

Setor 08. Respiratório, Urinário e Reprodutor/ Respiratory, Urinary and Reproductive

08.001

Can prenatal exposure to betamethasone induce late changes in androgen receptor gene expression in rats prostate and testis? Piffer RC¹, Garcia PC¹, Sankako MK², Castilho, ACS², Pereira OCM² ¹UNESP-Botucatu - Clínica Médica/Farmacologia, ²UNESP-Botucatu - Farmacologia

Introduction: Corticosteroid administration to pregnant women at risk of preterm delivery has been widely used in obstetrics as these hormones can improve fetal lung maturity in humans. Considering that corticosteroids can suppress fetal testicular testosterone (Lalau, Neuroendocrinology 43:289, 1990), which plays a very important role in the sex differentiation of the brain, this study aimed at assessing the wet weight of reproductive organs, plasma testosterone and androgen receptor (AR) gene expression in the prostate and testis of adult male rats prenatally exposed to betamethasone. **Methods:** Pregnant Wistar rats received either 0.1mg/kg of betamethasone- Sigma Co., USA, (Betamethasone Group) or saline (Control Group) by the intramuscular route on the 12th, 13th, 18th and 19th days of pregnancy (Piffer, Reprod Fertil Dev 21:634-9, 2009). Data collected from the adult offspring (90 days of age) included wet weight of reproductive organs (testis, epididymis, vas deferent, seminal vesicle with and without secretion, and prostate), plasma testosterone (radioimmunoassay: Coat-A-Count Total Testosterone Kit DPC, USA), and relative AR gene expression in the prostate and testis (RT-PCR), according to the protocols of the kits Trizol, SuperScript III and Taq DNA polymerase (Invitrogen[®]) and primers specific for AR and cyclophilin - gene constitutively expressed. Statistics: Students *t* test (mean±EPM), **p*<0.05, ***p*<0.01. This study was approved by the Committee of Animal Experiments # 429. **Results:** In adulthood, prenatal exposure to betamethasone caused no change in the wet weight of the epididymis, seminal vesicle, vas deferent, and prostate. However, it reduced testis wet weight (1.83±0.04 / 1.70±0.04*, g, n=10/group) and the amount of seminal vesicle secretion (0.44±0.03 / 0.36±0.01*, g, n=10/group), as well as testosterone plasma concentration (406.65±57.41 / 192.81±15.27**, ng/dl, n=10). When compared to the Control Group, the Betamethasone group showed greater relative androgen receptor gene expression in the prostate (0.27±0.04 / 0.41±0.04*, n=6/group), but not in the testis (1.03±0.32 / 1.23±0.22, n=6/group). **Discussion:** The poor testicular development observed probably resulted from an alteration in the hypothalamus-pituitary-gonad axis during the prenatal period, whereas the reduction in vesicle secretion was probably induced by the decrease in testosterone levels. The increase in AR gene expression seen in the prostate was probably compensatory as the wet weight of this organ remained unchanged. Therefore, it seems that the increase in AR expression associated with other factors, including transcription factors, was enough to ensure normal prostate development, despite reduction in plasma testosterone levels. In the testis, however, only an apparent increase in the expression of these receptors was observed. Thus, the intensity of the expression of these receptors did not accompany the development of the organ that showed reduced testosterone production. Financial support: CNPq # 142388/2004-1 and FAPESP # 2006/57038-6.

08.002

PI3K γ plays a critical role in bleomycin-induced pulmonary injury and fibrosis. Russo RC¹, Garcia CC¹, Guabiraba R¹, Barcelos LS², Sousa LP¹, Roffe E¹, Souza ALS¹, Cassali GD³, Pinho V⁴, Mirolo M⁵, Doni A⁵, Locati M⁵, Teixeira MM¹ ¹ICB-UFMG - Bioquímica e Imunologia, ²ICB-UFMG - Biofísica, ³ICB-UFMG - Patologia Geral, ⁴ICB-UFMG - Morfologia, ⁵Instituto Clinico Humanitas - Translational Medicine

