Session 08 − **Respiratory, Urinary and Reproductive**

08.001

NO association between vitamin D Receptor haplotype and preeclampsia in a Brazilian population. de Rezende V[1], Sandrim VC[1], Palei ACT[2], Cavalli RC[2], Luizon MR[1], Tanus-Santos JE[1] [1]FMRP-USP − Farmacologia, [2]FMRP-USP − Ginecologia e Obstetrícia

Introduction: One of the characterize of preeclampsia is low circulating levels of 1,25-dihidroxivitamin D3 [1,25(OH)2D3] and calcium (1). Vitamin D endocrine system has important influences on immune modulation that have been associated to preeclampsia (2-5). Recent studies suggest that genetic factors may have a strong influence on susceptibility to preeclampsia (6). This study aimed at examining if haplotype of three polymorphisms (BsmI, ApaI and FokI) in VDR gene are associated with preeclampsia. **Material and Methods:** One hundred and sixty two with preeclampsia and 213 healthy controls were genotyped for the FokI, BsmI and ApaI polymorphisms in VDR gene using polymerase chain reaction–restriction fragment length polymorphism (RFLP). Haplotype groups are estimated by Phase v2. Results: No significant differences were observed in the genotype and allele frequencies of the FokI, BsmI and ApaI polymorphisms between the cases and controls. Haplotype frequency in preeclampsia women: (H1: 13.1; H2: 19.1; H3: 11.7; H4: 20.9; H5: 5.7; H6: 10.7; H7: 7.5; H8: 11.3) . Haplotype frequency in healthy controls women (H1: 15.9; H2: 16.2, H3: 12.9; H4: 16.9; H5: 8.4; H6: 10.5; H7: 7.3; H8: 11.9) P were considered significant when <0.0063 (0.05/8 or 0.05/number of haplotypes). **Discussion:** Although the haplotype analysis has been valued as a more powerful approach than the analysis of single polymorphisms, our findings show that VDR genotype neither haplotypes would not predict about preeclampsia. References: 1-Tanaka Y; Proc Natl Acad Sci; v. 76 pp. 5035; 1979 2-Broughton Pipkin F; Biol Neonate ; v. 76 pp. 325; 1999 3- Seely E; J Clin End Met ; v 92; pp. 3402 ; 2007 4-Bodnar L; J Clin End Met; v. 92; pp. 3517; 2007 5-Hypponen E; Nutrition Reviews; v. 63 pp. 225 ; 2005 6-Medica I; Eur J Obstetric & Gynec Reprod Biol; v 131 pp.115; 2007 Key words: Preeclampsia , vitamin D receptor gene (VDR), haplotype. **Financial support:** CAPES, FAPESP, CNPq.

08.002

Effect of intrauterin undernutrition in rat vas deferens: altered Ca^{2+} homeostasis and implications in male fertility. Muzi-Filho H[1], Souza AM[1], Bezerra CGP[1], Boldrini LC[2], Takiya CM[2], Oliveira FL[2], El-Cheikh MC[2], Einicker-Lamas M[3], Vieyra A[3], Lara Morcillo LS[1], Cunha VMN[1] [1]ICB-UFRJ − Farmacologia Celular e Molecular, [2]ICB-UFRJ − Ciências Morfológicas, [3]IBCCF-UFRJ − Instituto de Biofísica Carlos Chagas Filho

