08. Respiratory, Urinary and Reproductive

08.001 Impact of early exposure to air pollutant in the innate response to allergic insult in mice. Santos KT[1], Florenzano J[1], Peron JPS[2], Teixeira SA[1], Câmara NOS[2], Muscará MN[1], Costa SKP[1] [1]ICB-USP – Farmacologia, [2]ICB-USP – Imunologia

Introduction: Early exposure to ambient pollutant 1,2-naphthoquinone (1,2-NQ) enhances animal susceptibility to airway inflammation, but the mechanisms remain unclear. Several studies suggest that particulate air pollution induces innate immune responses by cells and endotoxin-toll-like receptor (TLRs) pathways that activate transcription factors, inducing inflammatory cytokines production (He, *Toxicol*., 22(9):709, 2010). Early exposure to 1,2-NQ was evaluated in the murine lung innate immune response upon ovalbumin (OVA). **Methods:** Under approval of local Animal Ethics Committee (Number. 170, page 80, book 2), the innate immune response was evaluated by analyzing inflammatory cells and expression of TLRs in the lungs of wild-type (WT) and knockout mice (KO; $TLR4^{-/-}$ and $MyD88^{-/-}$). Neonate mice were exposed to 1,2-NQ or vehicle. After 8 weeks, animals were sensitized/challenged with ovalbumin (OVA). Samples were collected 24h after the last challenge or at 24h post 1.2-NQ. Bronchoalveolar lavage (BAL), bone marrow (BM), spleen and thymus cells population were analyzed by cells count and/or flow cytometry in addition to mRNA expression of TLRs and adaptors molecules in the lung. Data are mean ± SEM for n mice. Stats were by ANOVA plus Bonferroni's test. **Results**: At 24h post 1,2-NQ exposition, thymus of WT neonate mice shows increased CD8+ T lymphocyte population (2.1*±0.14 % of cells) and PDL-1 expression (0.38**±0.01 % of cells) compared to untreated WT mice (CD8+: 1.2±0.16 % of cells; PDL-1: 0.21±0.01 % of cells). 1,2-NQ treated WT mice spleens exhibited enhanced B-lymphocytes, NK1 cells, CD11c+ populations or co-stimulatory molecules (CD80, CD86, MHC-II). OVA treatment in prior exposed 1,2-NQ WT mice increased eosinophil counts in BAL (42±10.0** x 10^4 cells/BAL) and eosinophil maturation in (4.1±0.5* x 10^6 cells/BM) compared to allergic WT mice (BAL: 9.7±1.4 x 10^4 cells/BAL; BM: 2.1±0.3 x 10^6 cells/BM). However treatments with both 1,2-NQ plus OVA allergen in $TLR4^{-/-}$ and $MyD88^{-/-}$ KO mice did not evoke eosinophils influx in the lungs and in the BM. Lung mRNA expression of TLR2, TLR4 and TLR7 or TRIF, but not MyD88 was higher in WT treated mice, with or without OVA, compared to vehicle WT mice. Interestingly a clear increase of TRIF and MyD88 mRNA expression was seen in $TLR4^{-/-}$ mice exposed to 1,2-NQ in comparison to vehicle mice. **Discussion:** These findings show that the early exposure of 1,2-NQ exacerbates allergic pulmonary inflammation in an adult stage by TLR4 activation and for a signaling pathway MyD88 dependent and TRIF independent. We also speculate that the exaggerated inflammatory effect against the allergen in adulthood is resulted of 1,2-NQ interference in the maturation and function of innate immune system cells exposed to this air pollutant, leading to changes in the adaptive response. This might be one mechanism that contributes to common pulmonary illnesses in children living in heavy traffic areas. **Acknowledgments:** FAPESP, CAPES and CNPq for financial support, and Mrs. Maria Alice Barreto and Irene Maria Gouvea for technical support.

08.002 Nitric oxide and cyclooxygenase products are key factors in the antispasmodic effect of glucagon on airway smooth muscle contraction *in vivo.*
Insuela DBR, Daleprane JB, Almeida RR, Arantes ACS, Cordeiro RSB, Silva PMR, Martins MA, Carvalho VF Fiocruz – Inflamação