Introduction: Bleomycin-induced lung fibrosis in mice is the most used model to study Idiopathic Pulmonary Fibrosis (IPF). Bleomycin (BLEO), a chemotherapeutic, causes direct lung epithelial/endothelial damage, cytokine release, leukocyte recruitment, angiogenesis and interstitial collagen deposition. PI3Ks are central signalling elements in a diverse array of cell functions, including growth, proliferation, migration and survival. PI3K γ is the unique member activated by GPCRs, coordinates inflammation and is expressed by leukocytes and endothelial cells. PI3K γ plays a role in a variety of respiratory diseases, ranging from asthma to cancer. Here, we hypothesized if PI3K γ deficiency could prevent bleomycin-induced lung fibrosis. **Methods:** WT or PI3K γ KO mice received an intra-tracheal BLEO instillation (0.125U) (Protocolo CETEA/UFMG 146-06). We evaluated lethality, weight, leukocyte recruitment into airspace (BAL) and lungs by citospin and FACS, chemokine and cytokine by ELISA, mRNA transcripts by real-time PCR, western blot, cell respiratory burst by luminol-enhanced chemiluminescence assay zymosan-induced, TUNEL, OH-proline quantification and Gomori's trichrome stained morphometry. HUVECs were used in proliferation, wound closure scratch-induced and capillary-like organization *in vitro* assays, in presence of IL-8 and PI3K γ Inhibitor, AS605240. **Results:** BLEO-instilled PI3K γ KO mice showed increased survival and weight recovery, reduced lung collagen deposition, also confirmed by collagen morphometry, and focal fibrosis with preserved areas when compared to WT, which presented diffuse and dense fibrosis at day 16. It was associated to decreased TGF- β 1 and increased IFN γ and IL-10 levels, followed by reduction on pro-fibrogenic mRNAs (COL1 α 1, COL1 α 2, Fibronectin, α -SMA and von Willebrand factor) transcripts in PI3K γ KO at day 8. BAL analysis revealed impaired leukocyte influx (PMN, CD3+CD4+ and CD3+CD8+) into PI3K γ KO airspaces caused by lung interstitial leukocyte retention, even in the presence of increased CXCL1 and CXCL9 BAL levels. Reduced CCL2 levels were associated to decreased M ϕ numbers in PI3K γ KO BAL and lungs. Nitrite and total protein were found diminished in PI3K γ KO BAL, together with reduced lung cell activation measured by chemiluminescence assay, reduced AKT and I κ B α phosphorylation, and increased apoptotic cell numbers, but not in WT, 8 days post-BLEO. *In vitro*, HUVECs proliferation was dose-dependent inhibited by AS605240, that also inhibited the wound closure scratch-induced and capillary-like organization in presence of IL-8. **Discussion:** PI3K γ deficiency confers protection to bleomycin-induced lung fibrosis through the modulation of lung inflammatory activity, as well as angiogenesis, fibrogenesis and resolution, suggesting that PI3K γ plays a central role during lung fibrosis development. Moreover, PI3K γ can represent a critical target for therapeutic intervention in IPF. It's possible that PI3K γ inhibitors may be useful as a palliative drug during bleomycin cancer therapy. **Financial support:** CAPES, CNPq and FAPEMIG.

08.003

Sexual and reproductive performance in male rats exposed to perinatal androgen block with flutamide. Leonelli C¹, Garcia PC², Pereira OCM¹ ¹IB-UNESP-Botucatu - Farmacologia, ²FMB-UNESP-Botucatu - Clínica Médica

Introduction: The role of aromatized androgens in sexual hypothalamic differentiation and physiological and behavioral stabilization is already well described in the literature. However, evidence points to further pathways of action of these steroids. This suggests a synergism between the estrogen and *per se* androgens in the sexual differentiation of the brain. Thus, the present study aimed to investigate the existence of sexual and reproductive changes resulting from the absence of binding of androgens to its specific receptors in perinatal life. **Methods:** Pregnant Wistar rats were exposed to 15µg/kg/day of flutamide (Flutamide group) or peanut oil (control group) on the 19th day of pregnancy and on the first 5 days of postnatal life (sc). In adulthood, in the male offspring, male sexual behavior performance was assessed and the existence of female sexual behavior (mounting acceptance with or without lordosis) before castration and estrogen treatment. Moreover, the fertility parameters (mating index, fertility index, pre- and post-implantation loss, implantation rate and litter size) and sperm quality (concentration, morphology and vitality) were also evaluated. Data were analyzed using statistical tests of Mann-Whitney [median (IQ1-IQ3)] or Fisher's Exact Test (for comparison of proportions) N=10/group. p<0.05*. This study was assented by Ethical Committee for Animal Research from Biosciences Institute/UNESP-Botucatu (Protocol nº 69/07-CEEA, 14/12/07). **Results:** The perinatal exposure to flutamide impaired sexual performance of male offspring. Flutamide animals performed more mounts without intromission [0.0(0.0-0.0) / 7.5(6.0-9.5)*], showed higher latency for the beginning of sexual behavior [32.0(28.5-44.5) / 151.0(102.5-285.0)*] and lower number of animals reached the first ejaculation during the time test (100.0% / 36.4%*), and a larger number of animals to accepted mounts from non-experimental male (0.0% / 45.5%*), although they did not shown lordosis. During the fertility test, there were reduced rates of mating (100.0% / 60.0%*) and fertility [95.0% / 46.6%*] on Flutamide group. Moreover, this group shown more pre-implantation losses [0.0(44.5-47,5) / 67.0(64.5-87.5)*] and low rates of implantation [100.0(93.0-100.0) / 33.0(12.5-35.3)*] generating, therefore, smaller litters [13.0(6.0-13.0) / 3.0(1.0 – 4.0)*]. The rate of post-implantation losses did not differ between groups. The sperm quality was impaired in Flutamide, with lower sperm concentration [81.3(81.8-86.6) / 63.0(58.3-73.0)*] and more abnormal sperm [2.0(2.0-2.7) / 3.0(3.0-4.0)*], although there was not any difference in sperm motility. **Discussion:** The Flutamide group presented sexual and reproductive performance significantly impaired. These results are likely because of the treatment period, a critical period of sexual hypothalamic differentiation in rats, when occurs defeminization and masculinization process in the male hypothalamus. Thus, it is suggested that the deficiencies presented by Flutamide group are due to failed differentiation process, showing the importance of testosterone binding in this programming cerebral moment. **Financial support:** FAPESP 07/57521-1

08.004

Later reproductive effects and physical development in male rats exposed perinatally to flutamide. Leonelli C, Pereira OCM, IB-UNESP-Botucatu - Farmacologia