Introduction: It is known that multifactorial undernutrition modifies molecular structures responsible for intracellular Ca2+ homeostasis. The aims of this work were: 1 - investigate the tissue composition in the rat vas deferens (RVD); 2 - evaluate the expression and activity of protein kinase A (PKA) and protein kinase C (PKC) in RVD; and 3 - evaluate the effect of a1-adrenoceptor agonists and antagonists in the Ca2+-ATPase activity. **Methods:** According to this model pregnant Wistar female rats were fed with the Regional Basic Diet (RBD). After birth, the offsprings (20) were fed with a conventional diet (RBD-IU). In the control group, pregnant female rats and their offspring were fed with the conventional diet. When the rats reach 13 weeks-old they were sacrificed (CEUA DFBCICB 007) and the RVD were removed to perform: 1-histological observations (Hematoxylin and Eosin staining); 2-biochemical and Western Blotting assays to evaluate activity and expression of PKA and PKC; 3- the measure of Ca2+-ATPase activity (with a1-adrenoceptor agonists and antagonists). Results: The histological observations show global atrophy of all RVD, mainly of the mucosa (tissue layer that delimits the light organ). The PKA and PKC activities were increased in the RBD-IU group (250% and 616%, respectively; n=4; $p<0.05$), as well as the expression only of I isoform of PKC (66.0 ± 17.5%; n=4; $p<0.05$). However, there was none observed change in the expression of other isoforms of PKC and PKA. In both groups, the Ca2+-ATPase activity measured in the presence of an a1-adrenoceptor agonist (methoxamine), is smaller than in the control condition (56% of total in the control vs 33% of total in the RBD-IU group; n=3; $p<0.05$). However, the use of methoxamine and 5-metilurapidil (an a1-adrenoceptor antagonist) in the incubation medium promoted a reversal of the Ca2+-ATPase activity inhibition observed in the control group, while it remains inhibited in RBD-IU (92% of total; n=3; $p<0.05$). **Discussion:** Our data show that in RBD-IU group, the increase of PKA and PKC activity (protein related to Ca2+ mobilization), and the activation of a1-adrenoceptor signalization inhibit the Ca2+-ATPase activity and affect the Ca2+ uptake to the intracellular stores, possibly leading to reduction of the contractile RVD as a consequence. These data, combined with the global atrophy of the RVD, is possible linked to reduced reproductive capacity of these animals, as we showed previously. **Financial support:** Projeto Casadinho-CNPq; PROCAD-CAPES; FAPERJ Primeiros Projetos, Programa ALV

08.003

Functional characterization of erectile dysfunction in middle-aged rats. Silva FH, Monica FZT, Priviero FBM, Flores Toque HA, Antunes E UNICAMP − Pharmacology

Introduction: Epidemiologic studies have suggested that aging is an independent predictor of erectile dysfunction (ED). Aging is a complex process with multiple alterations in the physiological structure and functional responses of the organism. Structural alterations in the human cavernous tissue occur during aging, namely fibrosis and reduction in smooth muscle cells (SMCs), endothelium, and nerve content, which together contribute to vascular damage (Johannes et al., 2000). Previous studies showed that age-related ED is associated with reduction in eNOS activity in the mouse corpus cavernosum (Jim et al., 2006; Bivalacqua et al., 2007). In the present study we have further investigated the pathophysiological alterations of age-related ED looking at both relaxant and contractile machinery in RCC. **Methods:** The experimental protocols were approved by the Animal Ethical Committee of UNICAMP. Wistar rats were divided into two groups: (a) young (14-15 weeks) and (b) middle-aged rats (37-38 weeks). The erectile function was assessed by measuring the rise in intra-cavernous pressure (ICP) following cavernous nerve electrical stimulation. Concentration-response curves to the relaxing agents acethylcholine (ACh), sodium nitroprusside (SNP), glyceryl trinitrate (GTN), sildenafil, tadalafil and isoproterenol, as well to electrical-field stimulation (EFS; nitrergic relaxations) were obtained in RCC. Contracting responses to phenylephrine (PE) and EFS were also obtained in all groups. Results: A significant decrease in ICP ($P<0.05$) was observed in middle-aged compared with young rats (6 Hz: 15.6 ± 3 and 25.8 ± 3 mmHg, respectively). The nitrergic relaxations in middle-aged RCC were also significantly reduced ($P<0.05$) compared with young rats (32 Hz: 39.2 ± 4 and 53 ± 1.5%, respectively). The RCC relaxations to ACh, SNP, GTN, sildenafil and tadalafil in middle-aged rats were significantly lower (Emax: 37 ± 2%, 89 ± 3, 39 ± 2%, 60 ± 6% and 60 ± 5 %, respectively; $P<0.05$) in comparison with young rats (74 ± 3%, 104 ± 3%, 59 ± 7%, 93 ± 2% and 79 ± 6%, respectively). The RCC relaxations to isoproterenol remained unaltered in both groups. The phenylephrine-induced RCC contractile responses were significantly higher in middle-aged rats (Emax: 4.44 ± 0.38 mN; $P<0.05$) compared with control group (Emax: 3.48 ± 0.12 mN). EFS-induced contractions in middle-aged RCC were significantly higher ($P<0.05$) compared with young rats (32 Hz: 5.14 ± 0.3 and 3.6 ± 0.5mN, respectively) **Discussion:** Our findings showed erectile dysfunction in middle-aged rats is a consequence of impaired nitrergic-, endothelium-dependent, and endothelium-independent relaxations, along with increased sympathetic nerve transmission and a1-adrenoceptor-mediated contractile response. Whether such alterations reflect increased PDE5 activity is under current investigation. support: FAPESP and CAPES **References**: Bivalacqua TJ et al., Am. J. Physiol. Heart Circ. Physiol. 292:1340–51, 2007. Jin L et al., FASEB J 20:536–8, 2006. Johannes CB et al., J. Urol. 163: 460–3, 2000.