Aim: In our previous work we demonstrated that glucagon presents an antispasmodic effect on airway smooth muscle contractility *in vitro* and that this action occur in an indirectly way, through activation of its receptor present in epithelium with consequent release of nitric oxide and products of COX activation. In this study we investigated the antispasmodic effect of glucagon *in vivo* and if nitric oxide and products of COX activation are involved in this action. **Methods:** The animals were obtained from Oswaldo Cruz Foundation breeding colony and used in accordance with the guidelines of the Ethic Committee on Use of Laboratory Animals of the Oswaldo Cruz Foundation, License LW – 23/10. First, we evaluated the expression of glucagon receptor (GcgR) in lungs and tracheas in the presence or absence of epithelium by Western blott. Next, glucagon (1 µg/Kg, i.n.) was administered in mice and 1, 3 and 6h after this treatment the animals were exposed to methacholine-evoked airways obstruction in a non-invasive barometric plethysmography. We evaluated the dose-response of glucagon (0.1 - 10 µg/kg, i.n.) on the resistance and compliance of the airways induced by aerosolization of methacholine in an invasive barometric plethysmography. The administration of glucagon was realize 3h before the stimulation with methacholine and in some experiments the mice received i.p. a NOS inhibitor (L-NAME, 20 mg/kg) or a COX inhibitor (indomethacin, 10 mg/kg) 30 min before glucagon. A daily treatment with glucagon (0.1 - 10 µg/kg, i.p.) was performed once a day for 7 or 14 days, and glucose levels were assessed 24h after the last day of treatment. **Results:** GcgR was expressed in lungs and tracheas with intact epithelium but less expressed in tracheas that had the epithelium removed. an increase of bronchial spasm in a concentration-dependent manner. The intranasal administration of glucagon inhibited methacholine-induced bronchoconstriction 1h (saline i.n.: 67.03 ± 6.60 AUC of Penh; glucagon i.n.: 49.09 ± 3.05 AUC of Penh; mean ± SEM) and 3h after treatment (saline i.n.: 165.50 ± 21.51 AUC of Penh; glucagon i.n.: 82.14 ± 9.44 AUC of Penh; mean ± SEM) but not 6h (saline: 77.14 ± 11.78 AUC of Penh; glucagon i.n.: 69.75 ± 5.11 AUC of Penh; mean ± SEM). Yet, this effect was more prominent at 3h. All the doses of glucagon (0.1, 1 and 10 µg/kg) prevented the decrease in airways compliance promoted by methacholine, however, only the dose of 1µg/Kg inhibited the increase in airways resistance. Either L-NAME or indomethacin abrogated the antispasmodic effect of glucagon on airways resistance and compliance. Finally, the daily administration of glucagon did not interfere with the circulating levels of glucose. **Conclusion:** Our results describe a new extrahepatic action of glucagon *in vivo* that is an antispasmodic effect on airway smooth muscle contraction. This action is mediated by activation of the glucagon receptor present in the airways epithelium, which culminates in the release of nitric oxide and products of COX activation. **Financial support:** CNPq, FAPERJ and FIOCRUZ

08.003 Long-term treatment of BAY 60-2770, a soluble guanylate cyclase activator, prevents lower urinary tract dysfunctions induced by obesity. Alexandre EC, Leiria LOS, Silva FH, Calixto MC Monica, FZ, Antunes E FCM–Unicamp – Farmacologia

Introduction: Activation of the NO-cGMP signaling pathway results in inhibitory responses at the level of urethra and urethral sphincter, as well as of detrusor smooth muscle (DSM). Therefore, impairment of the NO-cGMP signaling pathway contributes to bladder overactivity. BAY 60-2770 is a novel described activator of soluble guanylate cyclase (sGC) that acts by NO- and haem-independent mechanisms (Stasch and Hobbs, 2009). Diet-induced obesity displays bladder dysfunction and overactive detrusor smooth muscle associated with hypercontractility in mice (Leiria et al., SBFTE, 2011). This study aimed to evaluate whether chronic oral intake with BAY 60-2770 prevents the lower urinary tract dysfunctions induced by obesity. **Methods:** The experimental protocols were approved by the Animal Ethical Committee of UNICAMP (CEEA-IB/UNICAMP, 2582-1). C57BL/6 mice fed for 10 weeks with standard chow (SCD) or high-fed diet (HFD) were given for 2 weeks BAY 60-2770 (1 mg/Kg) or vehicle (transcutol:cremophor:water) by daily oral gavage. The groups were divided as follows: SCD + vehicle, SCD + BAY 60-2770, HFD + vehicle and HFD + BAY 60-2770. Detrusor and urethra smooth muscles were mounted in organ baths containing Krebs solution, contractile and relaxing responses were measured. The potency (pEC_{50}) and maximal response (E_{max}) values were determined. Cystometric study in anaesthetized mice was performed in all groups. **Results:** In detrusor smooth muscle, contractions induced by carbachol (CCh, 0.001-30 μM), calcium chloride ($CaCl_2$, 0.001-30 mM) and potassium chloride (KCl, 1-300 mM) were significantly higher in obese (E_{max}: 3.1±0.76; 2.9±0.5 and 2.6±0.4 mN/mg respectively) compared with control mice (E_{max}: 1.6±0.3; 2.1±0.2 and 1.7±0.2 mN/mg, respectively; $P<0.05$). Long-term treatment with BAY 60-2770 in HFD mice prevented the enhanced contractile responses to these contractile agents. BAY 60-2770 did not affect the CCh-, $CaCl_2$- and KCl-induced contractions in control group. The cystometric study showed that non-void contractions and micturition frequency were 406% and 68% higher in obese mice ($P<0.05$), respectively, while co-treatment with BAY 60-2770 largely attenuated both of these parameters. In control group, BAY 60-2770 had no effect in the cystometric parameters. Relaxation induced by NO (acidified sodium nitrite) in pre-contracted urethra was reduced in obese mice (E_{max} :37.4±6.5%; $P<0.05$, n=5) in comparison with control mice (E_{max} :51.6±1.1% ; n=5). BAY 60-2770 normalized the NO-induced urethral smooth muscle relaxation in the obese group (E_{max}=48.9±3.7%; n=6). **Discussion:** Our findings show that long-term oral treatment with BAY 60-2770 prevents the lower urinary tract dysfunctions seen in obese mice, suggesting that sGC activators may be of beneficial value to treat overactive bladder. **Financial Support:** CNPq – Conselho Nacional de Desenvolvimento Científico e Tecnológico.