Background: Considering the possibility of extra pathway(s) for the elucidation of the defeminization and masculinization of the hypothalamus in perinatal life, excluding the reduction and aromatization of testosterone, this study aimed to evaluate possible late changes in male rats exposed to the androgen receptor blocker, flutamide, in the perinatal period. **Methods:** Pregnant Wistar female rats received 15µg/kg/day of flutamide (Flutamide group) or peanut oil (Control group) on the 19th day of pregnancy and on the first 5 days of postnatal life (sc). It was assessed: the body weight at birth, weaning, and puberty, the anogenital distance (AGD) at birth and weaning and the age at puberty installation. In adulthood (90 days old) animals were euthanized by an overdose of sodium pentobarbital for the assessment of the wet weights of testis, epididymis, vas deferens, prostate, seminal vesicle, and its secretion and gonad-somatic index (GSI) . Data were analyzed by statistical tests of "t" Welch Corrected (mean±SD) or Mann-Whitney [median (IQ1-IQ3)], N = 10, p<0.05*. This study was assented by Ethical Committee for Animal Research from Biociencias Institute/UNESP-Botucatu (Protocol nº 69/07-CEEA, 14/12/07). **Results:** The perinatal exposure to flutamide reduced significantly the body weight of male offspring at weaning (54.13±3.52 / 47.34±4.83* g), but at puberty shown an increase (221.32±15.20 / 236.53±26.83* g). The anogenital distance showed a reduction at birth (2.97±0.50 / 2.63±0.21* mm), remaining low at weaning (16.9±0.55 / 14.8±0, 45* mm). There was delay in the puberty installation [46(44.5-47.5) / 48(45.0-51.0)* days]. In adulthood, the wet weight of the epididymis was increased (0.63±0.08 / 0.71±0.07* g), while the weights of the prostate (0.48 ± 0.11 / 0.38±0.10* g) and testis (1.69 ± 0.15 / 1.58 ± 0.13g *) and the GSI [0.71(0.69-0,79) / 0.68(0.66-0.71)* g] were smaller. There were not any alterations in the wet weights of the other organs despite the treatment. **Discussion:** The blockage of testosterone binding to its receptor in the perinatal period affected physical and reproductive aspects of male offspring. The reduction of AGD at birth and weaning was early indication of the possibly reduced levels of testosterone. The delay for the puberty installation, an event mediated by testosterone, once again shows signs of reduced androgen levels, suggesting impairment in the hypothalamic-pituitary-gonads axis activation. The reduction in wet weight of organs androgen-dependent corroborates to this suggestion. Thus, it is suggested that the testosterone in the perinatal period acts by additional pathways through *per se* androgen action, with programmer function on later reproductive events, by modulating of the testosterone secretion level and/or by alterations in androgen receptors. **Financial support:** FAPESP 07/57521-1

08.005

Sinvastatina reduz o recrutamento de neutrófilos em pulmão de ratos submetidos à ventilação mono-pulmonar. Landucci ECT², Mussi RK², Leite, C F², Marangoni FA¹, Camargo E³ ¹UNICAMP - Farmacologia, ²UNICAMP - Cirurgia Torácica, ³UFS - Fisiologia

Objetivos: A ventilação mono-pulmonar (OLV) é uma técnica muito utilizada durante procedimentos cirúrgicos por oferecer boas condições de visualização das estruturas intratorácicas. Entretanto, pode-se atribuir a esta modalidade ventilatória função adjuvante no processo inflamatório, que repercute negativamente na recuperação do paciente pós-cirúrgico. Com o objetivo de modular esta resposta inflamatória, foi realizado este estudo experimental propondo a utilização de sinvastatina como possibilidade terapêutica antiinflamatória. **Métodos:** Ratos Wistar foram divididos em dois grupos: *Grupo OLV*, submetido ao protocolo de cirurgia torácica e OLV por 60 minutos, seguido de ventilação bilateral por igual período; *Grupo OLV+Sinvastatina*, submetido ao mesmo protocolo, porém com administração prévia de sinvastatina, na dose de 40 mg/kg, por 21 dias. Para caracterização da resposta inflamatória foram analisados, tanto no pulmão colapsado durante o ato cirúrgico como no pulmão contralateral a atividade da mieloperoxidase (MPO), relação peso úmido/seco, níveis de extravasamento de proteínas plasmáticas, além de medidas dos níveis séricos de PCR, TNF- α , IL-1 β , IL-6 e IL-10. **Resultados:** Foi encontrada redução significativa da MPO no pulmão expandido no grupo tratado ($p < 0,05$), sem diferenças significativas em relação ao pulmão colapsado. Não ocorreram diferenças significativas entre os grupos no que se refere a extravasamento de proteínas plasmáticas bem como relação peso úmido/peso seco. Quanto às dosagens séricas de citocinas, não foram detectados níveis de TNF- α , IL-1 β e IL-10 nos dois grupos. Tanto IL-6 quanto PCR apresentaram valores quantificáveis, porém sem diferenças significativas. **Conclusão:** O tratamento com sinvastatina foi útil ao reduzir a atividade neutrofílica no pulmão contralateral durante a ventilação mono-pulmonar. Os resultados sugerem que este modelo de ventilação mono-pulmonar não impõe consistentes alterações inflamatórias locais e sistêmicas na avaliação pós-cirúrgica imediata.