08.004

Pre-clinical evaluation of the isoniazid-derived compounds IQG-607 and IQG-639 in a mouse model of tuberculosis. Rodrigues-Junior VS[1], Santos Jr AA[1], Jader ABS[1], Souto AA[1], Calixto JB[2], Basso LA[1], Santos DS[1], Campos MM[1] [1]INCTTB-PUCRS, [2]UFSC − Farmacologia

Introduction: Tuberculosis (TB) continues to be one of the deadliest diseases in the world. The emergence of multi-drug-resistant strains of M. tuberculosis, the unbearable side effects of the available drugs and the frequent patient non-compliance in completing the therapy have increased the need for development of new effective agents. Isoniazid (INH) is the most prescribed drug for active TB and prophylaxis, and requires activation by the enzyme KatG. The M. tuberculosis enoyl-ACP reductase (InhA) has been shown to be the primary target for INH. Our group has published the rational design and synthesis of new isoniazid-derived compounds with possible anti-TB activity. Importantly, it was shown that these compounds do not require activation by KatG to bind its molecular target, InhA. We have also demonstrated that these INH analogs are able to inhibit in vitro the activity of wild-type and INH-resistant M. tuberculosis InhA. In addition, these compounds were active against cultures of M. tuberculosis H37Rv and two INH-resistant clinical isolates, showing a satisfactory efficacy in vitro. This work describes the pre-clinical evaluation of two pentacyano(isoniazid)ferrateII complexes, named IQG-607 and IQG-639, in a mouse model of TB. **Methods:** All the experimental protocols were approved by the local Animal Ethics Committee (CEUA 09/00094). Male Swiss mice were infected intravenously through the retro-orbital sinus, with 1 x 107 viable M. tuberculosis bacilli suspended in 0.2 ml of Middlebrook 7H9 medium. Treatment was started 5 days post-infection, and the compounds IQG-607 and IQG-639 (250 mg/kg) were administered orally, once a day, during 28 days. Separate groups of mice were treated at the same schedules of treatment with the reference drug INH (25 mg/kg; positive control) or saline solution (negative control). At the end of treatments, mice were sacrificed by isoflurane inhalation. The spleens and lungs were aseptically removed, and the spleen weight (in grams) was determined. These organs were homogenized and the colony-forming units (CFU) were determined after 28 days of incubation in solid medium, at 37 °C. Results and **Discussion:** M. tuberculosis-infected mice showed a marked enhancement of the spleen weight, when compared to the non-infected group. Either IQG-607 or INH significantly reduced M. tuberculosis-induced splenomegaly, with inhibition percentages of 50 and 58 %, respectively. On the other hand, IQG-639 failed to significantly affect this parameter. Moreover, CFU number was practically abolished in both spleens and lungs of IQG-607- and INH-treated groups, whereas IQG-639 was not capable of significantly modifying CFU counting. Additional experiments are being currently performed to determine the pharmacokinetic and toxicological parameters of these compounds. The promising activity of IQG-607 in M. tuberculosis-infected mice suggests that it is a good candidate for clinical development as a new anti-tuberculosis agent.

