration response curves to mirabegron were carried out in the absence and presence of nitric oxide synthase inhibitor (L-NAME, 100 µM) in tissues pre-contracted with phenylephrine (PE). In rabbit prostate, concentration response curve to PE was constructed in the absence and in the presence of mirabegron (1 µM). In some experiments, the relaxing response induced by mirabegron was also verified. Maximal response (E_{max}) and potency (pEC_{50}) values were determined. The experimental protocols were approved by the Animal Ethical Committee of UNICAMP (CEUA:3094-1and 2720-1) **Results**: Mirabegron (0.00001-30 µM, n=5) produced concentration dependent relaxation with pEC_{50} and E_{max} values of 6.08 ± 0.57 and 63 ± 5%, respectively in isolated CC from rat. Neither pEC_{50} (5.92 ± 1.34) or E_{max} (66 ± 10%) of mirabegron was altered in the presence of L-NAME (100 µM, n=5) in comparison to the control curve. In rabbit prostate, mirabegron (0.001-100 µM, n=4) also induced relaxation with pEC_{50} and E_{max} values of 6.2 ± 0.12 and 77 ± 2%, respectively. Prior addition of mirabegron (1 µM) produced a 2.4-fold rightward shift in PE (0.00001- 1 mM)-induced contraction (from 5.05 ± 0.05 to 4.67 ± 0.12, n=5, $P<0,05$) and a reduction of, approximately, 40 % of the E_{max} values (from 18 ± 0.77 to 10 ± 2 mN, $P<0.05$). **Discussion:** Our results showed that mirabegron relaxed isolated CC and prostate from rat and rabbit, respectively, suggesting that this new class of drug may be of great value to treat erectile dysfunction or to counteract the hypercontractility state seen in prostatic hyperplasia. **Financial Support:** CNPq

08.007 Effect of opiates agonist in isolated corpus cavernosum from *Callithirx sp*. Rodrigues RL, Antunes E, de Nucci G, Mónica FZ FCM-UNICAMP – Pharmacology

Introduction: Chronic usage of opiates can lead to erectile dysfunction mainly due to hypogonadism hypogonadotropic (Abs R., J. Clin. Endocrinol Metab., 85, 2215, 2000). On the other hand, the peripheral effects of opiates in the corpus cavernosum (CC) are controversial, since both detumesce and priaprism were observed (Al-Shaiji T.F., Curr Drug Saf., 6, 194, 2011; Hishmeh S., Orthopedics, 31, 397, 2008). To date, there are no studies that evaluated the role of opiate system in isolated CC. Since we observed that intramuscular administration of fentanyl induced penis tumesce in marmoset, the aim of this work was to study the peripheral effects of opiates agonists and antagonists in isolated CC from Callithrix sp. **Methods**: *Callithirx sp* were euthanized with ketamine (50 mg/kg) and xylazine (10 mg/kg). The CC was removed, cut into two strips, suspended in a 10ml organ bath containing Krebs solution at 37ºC and bubbled with a mixture of 95% O_2 and 5% CO_2. Following 60 min of equilibration period, the resting tension was adjusted to 5 mN. First, concentration response curves to fentanyl, loperamide and endomorphin-1 were carried out in the absence and presence of the non-selective opiate antagonist naloxone (10 µM) or nitric oxide synthase inhibitor (L-NAME, 100 µM) in tissues pre-contracted with phenylephrine (10 µM). Second, concentration response curve to phenylephrine (PE) or electrical field stimulation-induced contraction (EFS: 80V; 4-16 Hz, 10 seconds of stimulation) were also constructed in the absence and presence of µ-opiate agonist. Maximal response (E_{max}) and potency values (pEC_{50}) were determined and unpaired t-tests were used for statistical analysis. The experimental protocols were approved by the Animal Ethical Committee of UNICAMP (CEUA: 2022-1) and by SISBIO (16951-1). **Results**: The µ-opiate agonists fentanyl (0.001 -10 µM) and loperamide (0.001 -10 µM) produced concentration dependent relaxation in isolated CC with values of E_{max}=100 ± 10 % (n=3) and 63 ± 5 % (n=3) and pEC_{50} 5.7±0.2 and 6.9±0.2, respectively, while endomorphin-1 (0.001 -10 µM) did not produce significant relaxation (E_{max}=11±2 %, n=3). Neither naloxone (10 µM, n=4) nor L-NAME (100 µM, n=4) altered the relaxation induced by fentanyl. Both fentanyl (10 µM) and loperamide (1 µM) reduced by, approximately, 37% (n=3, $P<0.05$) and 27% (n=3, $P<0.05$), respectively the contractions induced by PE (0.01 - 300 µM). EFS-induced contractions were also reduced by 57 ± 10% (n= 4, $P<0.05$) in the presence of fentanyl (10 µM). **Discussion:** The relaxation induced by opiates agonists in isolated CC from Callithrix seems not to involve µ-receptor receptor activation or nitric oxide release, since naloxone or L-NAME did not affect this response. Since the contractions induced by PE or EFS were reduced by fentanyl or loperamide we may speculate that the relaxation induced by these substances could be in part due to alpha-adrenoceptor blockade. Further studies need to be carried out to verify whether opiates agonists interfere on the noradrenergic system. **Financial support:** Capes