08.006

Obesity enhances detrusor smooth muscle contractile responses in mice. Leiria LOS¹, Monica FZT¹, Calixto MC¹, Lintomen L¹, Saad MJA², Antunes E¹ ¹UNICAMP - Farmacologia, ²UNICAMP - Clínica Médica

Introduction: Clinical evidences have shown a relationship between obesity and disorders of urinary bladder such as incontinence and overactive detrusor smooth muscle. However, the physiopathology accompanying the urinary bladder disorders in obese individuals has been poorly investigated. The aim of the present study was to evaluate the functional alterations of detrusor smooth muscle in high-fat fed mice.

Methods: The experimental protocols were approved by the Animal Ethical Committee of UNICAMP (n° 1496-1). Detrusor smooth muscle (DSM) strips were prepared and mounted in 10-mL organ baths containing Krebs-Henseleit solution (pH 7.4, 37°C, bubbled with 95%O₂ and 5%CO₂). Cumulative concentration-response curves to the muscarinic agonist carbachol (1 nM to 30 µM), KCl and CaCl₂ were constructed. Frequency-response contractile curves to electrical field stimulation (EFS; 1-32 Hz) were also performed. **Results:** The diet-induced obese mice showed a significant increase in body weight and epididymal fat, as well as higher serum levels of total cholesterol and LDL, compared with mice receiving standard chow diet. The DSM contractile responses to carbachol were significantly higher in obese group (E_{max}: 11.2±1.8 mN/mg) in comparison with control group (E_{max}: 6.6±0.5 mN/mg). The CaCl₂-induced contractions obtained in Ca²⁺-free medium were also higher in obese animals compared with control group (E_{max}: 9.2±1.0 and 4.2±0.5 mN/mg, respectively). The EFS-induced contractile responses did not significantly differ between obese and control groups (E_{max}: 6.96±0.92 and 5.31±1.07 mN/mg, respectively), although tended to be higher in the obese group. In the presence of pre-incubated muscarinic receptor antagonist atropine (1 µM) combined with purinergic receptor antagonist suramin (100 µM), the EFS-induced contractions was higher in the diabetic group. **Discussion:** Our results indicate that DSM-induced contractile responses mediated by muscarinic receptors are enhanced in obese group, which may correlate with increased contractions in responses to CaCl₂. In addition, there is a non-adrenergic, non-cholinergic component (which does not involve the purinergic fibers), that is greater in the obese group and can be involved in the physiopathology of overactive bladder associated to obesity. **Financial Support:** CAPES and FAPESP

08.007

Detrusor smooth muscle (DSM) contractions alterations in streptozotocin-induced diabetes in mice. Leiria LOS¹, Monica FZT¹, Carvalho FDGF¹, De Nucci G², Antunes E¹
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Introduction: Urinary bladder dysfunction is a common complication associated with diabetes, but little is known about the physiopathology underlying this disorder in diabetic individuals. The present study aimed to evaluate the contractile responses of mice detrusor smooth muscle in vitro using the streptozotocin-induced diabetic model.

Methods: Male C57BL/6 mice ageing 8 weeks were used. The experimental protocols were approved by the Animal Ethical Committee of UNICAMP (n° 1861-1). DSM strips were prepared and mounted in 10-mL organ baths containing Krebs-Henseleit solution (pH 7.4, 37°C, bubbled with 95%O₂ and 5%CO₂). Cumulative concentration-response curves to the muscarinic agonist carbachol (1 nM to 30 µM), KCl and CaCl₂ were constructed. Frequency-response curves to electrical field stimulation (EFS; 1-32 Hz) were also performed. **Results:** Diabetic group showed increased glucose levels, bladder weight and lower corporal weight gain (P<0.05). The DSM contractile responses to carbachol were significantly higher in diabetic group in comparison with control group (E_{max}: 10.56±1.52 and 4.15±0.48 mN/mg, respectively). Pretreatment of DSM preparations with the Rho-kinase inhibitor Y27632 (1-10 µM) reduced the carbachol-induced contractions in the same extent in both groups. The KCl-induced contractions in diabetic animals were also higher than control group (E_{max}: 7.26±1.23 and 3.30±0.23 mN/mg, respectively). Similarly, the CaCl₂-induced contractions were higher in diabetic animals compared with control group (E_{max}: 7.05±0.72 and 3.88±0.53 mN/mg, respectively). EFS-induced contractions did not significantly change between diabetic and control groups. In the presence of the muscarinic receptor antagonist atropine (1 µM), the EFS-induced contractions tended to be higher in the diabetic group. **Discussion:** Our data indicate that DSM-induced contractions mediated by muscarinic receptors are greatly increased in diabetic group, probably due enhanced extracellular Ca²⁺ influx via L-type Ca²⁺ channels. In addition, the Rho-kinase pathway appears not be involved in the diabetic-induced overactive bladder. **Financial Support:** CAPES and FAPESP

BECLOMETHASONE ACTION ON MUCOCILIARY TRANSPORT

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Introduction - Mucociliary transport is an important defense mechanism of respiratory mucosa. Damage on ciliary system may trigger multiple implications: mucus stasis and bacteria colonization, increasing the incidence of respiratory infections. Thus, the dysfunction of mucociliary transport may deteriorate individual life quality. Complications that occur during asthma attacks were also associated with ciliary dysfunction and mucociliary clearance reduction. Anti-inflammatory drugs used on this process treatment appear do not act on mucociliary clearance. The improvement observed on mucociliary clearance after treatment with steroids probably results of its anti-inflammatory properties. The aim of this study was to evaluate the effect of beclomethasone on mucociliary transport velocity on frog's palates.

