ABSTRACTS

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07. Endocrine, Reproductive and Urinary Pharmacology

07.001 *Spirulina platensis* supplementation improves rat erections in a model of erectile dysfunction induced by hypercaloric diet. Souza ILL, Barros BC, Ferreira ES, Carvalho MTL, Interaminense LFL, Cavalcante FA, Silva BA DCF-UFPB

Introduction**:** Obesity is characterized by an excessive accumulation of adipose tissue located throughout the body and favor erectile dysfunction (ED) development (Santos, Rev Soc Bras Clín Méd, v.10, p.384, 2012). ED is defined as the inability to achieve or maintain a penile erection long enough to have a sexual intercourse (Costa, Drugs, v.72, p.2243, 2012). Recently, we establish a model for the study of promising bioactive substances to treat ED (Souza, SBFTE, 2016). In this view, *Spirulina platensis*, an alga, is highlighted as a potential functional food associated to vascular function improvement (Ferreira, SBFTE, 2016). Thus, we aimed to elucidate a possible erectile beneficial effect of this alga in a model of ED induced by hypercaloric diet. **Methods:** Wistar rats (8 weeks of age) were divided into control group (CG) that received a standard diet, obese group (OG) or obese treated with *S. platensis* 25, 50 or 100 mg/kg (OG25, OG50 and OG100, respectively). OG was fed with a hypercaloric diet during 8 weeks. Then, experimental obesity was evaluated throughout body mass gain, white adipose tissues (WAT) weight and body adiposity index (BAI). In addition, rats received apomorphine and penile erections were monitored. Results were expressed as mean and standard deviation of the mean and analyzed by one way ANOVA followed by Tukey's post-test and Pearson's correlation coefficient (r) (n=5). **Results:** OG (215.0±35.2 g) body mass gain was superior to CG (166.3±33.1 g), OG25 (148.6±45.8 g), OG50 (145.3±43.3 g) and OG100 (143.1±30.2 g). Also, epididymal WAT of OG (2.0±0.4 g/100 g) was higher than CG (1.0±0.4 g/100 g) and similar to OG25, OG50 and OG100. Meanwhile, retroperitoneal WAT of OG (3.5±1.0 g/100 g) was superior to CG (2.1±0.6 g/100 g), not being altered by treatments. Additionally, inguinal WAT was increased in OG (2.4±0.7 g/100 g) compared to CG (1.6±0.2 g/100 g). Interesting, OG25, OG50 and OG100 showed decreased mass of this tissue compared to OG (1.7±0.7; 1.6±0.5 and 1.5±0.5 g/100 g, respectively), being similar to CG. BAI of OG (2.2±0.3) was increased compared to CG (1.5±0.3), OG25 (1.6±0.4), OG50 (1.6±0.2) and OG100 (1.6±0.5). Erectile function of obese rats was impaired based on number of erections obtained in OG (0.5±0.2) that was lower than CG (2.5±0.2), not differing to OG25 and OG50. However, OG100 (1.7±0.8) improved the number of penile erection, become similar to CG. Also, latency to 1st erection in CG (12.8±1.9 min) was lower than OG (23.5±7.7 min). In OG25, this time was similar to OG, but OG50 (9.3±6.3) and OG100 (9.8±2.8) presented a decreased time, being similar to CG. Correlation between anthropometric and erectile parameters showed a moderate negative correlation between the number of erections and body mass gain (r=-0.48) or BAI (r=-0.43) and a weak positive correlation between the time to 1st erection and body mass gain (r=0.37) or BAI (r=0,30). **Conclusion:** Obesity induces body mass gain, adipose tissue accumulation and BAI increase, leading to an impairment of rat erectile function that is improved by the supplementation with *S. platensis*. Thus, providing an advance in sexual dysfunction field treatment. **Financial support:** CNPq, CAPES, PPgPNSB/UFPB **Research approval:** Ethical Committee on Animal Use/UFPB (0201/14).

07.002 Aldosterone-induced cavernosal tissue impaired function depends on the NLRP3 activation in hematopoietic cells. Fais RS, Nascimento TB, Ferreira NS, Mestriner F, Tostes RC, Carneiro FS FMRP-USP – Farmacologia

Introduction: Erectile function (EF) depends on the tone of the cavernous tissue (CT). Increased contraction and/or decreased relaxation responses contribute to erectile dysfunction. Erectile dysfunction and cardiovascular diseases share several mechanisms, like inflammation. There is growing evidence that hyperaldosteronism is a risk factor for the development of cardiovascular diseases and impaired EF. Aldosterone (Aldo) is an important inflammatory modulator. NLRP3 is an intracellular receptor of the innate immune system, responsible for the release of pro-inflammatory cytokines that induce inflammation and it is implicated in vascular dysfunction. Aldo induces NLRP3 activation in vascular beds. However, the role of NLRP3 in the modulation of cavernosal tonus is unknown. Therefore, we hypothesize that Aldo modulation in the basal tonus of the CT depends on NLRP3 activation. **Methods**: Male C57BL/6 (WT), NLRP3 ($NLRP3^{-/-}$) and interleukin-1β receptor ($IL\text{-}1R^{-/-}$) knockout mice were used. Bone marrow was transplanted from WT to WT (WT->WT) and $NLRP3^{-/-}$ (WT->$NLRP3^{-/-}$) or from $NLRP3^{-/-}$ to WT ($NLRP3^{-/-}$->WT) mice with 10 to 12 weeks treated with vehicle or Aldo (600 $\mu g.kg^{-1}.day^{-1}$ for 14 days) while receiving 1% saline to drink. Reactivity of CT was performed. All the experiments were approved by Ribeirao Preto Medical School ethics committee (CEUA 012/2013-1). **Results**: Aldo infusion reduces the endothelium-dependent relaxation to acetylcholine (ACh) (Emax WTvehicle: 76,6 ± 2,9; WTAldo: 58,0 ± 2,2; n=5), but not endothelium-independent relaxation to sodium nitroprusside (Emax WTvehicle: 82,9 ± 2,1; WTAldo: 82,9 ± 3,6; n=5). The absence of NLRP3 (Emax $NLRP3^{-/-}$vehicle: 73,8 ± 3,3; $NLRP3^{-/-}$Aldo: 84,0 ± 2,9; n=4) and IL-1β receptor (Emax $IL\text{-}1R^{-/-}$vehicle: 85,9 ± 3,1; $IL\text{-}1R^{-/-}$Aldo: 75,1 ± 3,3; n=3-5) prevents the impairment in ACh-induced relaxation. Also, bone marrow transplantation from $NLRP3^{-/-}$ to WT mice prevented Aldo-induced relaxation impairment, but the transplantation from WT to $NLRP3^{-/-}$ did not (Emax WT->WTvehicle: 98,2 ± 1.5; WT->WTAldo: 70.2 ± 3,2; $NLRP3^{-/-}$->WTAldo: 93,5,0 ± 10,9; WT-> $NLRP3^{-/-}$Aldo: 78,5 ± 5,6; n=3-6). **Conclusion**: NLPR3 mediates Aldo-induced impaired relaxation in the CT. In addition, NLRP3 in hematopoietic cells contributes to the effects of Aldo in CT functional responses. Thus, NLRP3 inhibition may represent a new therapeutic target to treat cavernosal dysfunction induced by Aldo. **Financial Support**: FAPESP, CRID, CNPQ, CAPES, FAEPA