08.008 Wistar audiogenic rat (WAR) displays erectile dysfunction: ***In vivo*** **assessment**. Rodrigues FL[1], Pereira MGAG[2], Garcia-Cairasco N[2], Tostes RC[1], Carneiro FS[1] [1]FMRP-USP – Pharmacology, [2]FMRP-USP – Physiology

Introduction: The association between epilepsy and erectile dysfunction (ED) has often been described, but not clearly defined. Wistar audiogenic rats (WARs), an inbred strain derived from Wistar rats susceptible to audiogenic epileptic seizures, represent an experimental model of epilepsy and have been frequently used to examine the pathophysiological mechanisms of this disease. Considering the indices of ED in epilepsy, we hypothesized that WARs display ED. In the present study, we determined the erectile function, as well as cavernosal function, contractility and relaxation, in male naïve WARs. **Methods**: All procedures were reviewed and approved by the Ethics Committee in Animal Research of the School of Medicine of Ribeirão Preto (protocol n^{o} 119/2011). Changes in the ratio of intracavernosal pressure/mean arterial pressure (ICP/MAP) after electrical stimulation of the cavernous nerve were determined. Cavernosal contractility was induced by electrical field stimulation (EFS) and phenylephrine (PE). In addition, nonadrenergic-noncholinergic (NANC) and sodium nitroprusside (SNP)-induced relaxation was determined. **Results**: WARs display a significant decrease in mean ICP/MAP responses (WAR: 0.58 ± 0.02 vs. control: 0.78 ± 0.02 at 20 Hz, $p<0.05$). Contractile responses to EFS or PE were not different between cavernosal strips from WAR and control rats. However, in the presence of L-NAME (10^{-4} mol/L) plus atropine (10^{-6} mol/L), contractile responses to EFS were increased in the WAR group (WAR: 2897 ± 267.6 vs. control: 1331 ± 483.1, mN/g of dry tissue, at 32 Hz, $p<0.05$). SNP-induced relaxations were not different between the groups. NANC-induced relaxations were markedly decreased in cavernosal strips from WARs (WAR: 63 ± 8.80 vs. control: 120 ± 2.45 percentage of relaxation, at 32 Hz, $p<0.05$). **Conclusion**: Increased sympathetic activity and decreased NANC-induced relaxation may contribute to ED in this experimental model of epilepsy. **Financial Support**: CAPES, FAPESP, CNPq and FAEPA.