08.005

Effect of chronic undernutrition in rat vas deferens: altered Ca^{2+} homeostasis and implications in male fertility. Muzi-Filho H[1], Bezerra CGP[1], Souza AM[1], Boldrini LC[2], Takiya CM[2], Oliveira FL[2], El-Cheikh MC[2], Einicker-Lamas M[3], Vieyra A[3], Lara Morcillo LS[1], Cunha VMN[1] – [1]ICB-UFRJ − Farmacologia Celular e Molecular, [2]ICB-UFRJ − Ciências Morfológicas, [3]IBCCF-UFRJ

Introduction: It is well known that diverse related molecular structures to Ca2+ homeostasis are altered by multifactorial undernutrition. The aims of this work were: 1-investigate the tissue composition and the sperm count in the rat vas deferens (RVD); 2-evaluate the expression and activity of protein kinase A (PKA) and protein kinase C (PKC) in RVD; and 3-evaluate the effect of a1-adrenoceptor agonists and antagonists in the Ca2+-ATPase activity. **Methods:** Twenty male Wistar rats proceeding from healthy mothers were randomically divided in 2 groups after weaning: RBD-C (the rats were fed with Regional Basic Diet) and control group (fed with conventional diet). When the rats reach 13 weeks-old they were sacrificed (CEUA DFBCICB 007) and the RVD were removed for carry out: 1- histological observations (Hematoxylin and Eosin staining); 2-citometry assays for evaluation of the cellular content and differentiation; 3-biochemical and Western Blot assays to evaluate activity and expression of PKA and PKC; 4-the measure of Ca2+-ATPase activity (with a1-adrenoceptor agonists and antagonists). Results: The histological observations show global atrophy of all RVD, mainly of the mucosa (tissue layer that delimits the light organ). In citometry assays, the sperm count in the RBD-C group is significantly smaller (90% of reduction; n=3; $p<0.05$) as well as haploid cells percent (35.9% of total) in comparison to the control group (51.5% of total; $p<0.05$). Moreover, it is seen an increase of the meiotic (11.9% in the RBD-C vs 8.5% in the control group; $p<0.05$) and mitotic cells (5.7% in the RBD-C vs 3.1% in the control group; $p<0.05$). The PKA and PKC activity is increased in the RBD-C group (264% and 193%, respectively; n=4; $p<0.05$), although it was not observed any alteration of expression for these proteins. Ca2+-ATPase activity, in the presence of methoxamine (a1-adrenoceptor agonist) is smaller than in the condition without the drug (36% vs 56% of total, respectively; n=4; $p<0.05$). The combination of methoxamine and 5-methyl-urapidil (a1A-adrenoceptor antagonist) produces a reversal of Ca2+-ATPase activity inhibition in the control, while it remains inhibited in RBD-C group (90% of total; n=4; $p<0.05$). **Discussion:** Altogether, in the RBD-C group, the increase of PKA and PKC activity and the activation of a1-adrenoceptor signalization spoil the Ca2+ reuptake to the intracellular stores, reducing the contraction capacity of RVD. Moreover, the sperm count in the undernourished group is smaller and the immature cells percentage is greater than in the control group, indicating deficiency of cellular maturation. These data, combined with the global atrophy of the RVD, is possible linked to reduced reproductive capacity of these animals, as we showed previously. **Financial support:** Projeto Casadinho-CNPq; PROCAD-CAPES; FAPERJ Primeiros Projetos; Programa ALV

08.006

Functional, molecular and morphological characterization of bladder dysfunction in diabetic mice. Leiria LOS[1], Carvalho FDGF[1], Franco-Penteado CF[2], Monica FZT[1], Claudino MA[1], Schenka A[1], Nucci G[1], Antunes E[1] [1]UNICAMP − Farmacologia, [2]UNICAMP − Hemocentro