07.003 CL 316,243 Fails to improve insulin sensitivity in diet-induced obese male mice offspring. Sousa E, Rodrigues AC ICB-USP – Farmacologia

Introduction**:** Epidemiological and animal models show that maternal obesity rises the probability of offspring obesity in adulthood. In parallel, studies showed that communication between adipose tissue and nervous system regulates energy expenditure, and is modified by obesity and cold exposure. However, there are no studies touching directly a possible influence of maternal obesity on neuroadipose communication. Since obesity is an intergenerational disorder, learning the effects of maternal obesity on offspring neuroadipose communication, which develops influenced by maternal metabolic status, means support future research for therapeutic approaches for obesity and comorbidities. Our aim was evaluating putative alterations in neuroadipose communication caused by maternal obesity, influencing offspring metabolism and capacity for adipose tissue browning in adulthood. **Methods:** Eight weeks-old female mice were randomly divided in two groups: high fat and high sugar diet or balanced diet. At the end of the sixth week, just before the mating, a glucose tolerance test was performed. At the day of detection of the vaginal plug, lean and obese female mice were single-housed and their respective diets were maintained through pregnancy and lactation. Offspring received ad libitum balanced diet from weaning to eight weeks, when energy expenditure was analysed by indirect calorimetry in a 24 hours period. After this analysis, offspring were randomly divided between two treatments: CL 316,243 (1mg/kg/day), a β3 adrenergic receptor agonist, or the vehicle, saline i.p. during 10 days. An insulin tolerance test (ITT) was performed on the seventh day of treatment. On the last day of treatment, energy expenditure was repeated. Both male and female offspring were studied. Data was analyzed by two-way ANOVA followed by Tukey post-hoc test. A p-value < 0.05 was considered statistically significant. **Results:** At the first day of diet, all females had similar body weights (mean for lean females group: 17.4 g, vs. 17.0 g for obese females group), with gradual increases each week. At the end of the sixth week, obese females were 4.9 g heavier than lean females ($p=0.0001$). Obese females also had impaired glucose tolerance after this period, showing a 57.7% increase in area under the curve of blood glucose through time ($p=0.0028$). At the first indirect calorimetry test, respiratory exchange ratio, which indicates ratio of carbohydrate to lipid oxidation, were not different between lean and obese offspring, with no influence of gender as well. After seven days of treatment, we performed an insulin tolerance test in the offspring. Female mice are more sensitive to insulin than male (glucose decay ratio, or KITT, for female mice: 22.26%/min, vs 3.02 %/min for male mice, $p=0.0079$). Obese female offspring is less sensitive to insulin than lean female offspring (lean offspring KITT = 22.27 %/min, vs obese offspring KITT = 15.90 %/min; $p=0.0396$). For female offspring, the treatment didn't change insulin sensitivity. However, for male offspring, CL 316,243 improved insulin sensitivity in lean dam pups, but obese dam pups didn't have this effect. **Conclusion:** A lack of properly response to CL 316,243 in obese dam male offspring, which indicates poor communication between sympathetic nerves and adipose tissue, is a putative cause for greater chance for obesity of these animals in adulthood, and can be a target for treatment of this metabolic disorder. **Financial Support**: FAPESP. CEUA process number 40/2016.

07.004 Protective effect of supraphysiological testosterone replacement in voiding dysfunction in ovariectomized rat is estrogen-independent. Bonilla-Becerra SM, Oliveria MG, Calmasini FB, Rojas-Moscoso JA, Tobar N, Antunes E
Unicamp – Farmacologia

Introduction and Aim: Postmenopausal women often exhibit urological complications that include urinary incontinence, urgency and nocturia (1). Testosterone replacement in postmenopausal women has been indicated mainly for sexual dysfunction and libido loss, but little is known about the androgen replacement in micturition dysfunction. In four-month ovariectomized (OVX) rats we showed recently that testosterone replacement at a supraphysiological dose largely reduces the lower urinary tract smooth muscle dysfunction (2). Since testosterone may be converted by aromatase (CYP19) to estrogen, we have now investigated the effects of letrozole (CYP19 inhibitor) in combination with testosterone on the lower urinary tract dysfunction of OVX rats. **Materials and Methods**: Two-month old Sprague–Dawley female rats (250-280 g) were submitted to bilateral ovary removal, whereas sham-operated rats were manipulated but ovaries were left intact. After three-month OVX, rats were divided into four groups: Sham, OVX, OVX plus testosterone undecanoate (10 mg/kg) and OVX plus testosterone undecanoate (10 mg/kg) and letrozole (2.5 mg/kg). The following parameters were evaluated: (i) Measurements of body, uterus, bladder, urethra and perigonadal fat weights, (ii) urodynamic studies (in vivo), (iii) in vitro carbachol-induced bladder contractions and (iv) in vitro angiotensin II-induced urethra contractions. **Results:** Ovariectomy significantly increased the total mass and fat mass, and diminished lean mass and uterus weight compared with Sham group ($p<0.05$). The bladder and urethra weights were not changed by OVX. Testosterone alone or in combination with letrozole did not modify these morphometric parameters. Urodynamic alterations in OVX rats were observed characterized by increases of basal pressure, threshold pressure, voiding frequency and post-voiding pressure, all of which were prevented by testosterone replacement. The combination of testosterone with letrozole did not modify the protective effects of testosterone alone. In OVX rats, carbachol-induced bladder contractions were reduced, whereas angiotensin II-induced urethral contractions were increased. Testosterone alone normalized these in vitro alterations; however, the reversal by testosterone of these in vitro alterations remained unchanged by concomitant treatment with letrozole. **Conclusion**: The protective effects of supraphysiological dose of testosterone in the lower urinary tract smooth muscle of OVX rats are not reversed by concomitant treatment with letrozole. These findings exclude that protective effect of testosterone is not secondary to estrogen synthesis. **References:** 1. Abram P et al. Neurourol and Urodyn, (29), 213-240; 2010. 2. Bonilla-Becerra et al. Lif Sci, (179), 120 -129; 2017 **Financial Support**: CNPq (146942/2016-7). All animal procedures were approved by the Ethical Committee on Animals Use CEUA/UNICAMP (CEUA; No. 3499-1).