08.004 Increased prostate smooth muscle contractions and reduced beta-adrenoceptor-mediated signal transduction in chronic Nitric Oxide (NO) deficiency model. Calmasini FB, Leiria LOS, Pissinatti L, Bau FR, Antunes E Unicamp –Pharmacology

Introduction: The prostate gland plays an important role in reproduction and secretion of several substances that are part of the seminal liquid and fertilization. Prostate consists of epithelium and stroma, the latter being composed by smooth muscle, which is responsible for tissue contraction. Benign prostatic hyperplasia (BPH) in elderly men is characterized by prostate enlargement and increased smooth muscle tone, thus contributing to overactive bladder. The NO-cGMP signaling pathway regulates smooth muscle contractility, but little is known about this signal transduction in the regulation of prostate smooth muscle (PSM). This study tested the hypothesis that prolonged NO deficiency leads to functional alterations in the PSM machinery. Therefore, in rats undergoing prolonged L-NAME intake (NO synthase inhibitor), we investigated the contractile responses to a_1-adrenoceptor, muscarinic and purinergic P2X1 activation, as well as the cAMP- and cGMP-mediated relaxing responses. **Methods:** The experimental protocols were approved by the Animal Ethical Committee of UNICAMP (nº 2424-1). Male Wistar rats were treated with the NO synthesis inhibitor L-NAME (20 mg/rat/day, 4 weeks). Concentration-response curves to the contractile agents phenylephrine (a1-adrenoceptor agonist; 1 nM-100 μM), carbachol (muscarinic agonist; 1 nM-100 μM), α,β-methylene ATP (P2X1 agonist; 1-10 μM), as well as to the relaxing agents isoproterenol (ISO) and sodium nitroprusside (SNP) were obtained in PSM. Neurogenic contractile responses were also obtained. Quantification of cAMP and histological analysis in prostates were performed. **Result:** Treatment with L-NAME promoted 30% increase ($P<0.05$) in prostate weight (n=10). Phenylephrine and carbachol-induced PSM contractions were enhanced in L-NAME-treated rats (E_{max}: 4.1±0.3 and 3.5±0.3 mN, respectively, $p<0.05$) compared with control rats (E_{max}: 3.2±0.2 and 2.5±0.2 mN, respectively, n=6). The PSM contractions induced by α,β-methylene ATP were also greater in L-NAME compared with control group (10 μM: 2.0±0.2 and 1.1±0.2 mN, respectively). Electrical field stimulation (1-32 Hz)-induced PSM contractions were also significantly greater in L-NAME (4 Hz: 2.5±0.3 mN) compared with control group (4 Hz: 1.6±0.2 mN). In vitro addition of L-NAME (100 μM) to the organ baths did not affect the PSM-induced responses. The PSM-induced relaxations in response to SNP remained unaltered, whereas ISO-induced relaxations were lower in L-NAME ($p<0.01$) compared with control group. The cAMP levels in prostate homogenate were 34% lower ($P<0.05$) in L-NAME compared with control group. Histology showed no great abnormalities between groups. **Discussion:** Our findings show that prolonged L-NAME administration causes increased PSM contractile responses to α-adrenoceptor, muscarinic and P2X1 receptor activation associated with reduced b-adrenoceptor-mediated signal transduction. It is likely that chronic NO-deficiency in rats mimics BHP in humans. **Financial support:** CAPES.