08.009 Influence of adrenalectomy and dexamethasone treatment on testicular morphology and sperm parameters in adult rats. Silva EJR[1], Vendramini V[2], Restelli AE[3], Bertolla RP[3], Kempinas WG[4], Avellar MCW[1] [1]Unifesp – Farmacologia, [2]Unifesp – Morfologia e Genética, [3]Unifesp – Cirurgia, [4]Unesp – Morfologia

Introduction: Glucocorticoids (GC's) are stress-induced steroid hormones that regulate several physiological functions, such as metabolism, immune response and reproduction. Natural and synthetic GC's are widely used as anti-inflammatory therapy for many inflammatory and immune diseases. Although several evidences indicated that high levels of GC's disrupt male fertility, their physiological roles in spermatogenesis and sperm maturation in the epididymis remain unclear. Our aim was to investigate the effects of bilateral adrenalectomy (ADX) alone or in combination with dexamethasone (Dex) treatment on the morphology of testis and quantitative and qualitative sperm parameters in rats. **Methods:** Male rats (90 days old) were sham-operated (control) or submitted to bilateral ADX. Control and ADX rats were sacrificed 1, 2, 7 and 15 days after surgery. Rats were also submitted to ADX, treated daily with Dex (5 µg/kg, i.p.) during 6 days and sacrificed 7 days after surgery. Testes were processed for paraffin embedding and submitted to histopathological and morphometric examination (n=5/group). Testicular and epididymal spermatozoa were isolated and submitted to quantitative (sperm count; n=4-5/group) and qualitative (motility, morphology, mitochondrial activity and DNA integrity; n=4-6/group) analyses. Data were analyzed by Student's t-test or by ANOVA followed by Tukey test ($p<0.05$). This study was approved by the Unifesp-EPM Research Ethics Committee (process n° 1255/05). **Results:** Testicular sperm count indicated a significant reduction in the number of homogenization-resistant spermatids (A) and in the daily sperm production (B) in 2- [p=0.029(A) and p=0.024(B)], 7- [p<0.001(A and B)] and 15-day [p=0.004(A) and p<0.001(B)] ADX rats in comparison to control groups. Epididymal sperm number was significantly reduced in the caput/corpus epididymis from 15-day ADX rats (p=0.011), and in the cauda epididymis from 2- (p=0.042) and 7-day (p=0.031) ADX rats. Sperm transit time was significantly increased in the caput/corpus epididymis from 7-day ADX rats (p=0.002). Dex treatment prevented the changes in testicular and epididymal sperm count induced by 7 days of ADX. Qualitative analysis of spermatozoa from the cauda epididymis indicated no changes in sperm motility, morphology, and mitochondrial activity among ADX and control groups. However, the alkaline comet assay revealed an increase in DNA fragmentation in spermatozoa collected from the cauda epididymis of 15-day ADX rats in comparison to control group (p=0.029). Testicular cross sections from 7-day ADX rats displayed more frequently damaged seminiferous tubules in comparison to control group, as indicated by a significant increase in the number of tubules with intraepithelial vacuoles, with sloughed germ cells detached into the lumen, and with multinucleated germ cells (p<0.001). These changes were not prevented by Dex treatment. **Discussion:** Our results indicated that spermatogenesis and post-testicular sperm maturation are under the influence of GC's, providing new insights into the importance of these hormones in male reproductive physiology. **Financial Support:** CAPES, CNPq, and FAPESP (Brazil); Fogarty International Center (USA).

08.010 Treatment with ipriflavone improves the expression of neuronal nitric oxide synthase in genital tissue in the ovariectomized rat model of menopause. Rodovalho GV[1], Martins TA[1], Rezende J[1], Sá RG[2], Leite R[1] [1]CiPharma-UFOP, [2]UFOP – Ciências Biológicas