07.005 Identification of a potential therapeutic target for abnormally invasive placenta. Botelho RM[1], Silva ALM[1], Souza LPG[1], Pires KSN[1], Santos JC[1], Gonçalves CM[1], Silva EMP[1], Oliveira HGS[1], Carmo JOS[1], Borbely KSC[1], Silva SV[2], Freitas VM[2], Borbely AU[1] [1]UFAL, [2]USP

Introduction: Abnormally invasive placenta (AIP), formerly known as placenta accreta, is a rather unknown pregnancy disease, which is reason of concern worldwide, since its increasing incidence and the possible outcomes, that can lead to massive obstetrical hemorrhage, uterine rupture and maternal death. It is believed to arise due an absence of maternal decidualization together with more aggressive invading trophoblast cells. Although surprising, no cure nor pharmacological treatment exist, with hysterectomy being the only solution to prevent the utterly bad outcomes, but also finishing the chances of a new pregnancy. Recently, our group showed versican V0 and V1 isoforms are produced only by invading trophoblast cells from AIP, thus characterizing the first specific target ever described. **Methods:** First trimester-derived HTR-8/SVneo cells were employed for versican gene silencing and its efficiency was accessed by RT-PCR, immunofluorescence and flow cytometry for versican V0, V1, V2 and V3 isoforms. Cell death was evaluated by annexin V - propidium iodide staining. Proliferation and cell cycle were evaluated by Ki-67 staining. Falloidin staining and cytokeratin, vimentin and alpha-tubulin stainings were performed to assess cytoskeleton organization. *In vitro* wound healing assay up to 72h and time-lapse video microscopy for 17h were employed to verify cell motility. Also, invasion through fibronectin-coated Transwell bipartite chambers was analyzed. Furthermore, phospho-focal adhesion kinase (FAK), phospho-Ras homolog gene family A (RhoA) and phospho-Rho associated coiled-coil containing protein kinase 2 (ROCK2) expression were verified by immunofluorescence and flow cytometry. **Results:** Versican V0 and V1 expression were detected in HTR-8/SVneo cells with 90% of silencing efficiency. Versican siRNA has not altered cell death, but slightly increased proliferation. Falloidin staining showed actin depolarization at cell periphery with reduction of p-FAK and vimentin expression. *In vitro* wound closure was greatly delayed, with only 51% after 72h and cell speed was reduced by half in comparison to control. In addition, invasion assay of versican-silenced cells had three times less invaded cells than control. Phospho-RhoA expression decreased 95% and phospho-ROCK2 decreased 12%. **Conclusion:** Versican silencing resulted in great changes in cytoskeleton organization and reduced RhoA and ROCK2 phosphorilation, as well as cell migration and invasion, which implies versican is a key regulator in trophoblast motility and a potential therapeutic target for AIP.

07.006 The role of adenosine receptor A2A on the hyperactivity of hypothalamus-pituitary-adrenal axis observed in diabetic animals. Buriche ALR, Magalhães NS, Silva PMR, Martins MA, Carvalho VF Fiocruz

Introduction**:** Type 1 diabetic patients and animals present a hyperactivity of hypothalamus-pituitary-adrenal (HPA) axis, which leads to an increased level of glucocorticoids. A2A receptor is up-regulated in hippocampus of streptozotocin-induced diabetic rats. In addition, the activation of A2A receptor is related to an increased production of glucocorticoids. According to these data, the present study was undertaken to evaluate the role of the adenosine receptor A2A over the hyperactivity of HPA axis observed in diabetic animals. **Methods:** Male Swiss-Webster mice were induced to diabetes by a single injection of alloxan monohydrate (65 mg/kg, i.v.). Seven days after diabetes induction, mice were treated with the specific A2A receptor antagonist SCH-58261 (0,1 mg/kg/day, i.p.) once a day during the 14 following days. Plasma Corticosterone quantification was done by means of radioimmunoassay. In the in vitro analysis, adrenocortical cells Y1 were incubated with normo (5mM glucose) or hyperglycemic (18.5 mM glucose) medium. In some assays, the Y-1 cells were stimulated with the selective agonist for the adenosine receptor A2A, CGS-21680 in the concentrations of 30, 100 or 300 nM. The number of fascicules formed was made 3 or 6 hours after stimulation with hyperglycemic medium or CGS-21680, respectively.**Results:** Our data showed that the antagonist for the adenosine receptor A2A, SCH-58261 reduced plasma corticosterone levels as well as adrenal hypertrophy observed in diabetic animals. However, SCH-58261, did not interfere with hyperglycemia noted in diabetic mice. In addition, our in vitro assays showed that hyperglycemic medium and the selective agonist for the adenosine receptor A2A CGS-21680 induced the formation of fascicles, the shape in which adrenocortical cells are organized in adrenal's *zona fasciculata*. **Conclusion:** The adenosine receptor A2A is involved in the hyperactivity of HPA axis observed in diabetic animals, since its antagonist was able to reduce the corticosterone levels and the adrenal hypertrophy seen in the diabetic animals. Besides, our data suggests that the adrenocortical cell organization in the tissue might be relevant for steroidogenesis, since the hyperglycemic medium and the selective agonist for the adenosine receptor A2A, CGS- 21680 induced the formation of fascicles. **Financial Support**: FAPERJ, CNPq and Instituto Nacional de Ciência e Tecnologia em Neuroimunomodulação (INCT-NIM). All the procedures were approved by the Committee on the Use of Laboratory Animals of the Oswaldo Cruz Institute (CEUA-IOC , license L – 027/2016)

07.007 Effects of bacterial endotoxins on sperm parameters in a rat model of epididymitis reveal potential pathogen-specific male fertility outcomes. Silva AAS[1], Mueller A[1], Kushima H[1], Ribeiro CM[2], Avellar MCW[2], Silva EJR[1] [1]Unesp-Botucatu – Farmacologia, [2]Unifesp – Farmacologia