Introduction: Lower urinary tract complications such as diabetic cystopathy or diabetic bladder dysfunction are among the most common complications of diabetes mellitus. The present study aimed to evaluate the functional, structural and molecular alterations of detrusor and urethral smooth muscles in diabetic mice, using the streptozotocin model. **Methods:** Male C57BL/6 mice ageing eight weeks were used. The experimental protocols were approved by the Animal Ethical Committee of UNICAMP. Detrusor smooth muscle (DSM) and urethral smooth muscle (USM) strips were prepared and mounted in 10-mL (or 5-mL) organ baths containing Krebs-Henseleit solution (pH 7.4, 37oC; 95%O2 and 5%CO2). Concentration-response curves to carbachol (muscarinic agonist; 1 nM to 30 μM), phenylephrine (α1-adrenergic agonist; 1 nM to 30 μM), α,β-methylene ATP (1-10 μM) or extracellular Ca2+ (3μM to 100mM) were constructed either in DSM or USM. Concentration-response relaxation curves to tadalafil (PDE5 inhibitor), SNP (NO donor) and BAY 41-2272 (NO-independent soluble guanylate cyclase activator) were performed in USM strips. Cystometric study in anesthetized mice was performed. Messenger RNA expression of muscarinic M3, M2 and P2X1 receptors, as well as of L-type Ca2+ channels were determined in DSM by real-time RT-PCR. A histological study was done in DSM. Results: The cystometric study in diabetic mice showed an increased bladder capacity, compliance, voiding frequency, non-void contractions and amplitude of void contractions compared with control mice. Histology analysis revealed increased detrusor volume and thickness of bladder wall. In DSM, carbachol-induced contractions were significantly higher in diabetic group (Emax: 5.06 ± 0.62 mN/mg; $P<0.05$) compared with control group (2.04 ± 0.23 mN/mg). Pre-incubation with the L-type Ca2+ channel blocker nifedipine (3 nM) prevented the increased carbachol-induced DSM contractions seen in the diabetic group (Emax: 2.29 ± 0.51 mN/mg). The Rho-kinase inhibitor Y27632 reduced the carbachol-induced DSM contractions in the same extent in both groups. The DSM contractions to either α,β-methylene ATP or extracellular CaCl2 were also higher in diabetic mice (1.84 ± 0.16 and 5.20 ± 0.66 mN/mg, respectively) compared with control group (0.68 ± 0.06 and 1.84 ± 0.23, respectively). Levels of mRNA for M3 receptor were significantly increased in diabetic animals, while M2, L-type Ca2+ channels and P2X1 were not altered. In USM, phenylephrine-induced contractions were significantly higher in diabetic mice (Emax: 132.9 ± 11.53%; $P<0.05$) compared with controls (Emax: 99.7 ± 4.24%). The SNP-induced USM relaxations were impaired in diabetic group (Emax: 52.3 ± 4.43%; $P<0.05$) in comparison with control animals (Emax: 86.2 ± 6.1%). Relaxant responses to tadalafil and Bay 41-2272 were not altered in USM. **Discussion:** Our data show that diabetic mice exhibit functional, molecular and histomorphological features of bladder dysfunction characterized by detrusor overactivity related to urethral resistance. Moreover, DSM-induced contractions mediated by muscarinic receptors are increased in diabetic group as a consequence of augmented M3 receptor expression and enhanced extracellular Ca2+ influx via L-type Ca2+ channels. **Financial Support:** CAPES and FAPESP

08.007

Relaxation of airways smooth muscle induced by glucagon is dependent of epithelial-derived factor. Insuela DBR, Coelho LP, Cruz CCD, Serra MF, Cordeiro RSB, Silva PMR, Martins MA, Carvalho VF IOC-FIOCRUZ − Fisiologia e Farmacodinâmica