08.005 The renin-angiotensin system (RAS) plays a major role in the voiding dysfunction of ovariectomized rats. Ramos-Filho ACS[1], De Almeida Faria J[1], Teixeira SA[2], Mónica FZT[1], Calmasini FB[1], De Nucci G[1], Muscará MN[2], Anhê GF[1], Antunes E[1] [1]Unicamp – Pharmacology, [2]ICB-USP – Pharmacology. **Introduction:** The loss of estrogen (E2) associated with menopause increase the risks for arterial hypertension, overweight and diabetes. Besides, E2 deficiency has been implicated in the etiology of lower urinary tract symptoms (LUTS) with 70% of women presenting urinary incontinence. E2 down-regulates the activity of renin-angiotensin system (RAS). It has been suggested the occurrence of a local tissue RAS in genitourinary tract where ANGII is synthesized independently of systemic RAS. Therefore, this work aimed to evaluate the role of RAS in the voiding dysfunction of ovariectomized rats. **Methods:** The experimental procedures were approved by the Animal Care and Use Committee of UNICAMP (protocol n^{o}. 2734-1). Female Sprague Dawley rats (250-300 g) were subjected to bilateral ovariectomy (OVX) and treated or not with 17β-estradiol (1 mg/kg/week) or losartan (30 mg/kg/day). Concentration-response curves to carbachol (CCh, muscarinic agonist), phenylephrine (PE, a_1-adrenoceptor agonist) and angiotensin II (ANGII) were carried out in isolated DSM and/or urethra. Relaxant responses to sodium nitroprusside (SNP) and BAY 41-2272 (NO-independent soluble guanylyl cyclase agonist), as well as cystometry in anaesthetized rats were also performed. The angiotensin converting enzyme activity (ACEa) and expression of AT_1/AT_2 receptors were carried out in urinary bladder and urethra tissues. **Results:** The cystometric study in OVX rats showed significant increases ($p<0.05$) in basal pressure (BP), capacity and intervals between voiding cycles, as well as decreases in voiding pressure compared with sham group. PE- and ANGII-induced urethral contractions were greater in OVX group (3- and 6-fold, respectively) compared with sham group. Reduced bladder contractibility to CCh in OVX group (24.7% reduction; $p<0.05$). The SNP- and BAY 41-2272-induced urethra and bladder relaxations did not significantly differ between sham and OVX animals. Significant increases in ACEa (2-fold) and AT1/AT2 protein expression (6-fold) in urethra of OVX group were observed ($p<0.05$). Losartan treatment prevented the cystometric, functional and molecular alterations in OVX rats. Replacement with 17β-estradiol also prevented the cystometric, functional and ACEa changes in OVX rats. *In vitro* incubation with 17β-estradiol (100 nM) did not affect the CCh- and ANGII-induced responses in urethra and bladder smooth muscles. **Discussion:** Our results show that local RAS activation and ANGII generation at the level of urethra are responsible for the micturition changes in E2 deficiency rats. It is likely that E2 deficiency promotes persistent urethra contractibility and hence low bladder responsiveness, decreasing voiding efficiency and increasing urinary retention. **Financial support:** FAPESP

08.006 Effect of multifactorial malnutrition in rat vas deferens: **Modulation of Ca^{2+}-ATPase by calmodulin**. Bezerra CGP[1], Souza AB[1], Muzi-Filho H[1], Einicker Lamas M[1], Vieyra A[2], Lara LS[1], Nascimento VM[1] [1]ICB-UFRJ –Farmacologia Celular e Molecular, [2]UFRJ – Biofísica

Introduction: It is generally accepted that the reproductive performance in the adult is determined by a wide varied of influences, including the nutritional status. We previously demonstrated that malnutrition during developmental periods of life impairs the reproductive profile of adult male rats. We proposed that this phenomenon is associated with *vas deferens* prostatic portion atrophy and adaptive changes in intracellular Ca^{2+} handling which are linked to protein kinase-mediated phosphorylation and increased expression of *alpha*1-adrenergic receptor[1]. The aims of present study were to evaluate the oxidative stress degree in the *vas deferens* and to determine the effect of *alpha*1-adrenergic signaling on Ca^{2+}-ATPase activity during chronic malnutrition. **Methods:** Male Wistar rats were chronically malnourished (CM) from weaning until 13 weeks of age using the model described as Regional Basic Diet[2]. After this period, control (n=5) and CM (n=5) male rats were sacrificed (CEUA DFCBICB007), the pair of vas deferens was removed and an enzymatic preparation (ultracentifuged homogenate) was obtained. Differences between the parameters studied in the control and CM groups were analyzed using unpaired Student's t-test. The significance was considered at $P< 0.05$. **Results :** The measurement of Ca^{2+}-ATPase activity in the control and CM groups showed that the distribution profiles of SERCA and PMCA activities relative to the total Ca^{2+}-ATPase activity were 68% - 32% (control) and 54% - 46% (CM), respectively. In CM group, it was observed a significant increase of lipid peroxidation by TBARS method (659%) and protein carbonylation (292%). However, the density of –SH free groups did not change. Methoxamine, an *alpha*1-adrenoceptor agonist inhibited total Ca^{2+}-ATPase activity from control and CM groups (36 and 56%, respectively). The concomitant addition of 5-methylurapidil (a specific *alpha*1-adrenoceptor antagonist) reverted the total Ca^{2+}-ATPase activity only in the control. This profile was the same for the SERCA activity. The exogenous addition of calmidazolium (calmodulin antagonist) in the incubation medium completely inhibited SERCA activity and partially inhibited PMCA activity (control: 31% and CM: 61%). **Discussion**: CM enhances oxidative stress in the *vas deferens* leading to the organ atrophy. Since 5-methylurapidil did not revert the inhibition promoted by methoxamine on Ca^{2+}-ATPase activity, we suggest a high adrenergic activation in the CM *vas deferens*. This event is mediated by Ca^{2+}/CaM regulation of SERCA rather than PMCA. **Sources of Research Support:** FAPERJ Primeiros Projetos, Programa ALV. 1. Bezerra, CGP *et al*. Effect of chronic malnutrition in rat vas deferens: morphological and biochemical analysis. XXVI Reunião Anual da Federação das Sociedades de Biologia Experimental, Resumo: 05-28, 2011. 2. Costa-Silva JH *et al*. Eur J Nutr 48: 43, 2009.