Introduction**:** Epididymitis, an inflammatory disease of the epididymis, is the most common cause of scrotal pain, being associated with loss of fertility and labor-hours. Bacteria such as *Escherichia coli (*Gram-negative) and *Enterococcus faecalis (*Gram-positive) are common causative pathogens of epididymitis. Despite the negative impact of epididymitis on reproduction, pathogen-specific fertility outcomes are little understood. We previously showed that the epididymis mounts a differential inflammatory response to endotoxins lipopolysaccharide (LPS) from *E. coli* and lipoteichoic acid (LTA) from *E. faecalis*. We hypothesized that these endotoxin-specific inflammatory responses in the epididymis differentially affects sperm parameters. Here, we investigated the effects of LPS- and LTA-induced epididymitis on sperm parameters in rats. **Methods**: Wistar rats (90 days, n=6-8/group) were anesthetized with ketamine/xylazin (100/10 mg/kg, i.p.) and submitted to an experimental model of epididymitis based on the retrograde injection of sterile-saline (control), ultrapure LPS (25 µg) or LTA (125 µg) into the lumen of the vas deferens. Rats were euthanized 1, 7 and 15 days after treatment and their testes and epididymides (caput/corpus – CP; and cauda - CD regions) were processed for sperm count. The following parameters were determined: homogenization-resistant spermatid number and daily sperm production (DSP) in the testis, and sperm number and transit time in the epididymis. CD sperm were processed for sperm motility evaluation by Computer-Assisted Sperm Analysis and stained with Panotic dye for sperm morphology. Quantitative parameters were analyzed by ANOVA followed by Bonferroni test; sperm motility and morphology were analyzed by Kruskal-Wallis followed by Dunn´s test ($p<0.05$ was considered significant). **Results:** Intravasal LPS or LTA treatment did not change testis and epididymis weight, as well as the testicular sperm parameters. Sperm count (10^6 x cells/g of organ) was significantly reduced in the CP at 1 day (<15.7%) and 7 days (<24.0%) after LPS treatment in comparison to their respective controls, and in the CD at 1 (<28.8%), 7 (<29.5%) and 15 days (<17.0%). In parallel, sperm transit time was accelerated in the CP at 7 days (>23.5%) after LPS treatment, and in the CD at 1 (>10.6%), 7 (>37.6%), and 15 days (>28.0%). Milder effects on epididymal sperm parameters was observed after LTA treatment, which significantly reduced sperm count in the CP and CD at 1 day (<22.3% and <19.8%, respectively), and accelerated sperm transit time in the CP at 1 day (>23.5%) only. Neither LPS nor LTA treatments affected sperm morphology and motility at 7 days. **Conclusions**: Our results showed that the inflammatory reaction in the epididymis to LPS challenge induced a more severe impairment on epididymal sperm parameters than LTA, suggesting distinct reproductive outcomes based on the etiological factor of epididymitis. **Financial Support:** Science without Borders Program (CSF/CNPq), CNPq, Fapesp, and PROPe-UNESP. Research Ethics Committee approval from IBB-UNESP: process #629; and UNIFESP-EPM: process #0310/12.

07.008 Tissue- and gender-specific expression profile of the male contraceptive target epididymal protease inhibitor (***Eppin***) **in mice**. Camara AC, Kushima H, Silva EJR IBB-Unesp – Farmacologia

Introduction: The *Eppin* (*Epididymal protease inhibitor*) gene encodes a cysteine-rich protein containing Kunitz-type and WAP-type four disulfide core protease inhibitor consensus sequences. In humans, EPPIN is specifically expressed in the testis and epididymis. EPPIN is found on the human sperm surface, where it plays a crucial role in the acquisition of sperm motility, which makes this protein a potential target for male contraception. The establishment of a mouse model to study EPPIN's functions *in vivo* will facilitate its development as a contraceptive drug target. Although EPPIN expression has been demonstrated in the mouse testis and epididymis, little is known about its expression profile in this species. Thus, this study was designed to fully uncover the expression of *Eppin* transcript in different organs of male and female mice, as well as to investigate the effects of sexual maturation on testicular *Eppin* expression. **Methods**: Adult (90 days old) male (n=5) and female (n=3) C57BL/6 mice were euthanized and the reproductive organs epididymis, vas deferens, seminal vesicle, prostate, ovary, oviduct, and uterus, as well as 19 non-reproductive organs from cardiovascular, digestive, endocrine, respiratory, urinary and central nervous systems were collected. Additional groups of sexually maturing male mice (20, 40, and 60 days old, n=5/group) were euthanized and their testis and epididymis were collected. Organs were processed for total RNA extraction, followed by first-strand cDNA synthesis. cDNA samples were used in conventional end-point PCR studies to detect mouse *Eppin* transcript, as well as in real-time PCR (qPCR) assays to evaluate its relative expression in the testis of sexually maturing mice. The mRNA levels of housekeeping genes *Rps18* (ribosomal protein S18) and *Ppia* (cyclophilin A) were used as endogenous controls. qPCR results were analyzed by ANOVA followed by Tukey test; $p<0.05$ was considered significant. **Results:** We confirmed the abundant expression of *Eppin* transcript in the testis and epididymis from adult mice, and further detected its expression in the vas deferens and seminal vesicle, as well as in the adrenal gland. Notably, we detected the expression of *Eppin* mRNA in the ovary, oviduct and uterus demonstrating for the first time its presence in the mouse female reproductive tract. The identity of PCR amplified products in positive samples was confirmed to be mouse *Eppin* (NCBI #NM_029325) by DNA sequencing. In support to the hypothesis that sexual maturation upregulates *Eppin* expression, we observed a significant increase on its relative expression in the testis from 40- and 90-day-old mice in comparison to 20-day-old mice (fold change 1.3 ± 0.1 and 1.4 ± 0.1, respectively). **Conclusions**: Our results revealed novel sites of *Eppin* expression in the adult mouse, suggesting it has roles in both male and female fertility, as well as in the adrenal gland physiology. Considering the positive regulation of *Eppin* by androgens, its expression may correlate with the local androgen production in the adrenal gland and ovary. Altogether, our results open new perspectives for the study of EPPIN's functions. **Financial Support:** FAPESP (2015/08227/0 and 2016/23025-7). CEUA-IBB/UNESP approval: process #703.