Introduction: Female sexual dysfunction (FSD) is a condition in which there are alterations in the processes involved in the female sexual response cycle. A major subcategory of FSD is female sexual arousal disorder (FSAD) that results in a series of vasocongestive and lubricative events resulting primarily from increased blood flow to clitoral, labial, and vaginal tissue. A decline in serum estrogen, which occurs in menopause, results in a significant decrease in the clitoral intracavernosal, vaginal, and urethral blood flow, thinning of vaginal mucosal epithelium and atrophy of vaginal wall smooth muscle. The influence of estrogen is related to the local action of nitric oxide (NO) in genital tissues. Experimental findings in the rat vagina demonstrated the dependence of neuronal nitric oxide synthase expression (nNOS) on estrogen presence. The conventional hormone therapy has proven effective in the treatment of FSD, however, this therapy is associated with an increased risk of the incidence of ischemic cardiovascular events, as well as venous embolism. For these reasons, there is great interest in the development of therapeutic alternatives that minimize the deleterious effects of hypoestrogenism on sexual function without causing side effects or contraindications. The fitoestrogen ipriflavone has demonstrated positive results in reverting established osteoporosis, also common in the menopause. However its action in the treatment of FSAD is unknown. The aim of this study was to investigate the therapeutic potential of the ipriflavone in the treatment of FSAD. **Methods:** All experiment was approved by Ethics Committee on Animal Use of Universidade Federal de Ouro Preto (protocol number: 2010 - 54). Adult female Wistar rats were ovariectomized and divided into 4 groups after 8 weeks: control (emulsion vehicle) and 3 groups treated with ipriflavone at the dose of 10, 30, 100 mg/kg during seven days, respectively. Protein extracted pooled from 3 rats in each group were submitted to Western Blotting technique to identify nNOS in vaginal and clitorial tissues preparations. Data were expressed as fold change over the respective control samples run on the same gel. **Results:** The ovariectomy completely abolished the expression of nNOS in the vaginal and clitoral tissues. Ipriflavone treatment at all doses increased the expression of nNOS in the vaginal (0.96; 0.99 and 0.94 fold at the dose of 10, 30, 100 mg/kg, respectively) and clitorial (0.89; 0.94 and 0.90 fold at the dose of 10, 30, 100 mg/kg, respectively) tissues. **Discussion:** Our results demonstrated that estrogen deficiency provided by ovariectomy induces a decreasing in nNOS production in the genital tissue. These finding suggest that dysfunction at the level of nNOS may be partly related to the decrease in the clitoral intracavernosal and vaginal blood flow depend of oxide nitric that occurred in FSAD. Ipriflavone treatment increases by almost 1 fold the expression of nNOS in both, vaginal and clitorial tissue, suggesting that ipriflavone could be a promising alternative treatment for female sexual arousal disorder in female rats. **Financial Agencies:** CAPES; FAPEMIG; UFOP

08.011 Acute aerobic exercise alters the contractile and relaxant responses on rat trachea. Brito AF, Souza ILL, Pereira JC, Silva AS, Silva BA UFPB