Methodology - Control group: palates immersed in Ringer^R solution and beclomethasone group: palates immersed in 8 ppm of beclomethasone solution diluted with Ringer^R. The palates were immersed in these solutions for four consecutive periods of 15 minutes. Mucociliary transport velocity was determined by measure of displacement velocity of autologous mucus on palate surface, before and after each exposure, using a stereoscopic microscope equipped with a graticule in one ocular. These results were expressed as relative basal transport velocity, obtained by dividing the transport velocity registered at 15, 30, 45 and 60 minutes by the velocity determined at zero time - the basal velocity. This work is part of a project approved by Ethics Committee on Animal Experimentation – N° 41/05. The values of relative velocity transport were compared by T test.

Results –

Groups	Immersion time (minutes)			
	15 mim.	30 mim.	45 mim.	60 mim.
Control	1,04±0,04	1,07±0,04	1,06±0,01	1,08±0,01
beclomethasone	1,06±0,01	1,08±0,01	1,08±0,01	1,10±0,01

Conclusion - Beclomethasone solution (8ppm) did not alter mucociliary transport velocity. This study requires the use of others beclomethasone concentrations to assess its effect on ciliary transport.

08.009

Salbutamol action on mucociliary transport. Correia VG, Gomes CS, Blanco EEA UEL - Ciências Fisiológicas

Introduction: Mucociliary transport is an important defense mechanism of respiratory mucosa. The propulsion of this system is the respiratory epithelium ciliary activity. Damage to the mucociliary apparatus causes mucus stasis with disturbances in ventilation and increase in air flow resistance and increases the risk of infections and inflammations. Thus, the dysfunction of mucociliary transport may deteriorate the individual life quality. Complications during asthma attacks were also associated with ciliary dysfunction and mucociliary clearance reduction. Sympathomimetic agonists, with relative selectivity on β_2 receptors, are used on asthma treatment. It was postulated that these substances beside promote relaxation of bronchi smooth muscles, increase mucociliar clearance. The aim of this study was to evaluate the effect of salbutamol, a β_2 agonist, on mucociliary transport velocity on frog's palates.

Methodology: Control group: palates immersed in Ringer solution and salbutamol group: palates immersed in 50 ppm of salbutamol solution diluted with Ringer. The palates were immersed in these solutions for four consecutive periods of 15 minutes. Mucociliary transport velocity was determined by measure of displacement velocity of autologous mucus on palate surface, before and after each exposure, using a stereoscopic microscope equipped with a graticule in one ocular. These results were expressed as relative basal transport velocity, obtained by dividing the transport velocity registered at 15, 30, 45 and 60 minutes by the velocity determined at zero time - the basal velocity. This work is part of a project approved by Ethics Committee on Animal Experimentation – N° 41/05. The values of the relative velocity transport were compared by T test.

Results:

Groups	Immersion time (minutes)			
	15 mim.	30 mim.	45 mim.	60 mim.
Control	1.09±0.17	1.08±0.16	1.09±0.14	1.10±0.12
Salbutamol	1.13±0.08	1.14±0.12	1.15±0.12	1.05±0.34

Conclusion: The results show that 50 ppm of salbutamol solution has a slight tendency to increase mucociliary transport velocity, but it is necessary to evaluate the effect of more concentrate salbutamol solutions.

08.010

Uso de anticoncepcionais orais entre mulheres adultas com queixas vestibulares.
Prezotto AO, Salgado RS, Paulino CA, Onishi, ET UNIBAN

Introdução: O uso regular de métodos contraceptivos entre universitárias com vida sexual ativa é bastante alto, entre eles os anticoncepcionais hormonais orais. Por sua vez, o aparelho vestibular (ou labiríntico) é um órgão com dupla função: a cóclea, responsável pela audição e o vestíbulo (ou labirinto), responsável pelo equilíbrio corporal. Os anticoncepcionais orais (estrógeno, progestágeno ou sua combinação) podem alterar o equilíbrio corporal e a audição, pois comprometem a homeostase dos fluidos labirínticos, na medida em que produzem alterações vasculares e de neurotransmissores. Portanto, esse trabalho avaliou o uso de anticoncepcionais orais em mulheres com queixas vestibulares. **Métodos:** Foi estudada uma amostra de 124 mulheres adultas, do município de São Paulo, pós-graduandas em nível de *lato sensu* e de diferentes idades. Uma delas tinha 20 anos (1%) e as demais estavam distribuídas nas seguintes faixas etárias: de 21 a 30 anos (48%), de 31 a 40 anos (27%), de 41 a 50 anos (15%) e de 51 a 60 anos (9%). O estudo foi do tipo transversal, descritivo e analítico, sendo realizado nas dependências de uma Universidade Privada de São Paulo, após aprovação pela Comissão de Ética (Protocolo nº 174-07). Para a coleta de dados foi utilizado como instrumento um questionário, previamente testado, referente às informações pessoais, profissionais, de saúde e sobre o uso de anticoncepcionais. **Resultados:** Todas as mulheres participantes da pesquisa relataram no mínimo duas queixas relacionadas às funções vestibulares, com maior prevalência para: cefaléia (81%), dores musculares e cervicais (77%), redução da concentração (71%), redução da memória (61%), tontura (56%), além de redução da audição, náuseas e insônia (47%), dificuldade de leitura (46%), cinetose (45%), zumbido e sudorese intensa (42%). Em relação aos contraceptivos orais, 47 mulheres (38%) relataram ser usuárias, por um período superior a 3 meses, de 20 diferentes especialidades farmacêuticas contendo anticoncepcionais, e 5 mulheres não informaram a especialidade utilizada. Dentre as usuárias destes medicamentos, a faixa etária predominante foi aquela de 21 a 30 anos de idade (74%), seguida pela faixa de 31 a 40 anos (19%). As especialidades relatadas apresentaram 9 diferentes formulações de anticoncepcionais, sendo 7 delas (78%) uma associação contendo estrógeno e progestágeno e 2 delas (22%) contendo apenas progestágeno. Dessas formulações, as mais prevalentes foram aquelas contendo as combinações medicamentosas: etinilestradiol + gestodeno (30%) e etinilestradiol + desogestrel (20%). Em relação às especialidades farmacêuticas referidas, houve predomínio de 5 delas, a saber: Diane-35 (30%), Femiane (25%), Yasmin (20%), Mercillon e Elani (ambas 15%). **Discussão:** O uso de anticoncepcionais hormonais orais durante um período de 6 meses (ou mais) pode causar nas mulheres diferentes reações adversas como alterações metabólicas, vasculares, auditivas, neurológicas, reprodutivas, entre outras; também pode desencadear vertigens. De fato, esses medicamentos estão associados a alterações funcionais da orelha interna, principalmente zumbido e tontura. Portanto, pode-se supor que o uso de anticoncepcionais nessas mulheres pode estar provocando, ou mesmo agravando, as queixas vestibulares aqui relatadas. **Apoio financeiro:** UNIBAN.