07.009 The guanine-based purines produced relaxation in isolated corpus cavernosum from mice. Nicoletti AS, Antunes E, Mónica FZ FCM-Unicamp – Farmacologia

The pathophysiological role of adenine-based purines (ABP) as adenosine triphosphate (ATP), adenosine diphosphate (ADP) and adenosine to trigger smooth muscle contraction or relaxation (Aronsson, et al, 2009) and platelet aggregation (Fuentes, et al,2014), among others is well established (Zimmermann, 2011). On the other hand, the extracellular role of guanosine triphosphate (GTP) and its breakdown products as guanosine diphosphate (GDP), 5'- guanosine monophosphate (5'-GMP) and guanosine (GUO) is less studied. Also, the cyclic guanosine monophosphate (cGMP) pumped out of the cell by the action of the multidrug resistance proteins could also be an important source of GUO. To date several studies have been showing that guanine-based purines have a neuroprotective effect both in vitro and in vivo (Molz, et al, 2011). Therefore, this study is aimed to evaluate the role of GTP, 5'-GMP and GUO in isolated corpus cavernosum (CC) from mice. **Method:** Male mice (12-weeks old) were anesthetized with isoflurane and exsanguinated. The corpus cavernosum was isolated, assembled in an organ bath and suspended to an isometric force transducer. Concentration-response curves(0.01, 0.1 and 1 mM) were obtained for GTP, 5'-GMP and GUO in tissues pre-contracted with phenylephrine (0.01 mM) and the potency (pEC_{50}) and maxima response (E_{max}) values were obtained. Data are expressed as mean + SEM, N= number of animals. **Results:** At 0.01, 0.1 and 1 mM, GTP, 5'-GMP and GUO produced concentration-dependent relaxation with the following values: GTP - 50. ± 7.8, 70.8 ± 12.2 and 122.1 ± 13.9 % (pEC_{50}: -3.497 ± 0.54, N=3), 5'GMP : 48.1 ± 7.7, 70.7± 6.8 and 101.3 ± 5.3 % (pEC_{50}: -3.837 ± 0.34, N=5) and GUO: 52.8 ± 5.2, 66.5 ± 8.3 and 142.6 ± 20.8 % (pEC_{50}: -2.908 ± 1.946, N=6), respectively. **Conclusion:** Our preliminary data suggest that the extracellular GTP, 5'-GMP and GUO may have a role in the erectile function. **References:** 1. Aronsson P., et al. Basic & Clinical Pharmacology & Toxicology, 107, 603–613 2. Fuentes, E. PLoS ONE 9(11): e112741, 2014; 3. Zimmermann, H. Purinergic Signal, Vol 6(4), pp 417–428, 2011; 4. Molz, S. Journal of Neuroscience Research 89:1400–1408, 2011. **Financial support**: CAPES. All animal procedures were approved by the Ethical Committee on Animals Use CEUA/UNICAMP (CEUA; No. 4201-1).

07.010 The androgen regulation on the confluence of innate immunity and epididymal morphogenesis Ferreira LGA[1], Ribeiro CM[1], Hinton BT[2], Avellar MCW[1]
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Introduction: The epididymis is essential for male fertility and the involvement of host defense peptides expressed in this organ with sperm function has become evident in recent years. The functionality of a normal epididymis depends prenatally on a fine-tune process of morphogenesis primarily orchestrated by androgens, during which the Wolffian duct (WD) originates this tissue. We recently identified that a component of the arsenal of innate immunity abundantly expressed in the adult epididymis, the β-defensin SPAG11C (sperm associated antigen 11 C), is negatively regulated by androgens and has a role in modulating the rat WD morphogenesis. Curiously, we also identified transcripts for other two β-defensins, *Defb1* and *Defb2,* in developing WDs, which are suggested as being involved in reproductive tasks in the adult rat. Herein we investigated the androgen regulation of these two β-defensins at mRNA level during the WD development to gain insights into their functionality in embryonic events that determine male fertility at adulthood. **Methods:** Total RNA was extracted from male Wistar rat WDs at embryonic days (e) 12.5-20.5 and from adult caput epididymis (120 days-old; positive control) for RT-qPCR. Isolated WDs (e17.5) were cultured ex vivo in the absence or presence of testosterone (T; 10 nM) and flutamide (10 μM), a competitive androgen receptor (AR) antagonist. Cultured WDs were used in RT-qPCR and their gross morphology was evaluated. **Results:** Transcripts for the tested β-defensins were detected in high abundance in the adult epididymis, as we expected. *Defb1* and *Defb2* mRNA levels increased (3.2- and 2.4-fold, respectively) in WDs between e17.5-e20.5. In rat, this time window comprises a surge in fetal plasma T levels, a decrease (2.5-fold) in *Spag11c* mRNA levels and the androgen-induced morphological differentiation of WD into epididymis. Influence of T/AR signaling on *Defb1* and *Defb2* transcripts were assessed by WD organotypic cultures under different androgenic conditions. T increased *Defb1* mRNA levels (3.4-fold) when compared to control tissues in the absence of this hormone. Although *Defb2* mRNA was not readily detected on WDs in the absence of T, this hormone incubation clearly induced its expression. The positive androgen effects on the expression of these two transcripts were abolished by flutamide, confirming the T/AR signaling participation. These results contrast with the negative androgen regulation we demonstrated for *Spag11c* mRNA in cultured WDs. **Conclusion:** The present data shed light on the differential androgen regulation of distinct β-defensins and suggest them as potential players in the WD morphological differentiation. To better understand the relationship between the components of the innate immunity and the epididymal morphogenesis, we are conducting studies to evaluate the effects of recombinant DEFB1 and DEFB2 on ex vivo WD development. **Financial Support**: CNPq/Science Without Borders (401932/2013-3, 1050066/2016-3, 101550/2016-2), NIH−NICHD #069654, FAPESP #2016/00164-1, # 2016/22118-1. Ethics Approval: CEUA Unifesp-EPM N. 1776201213.

07.011 MK 571, **a multi-drug resistance protein inhibitor potentiated the relaxing responses in bladder and prostate from mice** Bertollotto G, Alexandre EC, Antunes E, Mónica F Z Unicamp – Bioquímica e Farmacologia