08.007 Impairment of insulin-induced PI3-KINASE/AKT/ENOS pathway in urothelium as a cause of obesity-associated detrusor overactivity. Leiria LOS, Sollon C, Kinote A, Bau FR, Mónica FZT, Anhê GF, Antunes E Unicamp – Farmacologia

Introduction: Recently, we demonstrated an association between insulin resistance (IR) and overactive bladder (OAB) in high-fat fed obese mice (Leiria et al, Neurourol Urodyn, 30:1020, 2011). However, the direct action of insulin in bladder smooth muscle has not yet been studied. We aimed to investigate the role of insulin in the bladder and its relevance for the development of OAB in insulin-resistant obese mice. **Methods:** All human and animal procedures were approved by the Research Ethics Committee (761-CEP) and Animal Ethical Committee of Unicamp, respectively. C57BL6/J mice were fed with high-fat diet for 10 weeks to induce obesity. Concentration-response curves to insulin (1-100nM) were performed in human and mice isolated detrusor smooth muscle (DSM). Relaxing responses to insulin were repeated in the presence of L-NAME (NO synthase inhibitor), LY294002, wortmannin (PI3-kinase inhibitors) or 124005 (Akt inhibitor). Cyclic GMP levels were determined by ELISA kit assay. Western blot was performed in insulin-stimulated urothelium to detect eNOS (Ser1177) and Akt (Ser473) phosphorylation. Protein levels of TRB3 (pseudo-kinase inhibitor of Akt; used as a marker of endoplasmic reticulum (ER) stress-dependent IR), CHOP and ATF4 (proteins involved in the ER stress that regulates TRB3 expression) were also measured in urothelium. Cystometry was performed to evaluate urodynamic pattern of obese mice. We treated obese and lean mice with the ER stress inhibitor 4-Phenyl butyric acid (PBA; 250mg/kg/day, 4 days) to access whether the urothelium IR is due to ER stress. **Results:** Obese mice exhibited OAB, as evidenced by the greater void frequency and non-void contractions. Insulin produced concentration-dependently DSM relaxations in lean mice (E_{max}: 32±1.0%) that were greatly reduced in obese animals (E_{max}: 17±2.3, $p<0.01$; n=13). Insulin also relaxed human DSM (E_{max}: 26±2.0%, n=5). Inhibition of the PI3K/Akt/eNOS pathway impaired the insulin-induced relaxations in both murine and human bladders. Insulin stimulation produced a 2.5 fold increase ($p<0.001$) in the cGMP levels in lean mice (n=4), which was not observed in obese mice. The PI3K/Akt/eNOS pathway inhibitors prevented the increased cGMP levels in lean mice. Urothelium removal significantly reduced the insulin-induced DSM relaxations in human and mice ($p<0.01$). Insulin did not induce eNOS phosphorylation in denuded DSM, suggesting urothelium-dependence. Wortmannin prevented the increase of insulin-stimulated p-Akt and p-eNOS in urothelium of lean mice. In the urothelium, insulin-stimulated Akt and eNOS phosphorylation were impaired in obese mice ($p<0.001$). TRB3, CHOP and ATF4 protein levels were increased in obese group ($p<0.05$). PBA treatment improved insulin induced cGMP production in obese mice, as well as the pAkt, peNOS, TRB3, CHOP and ATF4 protein levels. Oral treatment with PBA normalized the greater voiding frequency and frequency of non-void contractions. **Conclusion:** Our data show that insulin relaxes human and mice DSM via activation of the PI3K/Akt/eNOS pathway in urothelium. ER stress-dependent IR in bladder urothelium of obese mice is likely to contribute to the OAB in these animals. **Financial Support:** Supported by Fundação de Apoio a Pesquisa do Estado de São Paulo (FAPESP).