08.011

Differences in bradykinin receptor-mediated bleomycin-induced lethality and pulmonary fibrosis in mice. Cordeiro BF¹, Russo RC¹, Garcia CC¹, Tavares, LP¹, Carvalho RT², Teixeira MM¹ ¹UFMG - Bioquímica e Imunologia, ²COLTEC-UFMG

Introduction: Bradykinin is an amino acid peptide derived from the plasma precursor kininogen during inflammation and tissue injury. Bradykinin acts through two G protein-coupled cell surface receptors, designated BdkBR1 and BdkBR2. In contrast to the constitutive expression of BdkBR2, BdkBR1 are not expressed in significant levels in normal tissues, but are expressed under inflammatory conditions. Both receptors are expressed by cell types such as the vascular endothelium, epithelial cells, neurons and leukocytes. Bleomycin (BLEO) is a cytotoxic drug used for treatment of lymphomas; the major side effect is lung toxicity correlated with fibrosis. In mice, BLEO is used as a model of Idiopathic Pulmonary Fibrosis. The objective of this study was to investigate the role of bradykinin receptors after BLEO administration in mice. **Methods:** C57Bl6/j (WT), BdkBR1^{-/-}, BdkBR2^{-/-} and BdkBR1BR2^{-/-} mice were intra-tracheal instilled with BLEO, 3.75U/Kg (sub-lethal protocol) or 6.25U/Kg (lethal protocol), approved by Ethics Committee, CETEA/UFMG-146/06. Loss of weight and lethality were evaluated during 21 days after BLEO administration. MPO and NAG assay were performed to quantify the neutrophils (PMNs) and macrophages (Mf) accumulation into lung tissue. Bronchoalveolar lavage (BAL) was performed to evaluate cell influx to airways. Total protein quantification by Bradford assay and nitrite quantification by Griess were performed in BAL fluid. Lung collagen deposition was assayed by hydroxyproline (OH-Proline) quantification. **Results:** WT and BdkBR1BR2^{-/-} mice presented the same lethality 21 days post-BLEO (3.75U/Kg) and the same weight loss, but with BLEO at dose of 6.25U/Kg, BdkBR1BR2^{-/-} showed increased lethality when compared to WT mice. However, BdkBR1^{-/-} displayed high susceptibility to BLEO, even at 3.75U/Kg or 6.25U/Kg doses, with 100% of lethality at day 14 and pronounced loss of weight. In contrast, BdkBR2^{-/-} is protected from lethality, displayed increased survival, 100% with sub-lethal and 90% with lethal protocols, and also presented weight recovery when compared to WT mice at day 21 post-BLEO. Under administration of lethal BLEO dose, reduced levels of OH-Proline were found in BdkBR2^{-/-} when compared to WT mice at day 21. Using the sub-lethal protocol we confirmed the reduced fibrosis in BdkBR2^{-/-}, and we also found increased levels of lung OH-Proline BdkBR1BR2^{-/-} when compared with WT. At day 7 after post-BLEO we observed reduced accumulation of PMNs into lung tissue (MPO) only in BdkBR2^{-/-} mice but no differences were found in the Mf numbers (NAG). BAL analysis revealed reduced PMNs and total protein levels, but increased nitrite levels only in BdkBR2^{-/-} mice 7 days post-BLEO. **Discussion:** Here we showed that different bradykinin receptors could establish the rules of susceptibility to lethality and lung fibrosis induced by BLEO. It's possible that the inflammatory events, which precede the lung fibrosis, can play a decisive role during this pathological process. A deeper investigation about these complex relations between different bradykinin receptors, during lung fibrosis development, will be performed to understand these mechanisms and their future pharmacological applicability. **Financial support:** CNPq and CAPES