Introduction: The levels of cyclic adenosine monophosphate (cAMP) or cyclic guanosine monophosphate (GMPc) are controlled by their rate of formation through the activation of adenylate cyclase and soluble guanylate cyclase (sGC) or particulate guanylate cyclase, respectively and their rate of degradation due to the actions of phosphodiesterases or multi-drug resistance proteins (MRPs) (Mujoo K. et al., 2011). To date MRP4, MRP5 and MRP8 are the subtypes known to pump cAMP or cGMP out of the cell. The role of these efflux transporters have been exploring in several tissues and cells including gut epithelial cells (Moon C. et al., 2015), pulmonary arteries (Hara Y. et al., 2011), platelets (Lien L. et al.,2014), skeletal muscle (Godinho et al., 2015) and heart (Sassi Y. et al., 2012). Since the cyclic nucleotides are important second messengers that control the reactivity of the urogenital tract organs, this study is aimed to characterize the effect of the non-selective MRP inhibitor, MK 571 on the relaxing responses induced by agonists that increase AMPc or GMPc in isolated bladder and prostate from mice. **Material and Methods**: Concentration-response curves to i) isoproterenol (0.001-30 µM), fenoterol (0.001-30 µM), mirabegron (0.000001- 1 mM), forskolin (0.01-100 µM), rolipram (0.001-100 µM), and 3-Isobutyl-1-methylxanthine (IBMX, 0.001-100 µM) in bladders pre-contracted with carbachol (1 µM) and to ii) sodium nitroprusside (0.001-100 µM) and isoproterenol (0.001-100 µM) in isolated prostate with phenylephrine (1 µM) were carried out. The intracellular levels of cAMP and cGMP in the bladder and prostate, respectively were also quantified. The potency (pEC_{50}) and maximal response (E_{max}) values were determined whereas the levels of cAMP and cGMP were expressed in pmol/mL. Data are expressed as mean ± SEM, N=number of animals. **Results**: In the bladder, isoproterenol, fenoterol and forskolin produced concentration-dependent relaxations with E_{max} values of 50±5.0, 59±6.1 and 81±2.6 % (N=05), respectively and these responses were significantly potentiated by 36, 37 and 24 % ($P<0.05$) in the presence of MK 571 (20 µM). The treatment with fenoterol generated a fivefold augment in the cAMP in the bladders in comparison with basal levels (from 4.6 ± 0.42 to 20.3 ±3.1 pmol/mL) and when MK 571 was co-incubated a 60% increase (32 ± 3.8 pmol/mL) in the AMPc levels was observed. MK 571 did not interfere on the relaxations induced by mirabegron, IBMX and rolipram. As for the prostate, the relaxation induced by SNP, but not that of isoproterenol, was significantly potentiated by MK 571 by 54 %, which was accompanied by greater levels of cGMP (from 1.14 ± 0.21 to 4.38 ± 0.39, $P<0.05$, N=06).**Conclusion:** MRPs inhibition, when coupled with agonists that augment the cyclic nucleotide levels, strongly potentiated bladder and prostate relaxation. These findings are of therapeutic interest because highlight the importance of MRP transporter in controlling the cAMP or cGMP levels in the urogenital tract organs. **Financial Support**: CNPq CEUA: n° 4202-1 **Ref:** 1.Mujoo K. et al Radic. Biol. Med. 51:2150-2157, 2011. 2. Moon C. et al The Journal of Biological Chemistry 290:11246-11257, 2015 3. Hara Y. et al The Journal of Clinical Investigation 121:2888-2897, 2011 4. Lien L. et al European Journal of Farmacology 737:159-167, 2014 5.Godinho et al Frontiers in Pharmacology 6:58, 2015 6. Sassi Y. et al The FASEB Journal 26: 1009-1017, 2012

07.012 Protein disulfide isomerase: **differential gene expression and androgen dependence in the rat epididymis**. Fernandes SG[1], Benham A[2], Avellar MCW[1]
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Introduction: The protein disulfide isomerase (PDI) family, a member of the thioredoxin superfamily of redox proteins, is composed of multifunctional molecular chaperones involved in protein post-translational modifications. To date, the PDI family contains 21 genes that code proteins differing in their amino acid sequence, molecular weight, tissue expression, and cellular processing. Given their vital role in protein-folding, specifically thiol-disulfide exchange, loss of PDI activity has been related to pathogenesis of numerous disease states, most commonly related to the unfolded protein response (UPR). Not all of them, however, display conserved functional domains and enzymatic activity. PDIs are reported to be secreted under physiological conditions in the male gonad and contribute to the ability of the spermatozoa to fertilize an egg. Some PDIs have been shown to bind steroid hormones in vitro. Their potential to bind/sequester sex steroid hormones and role in the male reproductive tissues is still scarcely known. **Aim**: Herein we evaluated the mRNA expression profile of PDIs in reproductive and non-reproductive tissues. We also studied the androgen dependence of selected *Pdi* genes in the epididymis, a vital organ of the male reproductive tract for sperm maturation. **Methods**: Reproductive (testis, epididymis, vas deferens, prostate) and non-reproductive tissues (adrenal, lung, liver, skeletal muscle and brain) were isolated from adult Wistar adult rats (120 days). Epididymis was divided in its regions (caput, corpus and cauda). Epididymal regions were also collected from control (sham-operated) and surgically castrated (7 and 15 days) adult rats. Semi-quantitative PCR or RT-qPCR assays were performed with tissue total RNA and primers against 20 *Pdi* genes. *Rpl19* and *Gapdh* were used as internal reference genes. Proper positive and negative controls were performed. **Results**: Our semi-quantitative PCR screening surprisingly revealed that all 20 *Pdi* genes tested were detected in the expected DNA product size in the adult caput epididymis. We selected ten of these *Pdi* genes (*P4hb*, *Pdip*, *Pdia3*, *Pdia5*, *Pdia6*, *Pdilt*, *Erp29*, *Erp44*, *Casq1*, and *Casq2*) for tissue mRNA expression studies. We observed that these transcripts were differentially detected in reproductive and non-reproductive tissues, as well in the epididymal regions. Curiously, *Pdilt* mRNA was not readily detected in corpus epididymis and heart. RT-qPCR analysis further revealed that the relative expression of *P4hb*, *Pdia3*, *Pdia5*, *Pdia6*, *Pdilt* and *Erp29* mRNA was significantly reduced in the caput epididymis of 7- and 15-days castrated rats when compared to control tissues, suggesting their potential dependence on androgen plasma levels. **Conclusion**: Our data expands the potential functional repertoire for PDIs in epididymal function, and open up the PDI family for exploitation as potential drug targets and biomarkers in the male reproductive system. **Financial support**: CNPq, FAPESP (#2015/50011-4, 2017/05261-8). **Ethics Approval:** CEUA UNIFESP-EPM # 5908210916.