Introduction: bronchospasm is one of the responses induced by exercise, which mainly in asthmatics and during this activity, presents an increased respiration rate. Nevertheless, there are scarce reports in physiological mechanisms for the occurrence of this phenomenon in response to physical exercise. Thus, this study aimed to evaluate the relaxation and contraction in rat trachea by means of concentration-response curves after swimming exercise at intensities of 3, 4 and 5% of its body weight. **Methods**: after project approval with certificate number 1101/11, we started a week of adaptation to swimming exercise, rats (*Rattus norvegicus*) weighing between 250-300 g and aged 12 weeks, underwent a session of forced swimming for 1 hour, having stuck to its torso a metal ring corresponding to 3 (n = 5), 4 (n = 5) and 5% (n = 5) of its body weight. The exercise was performed in a polyethylene tank with water at 28 ± 1 °C (Chies et al., Journal Smooth Muscle Research. v. 40, p. 249, 2003). Control group (n = 5) was subjected to the same place stress that the exercised animals, by acclimation, which were wet at the same place that the animals were exercised. After the exercise, the animals were euthanized and the trachea was removed and suspended in organ baths (5 mL) with Krebs solution, under tension of 1 g at 37 °C and gassed with carbogen. After a stabilization period of 60 min, a contraction was induced with carbachol (CCh) 10^{-6} M and during the tonic component, it was obtained a relaxant cumulative curve to aminophylline (AMF) (10^{-12} – 10^{-1} M), a phosphodiesterases inhibitor. After being washed, and rested by 30 min, a contractile cumulative curve was obtained with CCh (10^{-9} – 10^{-4} M), a muscarinic agonist. **Results:** according to the obtained data, all animals exhibited relaxant maximum effect (E_{max}) equal to 100%. However, the trained animals showed to be less sensible to AMF, being necessary additional concentrations when compared to control group. The relaxation curve induced by AMF (pD2 = 4.6 ± 0.3) in control group was attenuated significantly in the trained animals (pD2 = 3.1 ± 0.2, 3.2 ± 0.005, 3.1 ± 0.005) for 3, 4 and 5% of its body weight, respectively. The cumulative concentration-response curves to CCh (pD2 = 7.1 ± 0.06) in control group was shifted to the right, in a parallel manner in the trained animals (pD2 = 7.5 ± 0.009, 8.0 ± 0.003, 7.9 ± 0.02) for 3, 4 and 5% of its body weight, respectively. Additionally, the pD_2 values in 3 and 4% exercised groups presented statistical differences only in the cumulative concentration-response curves to CCh. **Discussion:** the acute aerobic exercise showed to modify the airway smooth muscle responsiveness, contraction and relaxation, in a manner not beneficial because reduced the potency of a relaxant drug (AMF) and increased the potency of a contractile one (CCh). These observations could be associated to increase response by pro-inflammatory mediators (kinins and interleukins) and oxidative stress (Kuchar et al., Respir Physiol Neurobiol. v. 13, p. 45 2013), indicating that, a possible supplement rich in antioxidants could reverse or prevent this situation. Therefore, we conclude that a single exercise session of swimming promotes contraction and relaxation alterations in rat trachea and the exercise intensities tested influence this response. **Financial support:** CNPq, CAPES, PgPNSB/UFPB.

08.012 Evaluation of the *in vitro* response of human cells exposure to environmental pollution by polycyclic aromatic hydrocarbons. Mattos MS, Kraemer LR, Freire BHL, Teixeira MM, Resende RR, Russo RC UFMG