08.012

Peripheral modulation of ejaculatory reflex: focus on α_1 -adrenoceptors. Kiguti LRA, Leonelli C, Pereira OCM, Pupo AS UNESP-Botucatu - Farmacologia

Introduction. Ejaculation is a complex reflex coordinated at spinal cord level consisting by two phases: seminal emission and expulsion. The former encompasses transport of sperm from epididymis and secretions from prostate and seminal vesicles until urethra which are expelled by urethral meatus during expulsion by rhythmic contractions of striated muscles. The sympathetic nervous system plays a crucial role in the seminal emission phase of ejaculation by controlling vasa deferentia (VD), seminal vesicles (SV) and prostate contraction through α_1 -adrenoceptors (α_1 -ARs) activation. In these tissues adrenergic (and non-adrenergic) contractions are dependent on influx of extracellular calcium and are very sensitive to organic calcium channel blockers indicating participation of L-type calcium channels. The objective of this study was to investigate the effects of α_1 -ARs antagonism and calcium channel blockade on VD and SV responsiveness and mating behavior. **Material and Methods.** *In vitro studies.* VD or SV were isolated, cleaned by adherent tissues and suspended in 10 ml organ baths under 1g resting tension to record of isometric tension development. Cumulative concentration-response curves to norepinephrine (NOR) were generated at 45 min interval in the absence and presence of Prazosin (PZS, 0.3-10nM), Tamsulosin (TAM, 0.1-100nM), (S)-(+)-Niguldipine (NGD, 0.3-30nM) or Nifedipine (NFD, 10-300nM). *Behavioral analysis.* Sexually experienced male rats received TAM (3 μ g/kg), NFD (50 μ g/kg), NGD (10 μ g/kg) or vehicle (200 μ l/rat) i.v. 15 minutes before behavioral analysis. Mating behavior was observed for 30 minutes after first mount or intromission (mount with penis intromission) and latency for the first mount or intromission (LMI), number of mounts and number of intromissions until ejaculation (NM and NI, respectively), ejaculatory latency (EL), refractory period (RP) and seminal plug weight (SPW) were evaluated. Comparisons of mating performances were done with Kruskal-Wallis followed by Dunn's test. **Results.** *In vitro studies.* PZS, TAM, NGD and NIF antagonized NOR induced contractions in the VD and SV. PZS shown a simple competitive antagonism at VD and SV characterized by rightward displacements of NOR concentration-response curves and NFD behaved as a classical insurmountable antagonist reducing contractions in both tissues. TAM and NGD shown an insurmountable behavior characterized by concomitant maximal response depression and rightward displacement of NOR concentration-response curves. *Mating behavior.* All animals treated with TAM, NIG or NIF presented sexual behavior and LMI and RP were not different to vehicle ($p>0.05$) showing that drug treatments do not affected sexual motivation. In addition, parameters related to performance (NM, NI and EL) were not modified by any drug ($p>0.05$ vs vehicle). TAM reduced SPW ($p<0.0001$ vs vehicle, NIF and NIG). **Conclusions.** The present results show that neither calcium channel blockade nor α_1 -ARs antagonism affect EL or ejaculation threshold. In addition they highlight the importance of α_1 -ARs activation in seminal emission process. Further analyses with a broader range of doses of antagonists are in progress. This research is supported by FAPESP (08/52587-7).

08.013

L-carnitine or L-arginine supplementation associated with physical exercise prevent the low urinary tract dysfunctions in hyperlipidemic rats. Ramos-Filho ACS¹, Priviero FBM¹, Gomes-Campos RA¹, Rojas-Moscoso JA¹, Valgas da Silva, CP¹, Antunes E¹, Zanesco A² ¹FCM-UNICAMP - Pharmacology, ²UNESP-Rio Claro - Physical Education