07.013 Tetrodotoxin-Insensitive Electrical Field Stimulation-Induced Contractions on *Crotalus durissus terrificus* Corpus Cavernosum. Campos R, Mónica FZ, Rodrigues RL, Rojas-Moscoso JA, Moreno RA, Cogo JC, Antunes E, de Nucci G Unicamp – Farmacologia

Introduction: Tetrodotoxin treatment (TTX) or other inhibitor of voltage-gated sodium channel abolishes the nitregic relaxation induced by electrical field stimulation (EFS) in rabbit, monkey and human corpora cavernosa preparations (Holmquist .,*J physiol*, 449,1992; Fernandes de Oliveira, *Urology*, 62, 581, 2003; Okamura *Am J Physiol*,274,1075, 1998). In contrast, TTX treatment does not inhibit the EFS-induced relaxation in rattlesnake *Crotalus durissus terrificus* corpus cavernosum (Capel *J Sex*, 8, 1616, 2011). Reptiles are the first amniotes to develop an intromitent penis, however until now the mechanisms involved in the electrical field stimulation-induced contraction on corpora cavernosa isolated from *Crotalus durissus* terrificus were not investigated. **Methods**: Crotalus and rabbit corpora cavernosa were mounted in 10 mL organ baths for isometric tension recording. Electrical field stimulation (EFS)-induced contractions were performed in presence/absence of phentolamine (10 μM), guanethidine (30 μM), tetrodotoxin (1 μM and 1mM), A-803467 (10 μM) and a modified Krebs solution (equimolar substitution of NaCl by N-methyl–D-glucamine). Immunofluorescence for tyrosine hydroxylase was also performed. **Results**: Electrical field stimulation (EFS; 8 Hz and 16 Hz) caused contractions in both Crotalus and rabbit corpora cavernosa. The contractions were abolished by previous incubation with either phentolamine or guanethidine. Tetrodotoxin (1 μM) also abolished the EFS-induced contractions of rabbit CC, but did not affect EFS-induced contractions of Crotalus CC. Addition of A-803467 (10 μM) did not change the EFS-induced contractions of Crotalus CC but abolished rabbit CC contractions. Replacement of NaCl by N- Methyl-D-glucamine (NMDG) abolished EFS-induced contractions of rabbit CC, but did not affect Crotalus CC. The presence of tyrosine hydroxylase was identified in endothelial cells only of Crotalus CC. **Conclusion**: Since the EFS-induced contractions of Crotalus CC is dependent on catecholamine release, insensitive to TTX, insensitive to A-803467 and to NaCl replacement, it indicates that the source of cathecolamine is unlikely to be from adrenergic terminals. The finding that tyrosine hydroxylase is present in endothelial cells suggests that these cells can modulate Crotalus CC tone. **Financial support:** This study was supported by Fundação de amparo a pesquisa do estado de São Paulo (FAPESP ; 2011/11828-4) and by Conselho Nacional de Desenvolvimento científico e tecnológico (CNPq ; 140731/2013-0). **Research approval:** All experimental procedures using *Crotalus durissus terrificus*, New Zealand white rabbit and the *Callithrix jachus* (marmoset) were approved by the Institutional Animal Care and Use Committee (CEUA/UNICAMP: 1655-1,CEUA/UNICAMP: 2720-1, CEUA 3811-1 respectively). The use of *Crotalus durissus terrificus* and *Callithrix jachus* was authorized by the Brazilian Institute for Environment (Sisbio: 18020-1 and Sisbio 16951-1, respectively).

07.014 Menthol improves obesity-associated mouse bladder dysfunction independently of TRPM8 activation. Oliveira M, Nascimento D, Alexandre EC, Calmasini FB, Zapparoli A, Antunes E FCM-Unicamp – Farmacologia

Introduction**:** A number of studies have investigated the effects of agonists and antagonists of the Transient Receptor Potential (TRP) family of ion channels on bladder function[1]. Menthol is a nonselective agonist of the TRPM8 and its instillation into the bladder is suggested to activate bladder sensory afferents[2], whilst inhibiting muscarinic contractions of the detrusor smooth muscle, possibly through an inhibition of voltage-dependent calcium influx[3]. Clinical and experimental studies have implicated obesity as a major contributing factor for voiding dysfunction and overactive bladder (OAB)[3], but no studies evaluated the role of TRPM8 on bladder dysfunction. Therefore, we evaluated here the acute effects of menthol on a mouse model of OAB induced by obesity. **Methods:** C57BL/6 male mice fed for 12 weeks with standard chow or high-fat diet were used. Cystometry was performed in urethane-anesthetized mice. Briefly, bladders were filled at a constant rate (0.6 mL/h) with menthol solution (100 µM) or vehicle (saline-DMSO 0.1%) and intravesical pressure was recorded for 45 min. In separate experiments, bladders from lean and obese mice were mounted in organ bath chambers containing oxygenated Krebs solution. After 45 min of stabilization, menthol (100 µM), icilin (selective TRPM8 agonist; 1 µM) or vehicle (DMSO 0.1%) were added to the organ baths and allowed to incubate for 20 min. Cumulative concentration-response curves to potassium chloride (KCl; 1mM – 300mM) and muscarinic agonist carbachol (CCh; 0.001 - 100 µM), as well as electrical-field stimulation (EFS; 1 - 32 Hz)-induced contractions were obtained. **Results:** Cystometric studies showed the obese mice infused intravesically with vehicle showed irregular micturition pattern, characterized by significant increases in voiding and non-voiding contractions (NVCs) frequencies compared with lean mice ($P < 0.05$), all of which were significantly reduced by menthol infusion ($P < 0.05$). In isolated bladders, contractions induced by carbachol and potassium chloride were significantly higher in obese (E_{max}: 21.44 ± 2.8 and 19.1 ± 2.9 mN, respectively) compared with lean mice (E_{max}: 11.46 ± 1.0 and 10.6 ± 1.1 mN, respectively; $P < 0.05$). Additionally, the EFS-induced contractions were greater obese compared with lean mice ($P < 0.05$). Pre-incubation with menthol in obese mice largely reduced KCl, carbachol and EFS-induced contractions ($P < 0.05$), driving the responses to the levels of lean mice. In contrast, pre-incubation with icilin had no significant effect on none of contractile responses, suggesting that menthol-induced effects are independent of TRPM8 activation. **Conclusion:** Our findings show that of menthol ameliorates the bladder dysfunction seen in obese mice, through TRPM8-independent mechanisms, suggesting that menthol may be of beneficial value to treat OAB. **References: 1.** Andersson et al. *BJU Int*. 106: 1114, 2010. **2.** Ramos-Filho et al. *PLOS One.* 9: e111616, 2015. **3.** Richter HE et al. *J Urol*. 183: 622, 2010. **Financial Support:** FAPESP, CAPES. **Disclosures:** All experimental protocols were approved by the Animal Ethical Committee of UNICAMP (CEUA/UNICAMP, 4156-1).