Introduction: Polycyclic aromatic hydrocarbons (PAHs) are a large group of diverse environmental organic pollutants formed, mainly, by incomplete combustion. Many of them are known to have mutagenic and carcinogenic potential. According to United States Environmental Protection Agency (EPA-USA), sixteen HPA types are considered priority pollutants and, recently, the European Union has set a limit of 1 ng. m^{-3} for benzo [a] pyrene in air in order to prevent and avoid the adverse effects of PAH. Such compounds may be found in environmental compartments such as air, soil and water. The main anthropogenic sources of these compounds are incomplete combustion of gasoline, coal combustion and tobacco. The PAH can be absorbed by inhalation, oral and dermal exposure. Considering the overall increase of tobacco use (WHO 2013)[1] and increase of fossil fuel combustion due to urban growth, it is extremely important to analyze the PAH effects in lung. Therefore, we evaluated the response of lung cells exposure to these pollutants **Methods:** We evaluated *in vitro* response of two immortalized human cell lineages: lung epithelial cells (A549) and Human monocytes (THP-1) exposure to PAH, as regards the production of IL-8, IL-1β TNF α and Nitric Oxide. Cytokine levels were measured by Enzyme Linked Immunosorbent Assay (ELISA) and nitric oxide levels were measured by Griess reaction. A549 and THP-1 cells were seeded in 24-wells plates with a cell density of 1 x 10^6 cell/well and cultivated in DMEM and RPMI 1640 medium, respectively, supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin and 1% glutamine. Cells were maintained in a humidified incubator with 5% CO2 at 37 °C. THP-1 cells were treated with PMA at 0,5 uM for 3 hours in order to differentiate them in macrophages. Once plated, cells were treated for 24 hours with PAHs in concentrations ranging from 0.01 µM, 0.1 µM, 1 µM. 3 µM and 6 µM. Data were analyzed by 1way ANOVA Tukey post test using the software GraphPad Prism 5.01 for Windows and expressed as mean ± standard deviation. The level of significance was set at $p < 0.05$. **Results:** PAH induced TNF production by THP-1 cells but no significant difference between the concentrations of PAHs administered. There was no difference in IL-8 production by THP-1 cells. In A549 cells, treatment with 1 uM of PAH induced higher production of IL-8 (1181±95 Pg/mL) than the other concentrations 0,01 (797±60,2 Pg/mL), 0,1 (1015±186,2 Pg/mL), 3(946,7±231,3 Pg/mL) e 6(755,8±56,94 Pg/mL). Treatment of cells with PAH did not alter the pattern of secretion of IL-1 in any cells. Treatment of monocytes with 1 uM of PAHs significantly increased NO production (1,861±0,77 mg/mL) compared to control (0,47±0,12 mg/mL). In A549 cells, however, the increase in NO production was induced by concentration of 3 µM (1,19±0,74 mg/mL) compared to control (0,72±0,50 mg/mL). **Discussions:** In the present study, we evaluated the response of only two cell lineages. A549 cells produce IL-8 in response to PAH. This may indicate that, in humans, chronic inhalation of such compounds may induce an inflammatory response since IL-8 is a chemoattractant for neutrophils. Furthermore the presence of nitric oxide in the lungs can lead to tissue damage. In the presence of oxygen, nitric oxide is oxidised to nitrate and, in high concentration, might cause tissue damage. The interactions between nitric oxide and superoxide anions may lead to the formation of peroxynitrite, that may generate tissue damaging hydroxyl radicals. Further studies will dissect the effects of PAHs in cell viability and other cells lineage. 1. http://www.who.int/mediacentre/factsheets/fs339/en/. **Acknowledgements:** CNPq, FAPEMIG, CAPES.

08.013 Inducible NO synthase plays a major role in obesity-associated overactive bladder. Leiria LO[1], Augusto TM[2], Teixeira SA[3], Muscará MN[3], Carvalho HF[2], Antunes E[1]
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Introduction: Obesity is a risk factor for lower urinary tract symptoms (LUTS), including overactive bladder (Leiria et al., 2013). However, the physiopathology of overactive bladder in adiposity conditions remains incompletely understood. In obese mice, increased iNOS expression is found in several peripheral tissues related to metabolic and vascular complications. In this study we aimed to access whether iNOS is involved in overactive bladder associated with obesity/insulin resistance. **Methods:** All animal procedures and the experimental protocols were according to the Ethical Principles in Animal Research adopted by the Brazilian College for Animal Experimentation (COBEA) and were approved by the institutional Committee for Ethics in Animal Research/State University of Campinas (CEEA-UNICAMP, protocol 2067-1). Male wild type (WT) C57BL/6 mice and iNOS knockout mice ($iNOS^{-/-}$) were fed with high-fat diet for 12 weeks to induce obesity. A separate group was treated with selective the iNOS inhibitor aminoguanidine (20 mg/kg/day) in the drinking water for three weeks. Concentration-response curves to carbachol (1-100 nM),KCl (1–300 mM) and CaCl (0.01–100 mM) were performed in mice isolated detrusor smooth muscle (DSM).Cystometric study was performed to evaluate urodynamic pattern. Imunofluorescence for iNOS as well as Western Blot for iNOS, p-JNK and p-IKK were also done in the bladders from obese and lean mice. **Results:** Obese mice exhibited higher body weight, epididymal fat mass and fasting glucose compared with lean group ($P<0.01$). Obese mice also showed insulin resistance that was abrogated by aminoguanidine treatment. The insulin resistance was not found in $iNOS^{-/-}$obese mice. iNOS activity and protein expression of p-JNK, p-IKK and iNOS were increased in bladder tissues of obese compared with control mice ($P<0.05$). Imunofluorescence revealed that iNOS expression was concentrated in DSM layer. Carbachol, KCl and extracellular Ca^{2+} all produced greater DSM contractions in obese mice (E_{max}: 3.63 ± 0.17, 3.74 ± 0.64 and 3.67 ± 0.16, respectively; $P<0.05$) compared with control group (E_{max}: 1.76 ± 0.21, 1.47 ± 0.21 and 1.56 ± 0.07, respectively). These enhanced DSM contractions in obese mice were normalized by aminoguanidine, and were not detected in$iNOS^{-/-}$ obese mice. The cystometric study showed that obese mice displayoveractive bladder, as evidenced by the increasedvoidingfrequency and non-voiding contractions. These alterations were not observed in aminoguanidine-treated and $iNOS^{-/-}$ obese mice. **Conclusions:** Our data indicate that obese mice presents overactive bladder associated with increased iNOS expression/activity, and that both genetic or pharmacological inhibition normalize bladder contractility and urodynamic profile. **Financial Support:** Fundação de Apoio a Pesquisa do Estado de São Paulo (FAPESP)
References: Leiria LO. J Physiol, 591: 2259-73. 2013.