Introduction: Hyperlipidic (HD) diet cause lower urinary tract (LUT) dysfunction and physical exercise (EX) is recommended for weight control and prevention of the injuries caused by HD intake. In addition to the EX, supplementations with fat burners are often taken to speed up the losing weight process. L-carnitine (L-Car) is a supplement that regulates body composition associated with lipid metabolism and L-Arginine (L-Arg) is a non-essential amino acid playing a critical role in many organism functions. We aimed to evaluate the effects of EX and oral supplementation with L-Car and L-Arg, in the LUT of rats fed with HD. **Methods:** Experimental procedures were approved by the Animal Care and Use Committee of the State University of Campinas (Protocol # 1307-1). Male Wistar rats were divided into 7 groups: (1) HDSD: HD sedentary (SD); (2) HDEX: HD exercised (EX); (3) NDEX: EX with normolipidic diet (ND); (4) HDSD L-Car: HD, SD, receiving L-Car; (5) HDEX L-Car: HD, EX, receiving L-Car; (6) HDSD L-Arg: HD, SD, receiving L-Arg; (7) HDEX L-Arg: HD, EX, receiving L-Arg. After 4 weeks of HD intake, rats were orally supplemented and submitted to an EX program of running on a treadmill, in sessions of 60 min, 5 days/week, during 4 weeks. Rats were sacrificed and the detrusor smooth muscle (DSM) was mounted in organ bath, connected to force transducers. Changes in isometric force were recorded in a PowerLab data acquisition system. Concentration-responses curves to carbachol (CCh; 0.001-100 μ M), non-cumulative concentration response curves to α - β -methylene-ATP (α - β -mATP; 1, 10, 100 μ M), and frequency-response curves (1-32 Hz) to electrical field stimulation (EFS) were performed. Data were normalized by the strips weight (mN/mg). **Results:** CCh-elicited maximal contractions were increased in the HDSD group (4.74 ± 0.56 mN/mg) compared to the HDEX group (3.21 ± 0.52 mN/mg). However, the maximal response (E_{MAX}) to CCh in the HDEX group was higher than the E_{MAX} of the NDEX group (1.80 ± 0.44 mN/mg). L-Car and L-Arg did not affect the E_{MAX} to CCh in HDSD rats but produced a further decrease in the E_{MAX} to CCh in HDEX rats compared to the non-supplemented HDEX group (HDEX L-Car: 2.75 ± 0.19 mN/mg and HDEX L-Arg: 2.38 ± 0.19 mN/mg). Contractions induced by each concentration of ATP were higher in HDEX rats (0.40 ± 0.05 ; 1 ± 0.09 ; 1.47 ± 0.20 mN/mg, for 1, 10 and 100 μ M of ATP, respectively) compared with NDEX rats (0.13 ± 0.03 ; 0.30 ± 0.09 ; 0.54 ± 0.17 mN/mg, for 1, 10 and 100 μ M of ATP, respectively). L-Car did not prevent this increase in the HDEX L-Car group while L-Arg partially reduced this increase (HDEX L-Arg: 0.22 ± 0.07 ; 0.47 ± 0.16 ; 0.80 ± 0.18 mN/mg, for 1, 10 and 100 μ M of ATP, respectively). EFS-elicited maximal contractions were higher in HDEX (3.66 ± 1.17 mN/mg) and HDSD (3.04 ± 0.62 mN/mg) compared with NDEX (1.96 ± 0.90 mN/mg). Neither L-Car nor L-Arg supplementation prevented this increased response in either SD or EX groups. **Discussion:** EX decreased the augmented CCh-induced contraction observed in HD group. Additionally, L-Car or L-Arg caused a further reduction of this response in EX rats, suggesting that supplementation with L-Car or L-Arg might increase the benefits of the EX. **Financial support:** CNPQ/FAPESP

08.014

Interferência do tratamento com álcool na viabilidade espermática de ratos wistar. Pereira JD¹, Nichi M², Barnabe VC², Aivazoglou LU¹, Souza BP¹, Jurkiewicz A¹, Jurkiewicz NH¹ ¹UNIFESP - Farmacologia, ²USP - Reprodução animal

Objetivos: Considerando a possível influência do consumo do álcool na reprodução, pretendemos verificar como o tratamento crônico por via oral com álcool interfere na morfologia e viabilidade espermática de ratos Wistar. **Materiais e Métodos:** Ratos Wistar, com idade entre 90-120 dias, foram cronicamente tratados com etanol por 70 dias *per os* com etanol 25% e comparados a um grupo controle, tratado em igual volume com água destilada. Após sacrifício, os testículos e epidídimos foram pesados e o sêmen coletado. Os parâmetros observados consistiram na avaliação de motilidade (% de espermatozóides móveis observados em microscópio convencional), vigor espermático (movimento progressivo retilíneo dos espermatozóides numa escala de 0 a 5), morfologia espermática, avaliação da integridade da membrana acrossomal (proporcional à coloração pela Reação com corante de Pope), integridade da membrana plasmática (coloração de Eosina-Nigrosina) e medida da atividade mitocondrial (quanto maior a intensidade da coloração com DAB maior a atividade mitocondrial da peça intermediária, graduada em DAB I [maior atividade], DAB II [predominância de segmentos corados em relação aos não corados], DAB III [predominância de segmentos não-corados] e DAB IV [coloração nula - sem atividade]. **Resultados e Discussão:** Verificamos que entre os animais tratados com etanol (pelo menos 5 animais por grupo) ocorreu maior número de espermatozóides com a cauda enrolada (C= 2,8 ± 0,1%; Etanol= 4,6 ± 0,7%) e com cauda dobrada (C= 2,8 ± 0,1%; Etanol= 4,6 ± 0,7%). O número de espermatozóides com total potencial de membrana mitocondrial (DAB I) foi significativamente menor para o grupo tratado com etanol (C= 47,5 ± 5,5%; Etanol= 30,6 ± 3,9%). O número de espermatozóides com parcial potencial de membrana mitocondrial (DAB II) foi significativamente maior para o grupo tratado com etanol (C= 45,2 ± 5,5%; Etanol= 58,4 ± 2,05%). **Conclusões:** Os resultados mostram que, no protocolo utilizado para tratamento de ratos Wistar, houve indução de maior número de defeitos relacionados à cauda e peça intermediária do espermatozóide. É relatado em literatura (BARROS et al.; Italian Journal of Animal Science, v. 6, p. 772-774, 2007) que danos iniciais de mitocôndria poderiam levar a uma liberação aumentada de espécies reativas de oxigênio que, por sua vez, levariam a danos de membrana e alterações morfológicas, assim como danos ao acrossomo e fragmentação de DNA. A diferença encontrada no DAB II indica a ocorrência de maior estresse oxidativo, o que pode ocasionar comprometimento da mobilidade e a qualidade do espermatozóide. Estudos em andamento visariam comprovar esta hipótese. **Aprovado pelo CEEA-UNIFESP:** Protocolo nº 1650/04. **Apoio Financeiro:** CAPES, CNPq e FAPESP.