Introduction: Glucagon is a 29-amino acid peptide hormone that is secret by the pancreatic α-cells. This hormone counteracts insulin actions by stimulating hepatic glucose synthesis and mobilization, carrying in an increase of blood glucose levels (Nussdorfer et al., Peptides, 21, 309, 2008). Moreover, glucagon has several effects in extrahepatic tissues too, as adipose tissue, kidney and heart (Love et al., Chest, 114, 323, 1998). Previous studies suggested that glucagon reduces methacholine-induced bronchospasm in asthmatic patients (Melanson et al., Am J Emerg Med, 16, 272, 1998). In this work, we investigated the effect of glucagon on airway smooth muscle contractility and possible mechanism involved. **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 (CEUA-FIOCRUZ, protocol 0085–02). A/J mice tracheal and bronchial rings were mounted in tissue baths filled with Krebs´ solution, and the contractile response to carbachol was measured in the presence or absence of glucagon (0.1 or 1.0 microM). In some experiments, we previous incubated the tracheal rings with glucagon receptor antagonist (des-His1-[Glu9] glucagon amide) 30 min before glucagon treatment. The epithelial cells of some of these tracheas were removed mechanically by rubbing the internal trachea surface with a fine silver wire before glucagon treatment. We also tested the effect of glucagon (10 ng/kg, i.n. or i.p.) on methacholine-evoked airways obstruction in A/J mice using barometric plethysmography. The treatment was realized 1 or 3 hours before the stimulation with methacholine. Results: Glucagon inhibited epithelium-intact tracheal or bronchial ring contraction induced by carbachol stimulation in a dose-dependent manner. The relaxation effect of glucagon was inhibited by glucagon receptor antagonist (des-His1-[Glu9] glucagon amide) at concentration of 10 microM. Besides, the relaxation effect of glucagon was abrogated by epithelium removal. Animals that received aerosolized methacholine administration presented an increase of airway contraction in compared with animals that received aerosolized saline (from 0.56 ± 0.049 to 3.00 ± 0.42 Penh, respectively; mean ± SEM). The intranasal administration of glucagon was able to inhibit the bronchoconstriction induced by methacoline with 1 hour of treatment (1.85 ± 0.06 Penh, mean ± SEM), while that intraperitoneal administration of glucagon didn't inhibit this phenomenon (3.21 ± 0.64 Penh, mean ± SEM). Finally, in animals treated with either intranasal or intraperitoneal administration of glucagon, we found a marked attenuation in the elevation of bronchial spasm induced by aerosolized methacholine 3 h after the treatment (control: 10.00 ± 1.7 Penh; glucagon i.n.: 5.20 ± 0.35 Penh; glucagon i.p.: 4.98 ± 0.50 Penh; mean ± SEM). **Discussion:** Our results showed that the relaxation effect of glucagon is dependent of its classical receptor and seems to be mediated by an epithelial-derived factor. Supported by: CNPq, FAPERJ and FIOCRUZ.

08.008

Role of CXCR2 in a model of pulmonary infection with *Klebsiella pneumoniae*. Paula TP[1], Arifa RDN[1], Ávila TV[2], Fagundes CT[3], Cruz RC[1], Werneck SMC[1], Karklin YC[1], Baltazar LM[1], Madeira MFM[1], Campi PS[1], Pinho V[3], Teixeira MM[3], Souza DG[3] [1]UFMG − Microbiologia, [2]UFPR − Farmacologia, [3]UFMG − Bioquímica e Imunologia

Introduction: *Klebsiella pneumoniae* is a gram negative bacteria with clinical importance related with nosocomial infections like pulmonary pneumonia. It is well described that neutrophils plays an important role in this pathology. Neutrophil recruitment is headed by several inflammatory mediators, between them, great attention is given to CXCR2 related chemokines. Then, our aim was study the role of CXCR2 in pulmonary infection induced by Klebsiella pneumonia in a murine model. **Methods:** This project was previously approved by CETEA/UFMG. Female C57/BL6 (8-12 weeks) were infected with *Klebsiella pneumoniae* ATCC-27736 after anesthesia with xylazine (0,02 mg/ml)/ ketamine (50 mg/ml). The trachea was exposed and 30 µL of a suspension containing 3 x 104 or 106 CFU of K. pneumoniae or saline was administered. The skin incision was closed with surgical staples. The animals were sacrificed 5 and 7 days after infections and some groups are treated with antagonist DF2162 by oral gavage 24h before the infection and each 24 hours in subsequent day. Lungs were harvest and collected to analyse the levels of MPO, NAG, lipoperoxidation and cytokines concentration. Bronchoalveolar lavage (BAL) was made to access number of bacterium and inflammatory mediator production. Results: First, we performed lethality experiments to this we choose two different inoculums: 104 and 106 CFU/ml. With inoculation of 3 x 106 twenty percent of mice started die in the 5 th day and 100% were died until 6 days after infection. With the 3 x 104 CFU/ml, mice started to die at 6th day (30%) and continue until the 8th day (100%). Seven days after infection with 104 CFU/ml, mice presented increase of hematocrit percentage, activity of MPO, LPO and NAG in lungs and number of neutrophils in BAL when compared with not infected mice. The treatment with DF2162 decreased the number of neutrophils, but was not able to alter another parameters assessed. Interestingly, DF2162 decreased the bacterial load recovery in the lungs. Conclusion: Our results demonstrated a discrete role of CXCR2 in model of pulmonary infection with *Klebsiella pneumoniae*. Finantial support: CAPES, CNPq and FAPEMIG