08.014 The NADPH oxidase inhibitor apocynin prevents sympathetic hyperactivity and down regulation of soluble guanylyl cyclase in corpus cavernosum from middle-aged rats. Silva FH[1], Leiria LO[1], Davel APC[2], Claudino MA[3], Toque HA[4], Antunes E[1] [1]Unicamp – Pharmacology, [2]Unicamp – Anatomy, Cellular Biology, Physiology and Biophysics, [3]USF – Laboratory of Multidisciplinary Research, [4]Georgia Health Sciences University – Pharmacology and Toxicology

Introduction: The nitrergic neurotransmission negatively modulates the sympathetic transmission. Erectile dysfunction in middle-aged rats has been associated with decreased NO bioavailability in erectile tissue due to increased oxidative stress (Silva et al., 2013). Thus, we hypothesized that increased oxidative stress impairs the biological activity of NO to modulate the sympathetic neurotransmission in rat corpus cavernosum (RCC) from middle-aged. **Methods**: The experimental protocols were approved by the Animal Ethical Committee of UNICAMP (n° 2110-1). Male Wistar young and middle-aged rats (2.5 and 10 months, respectively) were treated orally with apocynin during 4 weeks (85 mg/rat/day). RCC contractions induced by electrical-field stimulation (EFS) and phenylephrine were evaluated. Measurement of reactive oxygen species (ROS) and protein expression for tyrosine hydroxylase (TH) and α1 / β1 subunits of soluble guanylyl cyclase (sGC) in RCC were also performed. **Results:** The average weight of dry cavernosal strips from middle-aged rats did not differ significantly from young group (122±4 and 116±4 mg, respectively). EFS-induced contractions in middle-aged RCC were significantly higher ($P<0.05$) compared with young rats (32 Hz: 5.5±0.3 and 3.6±0.4 mN, respectively). Contractions to phenylephrine (10^{-8} to 10^{-4} M) were also higher in middle-age group (E_{max}: 4.4±0.3 mN) compared with young group (E_{max}: 3.5±0.1 mN). ROS intensity and TH expression were significantly higher ($P< 0.01$) in RCC from middle-age (62% and 100% increase, respectively) compared with young group. Protein levels of α1 and β1 subunits of sGC were decreased in RCC from middle-aged rats compared with young group (44% and 62%, respectively). Oral treatment with apocynin fully restored the functional and molecular alterations in middle-aged group, with no significant changes in the young rats. **Discussion:** Increased sympathetic neurotransmission accompanied by up-regulation of TH are observed in RCC from middle-aged rats. Downregulation of GCs in RCC of middle-aged rats was also observed. The reversal of these alterations by apocynin demonstrates that excess of superoxide anion contributes to its pathophysiology in corpus cavernosum at the middle-age. **Reference:** Silva FH et al. .Superoxide anion production by NADPH oxidase plays a major role in erectile dysfunction in middle-aged rats: prevention by antioxidant therapy. J Sex Med. 2013;10:960-71.