08.008 Epidermal growth factor receptor play important role in the spontaneous contractions of the cauda epididymis in castrated male adult rats. Agati LB[1], Kiguti LR[2], Godinho RO[1], Avellar MCW[1] [1]Unifesp – Pharmacology, [2]IBB-Unesp – Pharmacology,

Introduction: Agonists of G-protein– coupled receptors (eg, adrenoceptors and angiotensin receptors) signal, at least in part, through matrix metalloproteinases (such as matrix metalloproteinase [MMP]-7) that transactivate the epidermal growth factor receptor (EGFR). Previous studies from our laboratory have shown that EGFR and its activated form (pEGFR) are immunolocalized in the epithelial and interstitial cells along the epididymis from intact Wistar rats. After surgical castration, a significant increase in the immunolocalization of EGFR and pEGFR was observed in the smooth muscle cells surrounding epididymal tubules from the cauda region, an effect reversed by testosterone treatment of the animal. The present study was designed to confirm the involvement of EGFR activation in the contractile response of cauda epididymal tubules from intact and castrated rats. Methods: All the experimental procedures described here were approved by our institutional ethics committee (CEP 0921/6 and 0703/07). Control and surgically castrated (7 days) adult male Wistar rats (90 d) were used. Tubules from the cauda epididymis were isolated and mounted in 2 ml organ baths with nutritive solution (30^{o}C) under 1.0 g of tension for record of isometric contractions. The effects of the EGFR agonist (EGF, 100 ng/ml) and antagonist (AG1478, 20μM) on the spontaneous contractions recorded in control or castrated tissues were evaluated. **Results:** EGF induced no contractile effects in cauda tubules from control rats. The spontaneous contractions observed in cauda tubules from castrated rats were increased (frequency of spikes and maximal tension) during the incubation of the tissue with EGF (100ng/ml) for 30 min. Incubation of EGFR antagonist AG1478 (20μM) displayed insurmountable antagonism on both the castration-induced spontaneous contractions and on the EGF-induced effects on these contractions. **Discussion:** The results confirm that the high levels of EGFR/pEGFR immunolocalized in the smooth muscle layer of cauda epididymis from castrated rats correspond to functional receptors. They also reveal that the effects of EGFR activation on the pattern of spontaneous contractions in the epididymis are under androgen control. More studies are being conducted to elucidate the cross talk between EGFR signaling and alpha1-adrenoceptor, an important G-protein coupled receptor involved in the smooth muscle contraction in the epididymis. **Financial support:** CAPES, CNPq, Fogarty International Center (UNIFESP/UNC).

08.009 Evaluation of ionic substitution on [^{3}H]-noradrenaline release in rabbit isolated corpus cavernosum. Rodrigues RL, Mónica FZT, Antunes E, De Nucci G
FCM-Unicamp – Farmacologia

Introduction : The quantification of noradrenaline spillover from electrically stimulated sympathetic nerves can be obtained by direct detection of the amount released, which can be achieved by the detection of β-radiation by preparations preincubated with [^{3}H]noradrenaline. The method is useful to study the presynaptic effects of drugs and ionic replacement in the sympathetic neurotransmission in peripheral tissues. The aim of this work was to standardize the quantification of [3H]-noradrenaline release after electrical field stimulation (EFS) in isolated corpus cavernosum (CC) from rabbit and then to verify the effects of ionic replacement in this outflow. **Methods**: Male New Zealand White rabbits 3.0 kg) were euthanized with ketamine (70 mg/kg) and xylazine (10 mg/kg). The CC was removed and the strips were incubated for 2 hours in Krebs' solution continuously aerated with carbogen at 37^{o}C, containing 0.4 μM [^{3}H]noradrenaline (specific activity 14,8 Ci/mmol) and 1μM seleginine (MAO-B inhibitor). The tissues were suspended in a 10 ml organ bath containing Krebs' solution with 1 μM desipramine (inhibitor noradrenaline uptake). After the equilibration period, samples of 0.2 ml were repeatedly taken from the organ bath to determine the basal levels. The strips were subjected to two periods of EFS (16 Hz; 50 V; 0.5 ms pulse width; 0.2 ms delay, 5 minutes of stimulation). The first EFS was carried out in normal Krebs whereas the second one in solutions where equimolar NaCl was replaced by N-methyl D-glucamine, in the absence of extracellular calcium or tetrodotoxin (TTX, 1 μM). Release of [^{3}H]noradrenaline was expressed as DPM/min or as a percentage of the amount of radioactivity in the tissues at the sample collection time (fractional release). Data are expressed as mean±SEM. The experimental protocols were approved by the Animal Ethical Committee of UNICAMP (CEUA-IB/UNICAMP, 2720-1). **Results**: After 60 minutes of stabilization the radioactivity taken by the tissues was 253987±17208 DPM. The basal tritium efflux measured was 1472±110 DPM/min, which remained relatively constant throughout the experiment. EFS elicited an increase in [^{3}H]-noradrenaline release by approximately a factor 4.6±0.5. In the presence of sodium channel blocker TTX (1 μM) the [^{3}H]-noradrenaline release was significantly reduced by 68%. The replacement of NaCl by N-methyl-D-glucamine or the absence of extracellular calcium reduced EFS-induced [^{3}H]-noradrenaline release by 59% and 98%, respectively. **Discussion**: Our findings show that EFS in isolated CC from rabbit induced neurotransmitter release that is mainly due to sodium influx as the addition of TTX or sodium replacement significantly reduced noradrenaline outflow. This is the first study to show the effects of ionic replacement in isolated CC. **Financial Support**: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)