08.009

Alpha-1 adrenoceptor mediated contractions of the rat bladder neck. Pacini ESA, Pupo AS UNESP Botucatu − Farmacologia

Introduction: The urinary bladder is functionally and anatomically divided into two parts: bladder body and bladder base. Adrenoceptors are present in all areas of the urinary bladder and play distinct roles. During the storage phase, bladder body adrenoceptor activation relaxes detrusor smooth muscle allowing accumulation of urine whereas bladder base adrenoceptor activation results in contraction preventing urine flow through the urethra. Lower urinary tract symptoms are frequent in the general population and drugs which interact with adrenoceptors affect the smooth muscle tone of the urinary bladder. Thus, the purpose of the present study was to investigate the nature of the adrenoceptors involved in the contractions of the rat bladder neck in response to norepinephrine (NE). **Methods:** Adult male Wistar rats (16-20 weeks) were killed by decapitation and the whole bladder was excised. One ring of the bladder neck was cut and suspended in an organ bath containing 10 ml of modified Krebs solution under a resting tension of 1-1.3g. Concentration-response curves to NE were obtained in absence and presence of cocaíne (6 mM); corticorterone (1 mM, 10 mM); propanolol (0,1 mM) or yohimbine (0,1 mM) to determine the importance of neuronal uptake and extraneuronal uptake and the involvement of b- and a2-adrenoceptors, respectively. To antagonize a1-adrenoceptors concentration-response curves to NE were constructed in presence of prazosin (3 to 100 nM), a selective a1-adrenoceptor antagonist. All proceedings were approved by Comissão de Ética da Experimentação Animal (174-CEEA). **Results and Discussion:** The rat bladder neck contracted in response to NE with pD2 = 4.62 ± 0.09 and Emax = 210 ± 10 mg (n=17). Cocaine significantly shifted the concentration-response curves to NE to the left by ~8-fold without affecting the Emax ($P<0.05$). Corticosterone, propranolol and yohimbine did not affect NE concentration-response curves. Prazosin produced rightward displacements in the NE concentration-response curves showing classical competitive antagonism (pA2=8.62 ± 0.04) with slope in the Schild plot not different from the unity (slope=0.93 ± 0.09). The apparent affinity estimated for prazosin in the bladder neck was ~3-fold lower than its affinity in the rat vas deferent (pA2=9.10 ± 0.04, $P<0.05$), a tissue whose contractions to NE are mediated by the predominant activation of a1A-adrenoceptors. The slightly lower affinity for prazosin in the rat bladder neck may be due to the possible involvement of an “alternative” a1-adrenoceptor subtype named a1L. The a1L-adrenoceptor pharmacology is characterized by low affinities for prazosin, RS-17053, 5-Methylurapidil and BMY 7378 and high affinity for tamsulosin and silodosin. Therefore, additional experiments with selective antagonists are necessary to determine the nature of the a1-adrenoceptor subtype involved in the contractile responses of the rat bladder neck. In conclusion, these results suggest that neuronal uptake is an important mechanism of NE removal in the bladder neck and that a1-adrenoceptors mediate the contractions of this tissue to NE with no influence of b- or a2- adrenoceptors. **Financial Support**: CAPES