08.015 Castration-induced impairment of rat internal pudendal artery reactivity is associated with vascular remodeling. Lopes RAM[1], Neves KB[2], Silva MAB[1], Carneiro FS[1], Tostes RC[1] [1]FMRP-USP – Pharmacology, [2]FCFRP-USP

Introduction: Testosterone deficiency and erectile dysfunction (ED) are strongly associated. Androgen replacement in hypogonadal men restores erectile function. Many studies have shown that inadequate penile arterial flow is one of the major causes of impotence. Unilateral arterial ligation-induced occlusion of the internal pudendal artery (IPA) impairs the responsiveness of corporal smooth muscle, leading to ED. The present study tested the hypothesis that castrated rats display increased IPA contractions to phenylephrine (Phe) and electrical field stimulation (EFS) as well as impaired acetylcholine (Ach)-induced vasodilation as a contributing mechanism for ED. **Methods:** Male Wistar rats(12 weeks old) were studied 30 days after castration (Cast). Functional (isometric contraction and vasodilation) and structural properties of rat IPA (2mm) were determined in a DMT wire myograph and DMT pressure myograph system, respectively. **Results:** Castrated rats exhibited impaired vascular contractility represented by decreased Phe- [Control: 175.4 ± 4.6 *vs* Cast: 134,9 ± 11; Emax] and EFS-induced [Control: 213,0 ± 7.1 *vs* Cast: 137 ± 6.1; 12Hz] contractions and decreased Ach- [Control: 79.7 ± 2.2 *vs* Cast: 49.8 ± 2.8; Emax] and EFS-induced [Control: 54.6 ± 2.1*vs* Cast: 35.5 ± 1.6; 12hz] vasodilatations. No differences were found in sodium nitroprusside-induced vasodilatation. The decreased EFS-induced contraction in IPAs from Cast rats was not abolished by a selective α_2-adrenoceptor antagonist (RX821002 10^{-8} mol/L), an inhibitor of the norepinephrine transporter (desipramine 10^{-7} mol/L), a ROCK inhibitor (Y27632 10^{-6} mol/L), a cyclooxygenase inhibitor (indomethacin $3x10^{-6}$ mol/L), an analog of arginine that inhibits NO production (L-NAME 10^{-4} mol/L) or a superoxide scavenger (tiron10^{-4} mol/L). IPAs from Cast rats exhibited decreased internal diameter [Control (µm): 499,9 ± 35.9 *vs* Cast: 413 ± 14.8; at 60 mmHg], external diameter [Control (µm): 669.4 ± 40.1 *vs* Cast: 552.0 ± 30.0; at 60 mmHg], thickness of the arterial wall [Control (µm): 81.2 ± 6.4 *vs* Cast: 55.8 ± 7.5; at 80 mmHg] and cross-sectional area [Control (μm^2): 157217 ± 12533 *vs* Cast: 116383 ± 12424; at 30 mmHg]. No differences were observed in the wall:lumen ratio. **Discussion:** Unlike our initial hypothesis, IPAs of Cast animals exhibit hyporresponsiveness to contractile stimulation. Impaired EFS-induced contractile responses are not due to changes in pre-synaptic mechanisms, or abnormal RhoA activity, nitric oxide or COX pathways. The structural changes indicate hypotrophic vascular remodeling which may be associated with the vascular dysfunction found in these animals. In conclusion, castration-induced vascular dysfunction is associated with IPAs remodeling. Financial support: FAPESP - 2012/12178-6. Protocol of Animal Use Ethic Committee: 068/2013