08.010 Ultrastructure and functional anatomy of the hemipenis of *Crotalus durissus terrificus*. Pissinatti L[1], Porto M[1,2], Oliveira MA[1,3], Rojas-Moscoso JA[1], Cogo JC[3], Metze K[4], Antunes E[1], Nahoum C[1], Mónica FZT[1], de Nucci G[1,5] [1]FCM-Unicamp – Pharmacology, [2]IBMR, [3]Univap – Research and Development, [4]FCM-Unicamp – Pathology, [5]ICB-USP – Pharmacology

Introduction: A detailed histological description of the structure and geometry of the corpus cavernosum of the South American rattlesnake *Crotalus durissus terrificus* using scanning electron microscopy and light microscopy techniques is presented. The results were compared with the structure and function of the mammalian penis. **Methods:** All experimental procedures were approved by an institutional Animal Care and Use Committee (CEUA-UNICAMP: 1655-1) and also by the Brazilian Institute of Enviroment (Sisbio: 18020-1). Male adults of Crotalus durissus terrificus (weighting 550 ± 50g) were obtained from Centro de Estudos da Natureza - UNIVAP. Animals were first anesthetized with isoflurane, followed by ketamine/xylazine (100 and 70 mg/kg). The hemipenis was exposed by manual pressure on the caudal vein. For scanning electron microscopy, the everted hemipenis was fixed by infusion of a fixative solution containing 2.5% of glutaraldehyde diluted in cacodylate buffer 0.1M pH 7.2 for 2 hours. For light microscopy, the hemipenis was fixed as above and pieces were embedded in paraffin. **Results:** The hemipenis of *Crotalus* consists of two concentric cylinders named corpus cavernosum. Smooth muscles are present forming the sinusoids of corpus cavernosum. Sphincters made of smooth muscles are found beneath the tunica albuginea and between the corpus cavernosum. The retractor muscle is a skeletal muscle with many sinusoids transversally arranged, and a central sinus that bring blood to hemipenis. The minor retractor muscle should be renamed as propulsor muscle, since it is involved in organ extrusion. Unlike mammals, the sinusoidal walls of hemipenes are not organized with the smooth muscle intertwiningly with a fibrous connective tissue skeleton, but by radially oriented smooth muscles. **Discussion:** Our results show that *Crotalus durissus terrificus* hemipenes shares few similarities with human penis. Both are fleshy cylinders that are flexible and both contain a hydraulic skeleton that are filled with fluid before copulation to enlarge the penis. In the human penis (like other mammals), the sinusoidal walls are formed by the smooth muscle intertwiningly with a fibrous connective tissue skeleton. On the other hand, in Crotalus hemipenis there is not an arrangement of sinusoidal walls similar to mammals. Instead, the muscle projections are radially arranged, there are few fibrous connective tissue and probably functions to support and attach the two corpus cavernosum while the organ is everted. To date, no information on the erection mechanism in turtles and crocodilian is available to compare with snakes and mammals. **Financial Support:** Capes

08.011 Activation of NO/GMPc/PKG pathway by histamine modulates the noradrenaline induced contraction in rat testicular capsule. Silva Junior ED, Rodrigues JQD, Jurkiewicz A, Jurkiewicz NH Unifesp – Farmacologia

Introduction: The rat testicular capsule presents a physiological response to noradrenaline released by sympathetic nerves (Jurkiewicz, Eur J Pharmacol, 543, 141, 2006). However, the effects of modulatory agents, such as histamine, have not been investigated. Therefore, the aim of our study was to evaluate the modulatory activity mediated by histamine on contractions evoked by exogenous noradrenaline. **Methods:** Adult Wistar rats, 3-4 months-old and weighing 300-370 g were used. The isolated testicular capsule was mounted in an organ bath. Time-effect curves for exogenous noradrenaline (10^{-4} M, for 2 minutes) were performed in the presence of histamine (10^{-7} – 10^{-5} M, pre-incubated for 3 minutes). Antagonists acting at the different histamine receptor subtypes (H_1, H_2 and H_3) were tested. Furthermore, inhibitors of NO/GMPc/PKG signaling pathway were also used in the effects produced by histamine on noradrenaline induced contraction. The results were obtained from at least 5 experiments. All experimental procedures were approved by the Ethics Committee of UNIFESP (protocol number 0518/10). **Results:** Repetitive time-effect curves for noradrenaline (NA) 10^{-4} M produced a sensitization of rat testicular capsule. Thus, we used a temporal control to evaluate the effects of histamine on NA-induced contraction. Histamine 10^{-6} M prevented the sensitization caused by repetitive time-effect curves for NA and at this concentration the contractions elicited by NA were decreased by about 20%. Ketotifen 10^{-6} M was able to nullify the effects produced by histamine on NA-induced contraction. Cimetidine 3.10^{-5} M (H_2 receptor antagonist) partially blocked the effects elicited by histamine in this experimental protocol. Additionally, thioperamide (H_3 receptor antagonist) was unable to block the effects produced by histamine on NA-induced contraction. The reversible inhibitor of nitric oxide synthesis L-NAME (10^{-6} M) was able to block the effects produced by histamine. On the other hand, nitroprusside (10^{-4} M, pre-incubated for 3 min), a donor of NO, potentiated the effects produced by histamine by about 17%. The soluble guanylate cyclase inhibitor LY83583 (10^{-7} M, pre-incubated for 30 min) totally blocked the effect produced by histamine and the non-specific inhibitor GMPc phosphodiesterases IBMX (10^{-5} M, pre-incubated for 30 min) potentiated the histamine modulatory effects by about 33%. The inhibitor of cGMP-dependent protein kinase (PKG) KT5823 (10^{-6} M, pre-incubated for 30 min) was able to nullify the effects produced by histamine on NA-induced contraction. **Discussion:** The data altogether suggest that the effect produced by histamine on NA-induced contraction was due to interaction with H_1 receptors and consequently activation of NO signaling pathway. **Financial support:** CAPES, CNPq and Fapesp.

08.012 Pharmacological characterization of bronchial smooth muscle function in middle aged rats. Bau FR, Silva FH, Mónica FZT, Antunes E, De Nucci G Unicamp – Farmacologia

Introduction: Aging is a complex process with multiple alterations in the physiological structure and functional responses of the organism. With advancing age a significant decrement in the functional capacity of the respiratory system occurs. This alteration is related to structural and functional changes in respiratory tissues. Therefore, this study aimed to pharmacologically characterize changes in contractile and relaxant responses of bronchial smooth muscle (BSM) of middle-aged rats. **Methods**: The experimental protocols were approved by the Animal Ethical Committee of UNICAMP (n° 2110-1). Male Wistar rats were divided into two groups, namely young and middle-aged rats (2.5 and 10 months, respectively). The contraction was assessed by concentration response curves (CRC) to carbachol (CCh; 0.001 – 100 μM) in absence and presence of Rho-kinase inhibitor (Y-27632; 1 μM) or PKC inhibitor (GF-109203X; 1 μM). Relaxation responses were evaluated through CRC to nitroprusside sodium (SNP; 0.01 – 100 μM) and Isoproterenol (ISO; 0.001 – 10 μM) in tissues pre-contracted with CCh. The results are expressed as mean ± SEM of pEC_{50} and maximal response (Emax). **Results**: The potency values (pEC_{50}) of CCh was significantly lower in middle-aged rats (5.88 ± 0.03, n=5, $P<0.05$) in comparison with young rats (6.38 ± 0.02; n=5) with no difference in Emax. Treatment with Y-27632 produced a significant reduction in pEC_{50} in young (5.72 ± 0.03; n=9; $p<0.05$) and middle-aged rats (5.67 ± 0.03; n=9; $p<0.05$), while Emax was only decreased in middle-aged rats (16.39 ± 3.05 vs 9.93 ± 1.16 mN; n=5-9; $p<0.05$). Similarly to Y-27632 incubation, GF-109203X also produced a significant reduction in pEC_{50} in young (5.68 ± 0.03; n=8; $p<0.05$) and middle-aged rats (5.76 ± 0.03; n=8; $p<0.05$), whereas Emax was only decreased in middle-aged rats (control; 18.42 ± 3.50 vs 11.38 ± 2.09 mN; n=5-9; $p<0.05$). The pEC_{50} values for ISO was significantly reduced in middle-aged rats (7.44 ± 0.03 vs 6.72 ± 0.03; n=12; $p<0,05$) without affecting the Emax (19.62 ± 4.78 vs 19.09 ± 2.07 %; n=12). On the other hand, the relaxation induced by SNP was significantly lower in middle-aged-rats (28.19 ± 1.70 %; n=8; $p<0.05$) when compared with young rats (46.70 ± 8.02%; n=11). **Discussion**: Our results show a reduction in contraction and relaxation responses in BSM of middle-aged rats. This decrease in contractility of BSM seems to be related to Rho-kinase and PKC function, whereas the reduction in ISO and SNP responses suggest that the decrease in both cAMP and cGMP levels in BSM is involved in the aging process. **Financial support**: CNPq