07.015 Inhibition or deletion of toll like receptor 4 (TLR4) confers protection against cyclophosphamide-induced mouse cystitis. Oliveira MG[1], Calmasini FB[1], Alexandre EC[1], Mónica FZ[1], Soares AG[2], Costa SKP[2], Antunes E[1] [1]FCM-Unicamp – Pharmacology, [2]ICB-USP – Pharmacology

Introduction: Interstitial Cystitis/Bladder Pain Syndrome (IC/BPS) is a multifactorial chronic inflammatory disease characterized by suprapubic pain, discomfort, urgency and excessive urinary frequency, which profoundly impairs patient's quality of life. Despite efforts to understand the etiology of IC/BPS, this condition remains poorly understood, and therefore no fully effective treatment has been developed to date[1]. Changes in innate immune function contribute to the onset and progression of numerous chronic inflammatory diseases, and Toll-Like Receptors (particularly TLR4) are fundamental in this process[2]. However, there are still no studies in the literature addressing the contribution of TLR4 receptors in IC/BPS. The aim of the present study was to investigate the contribution of TLR4 signaling pathway in the development of cyclophosphamide (CYP)-induced cystitis. **Methods:** We performed experimental studies using C57BL/6 (wild-type, WT) and/or TLR4 knockout ($TLR4^{-/-}$) male mice (12 weeks old), treated with a single intraperitoneal injection of CYP (300 mg/kg) or saline (5 mL/kg, control), and then evaluated 24 h after injection[3]. In separated groups, WT animals were pre-treated with the TLR4 antagonist resatorvid (10 mg/kg, i.p.) 1 h prior CYP-injection. Measurements of TLR4, TRIF and MyD88 mRNA expressions were determined by RT-PCR. For micturition pattern analysis, mice were placed in cages individually and urine output was collected for 3 h on filter paper covering the cage bottom. Cystometry was performed in urethane-anesthetized mice. Briefly, bladders were filled at a constant rate (0.6 mL/h) and intravesical pressure was recorded for 45 min. **Results:** In isolated bladder of CYP-injected mice, RT-PCR analysis revealed significant increases ($P < 0.05$) of TLR4 itself and its downstream adaptor proteins MyD88 and TRIF ($P < 0.05$). In WT mice, the micturition pattern analysis showed a marked increase in the number of spots and a reduction of micturition volume in CYP-injected mice (9 ± 1.7 spots and 328 ± 38 µL) in comparison to saline group (2.4 ± 0.5 spots and 779 ± 56 µL). TLR4 deletion significantly reduced the number of spots (3.5 ± 0.9 spots, $P < 0.05$ in relation to CYP-injected WT mice) but had no effect in volume (262 ± 90 µL). The pre-treatement with resatorvid significantly reduced the spots and increased the micturition volume (1.7 ± 0.2 spots and 359 ± 98 µL). Cystometric studies showed significant changes in several micturition patterns in CYP-injected WT mice compared to saline ($P < 0.05$), including bladder capacity, micturition interval, voiding and NVCs frequencies, all of which were significantly prevented by TLR4 deletion ($P < 0.05$) or pharmacological inhibition ($P < 0.05$). **Conclusion:** Our results showed that CYP administration causes pronounced urinary bladder inflammation and overactivity, which was accompanied by an up-regulation of TLR4 signaling pathway. TLR4 deletion or pharmacological inhibition prevents CYP-induced cystitis and ameliorates voiding dysfunction. Thus, TLR4 receptors may provide a potential therapeutic target for IC/BPS. **References:** 1. Doggweiller R et al. *Neurourol Urodyn*. 26, 2016. 2. Schrepf A et al. *Pain*. 155 (9): 1755, 2014. 3. De Oliveira MG et al. *Am J Physiol Ren Physiol.* 311 (1): F85, 2016. **Financial Support:** FAPESP, CAPES. **Disclosures:** CEUA/UNICAMP nº 4121-1.

07.016 Deciphering Client Binding to PDILT, a Protein Disulfide Isomerase Required For Male Fertility. Alexander M[1], Benham A[1] - [1]Department of Biosciences, Durham University, UK

Introduction: Males require a specialised set of testis-specific chaperone proteins to ensure that sperm can bind to eggs (1). One of these proteins is an atypical Protein Disulfide Isomerase homologue called Protein disulfide isomerase-like protein of the testis (PDILT). PDILT lacks the catalytic cysteines present in the PDI redox active sites and doesn't have oxido-reductase activity (2). However, PDILT does have two cysteines, at position 135 and 420, that are conserved across species, suggesting that these residues are key for PDILT function. PDILT also possesses an N-terminal insertion and a C-terminal extension (2). When PDILT is knocked out in mice, their sperm fail to migrate and to bind to the zona pellucida of the female egg, impacting male fertility (3). The aim of this work is to understand how the structure of PDILT is able to facilitate its function using a mutagenesis and transfection approach. **Methods:** cDNAs were either generated in house by cloning and site-directed mutagenesis (1) or obtained as gifts. HT1080 cells were grown in 6-cm dishes and then transfected with a range of PDILT cDNAs including PDILT-wild-type, PDILT-C135A, PDILT-C420A, PDILT C135A/C420A, PDILT-DEL498-580, PDILT-DEL520-580, PDILT-DEL498-580/C135A/C420A and PDILT-DEL520-580/C135A/C420A. The transfectants were lysed and then analysed by SDS-PAGE and western blotting under both reducing and non-reducing conditions. For immunofluorescence analysis, HT1080 cells were also grown on coverslips and transfected with PDILT constructs. These coverslips were permeabilized, fixed, incubated with PDILT primary antibodies, the relevant secondary antibodies and costained with DAPI. These coverslips were then mounted onto slides with Vectashield and analysed by fluorescence/confocal microscopy. **Results:** SDS-PAGE and western blotting data show that residues C135 and C420 of PDILT govern the proteins' ability to form high molecular weight complexes with partner proteins. The two cysteine-to-alanine single substitutions caused a decrease in the range of complexes, with the dual substitutions causing a complete abolishment. The C-terminal tail-domain deleted mutants interacted with a wider range of clients. When the two cysteines are substituted along with the tail deletion, no complexes formed. The immunofluorescence data confirms that PDILT and the PDILT mutants localize to a perinuclear site, consistent with the ER. **Conclusion:** The substitution of C135 and C420 for alanine in PDILT caused a loss of disulfide–dependent protein interaction, suggesting that these two cysteines residues are important for client binding. Data from the C135A/C420A PDILT mutant show that these two cysteine residues are necessary and sufficient for all detectable disulfide-dependent interactions between PDILT and other proteins. The C-terminal tail domain deletion mutants were trapped more strongly than wild-type PDILT with partner proteins. This result suggests that the C-terminal tail domain regulates the ability of client proteins to interact with the PDILT protein and may prevent spurious interactions with bystander proteins. Future experiments will identify the PDILT interacting partners by mass spectrometry. References: 1) Ikawa M, et. al. J Clin Invest. 120: 984-94 (2010), 2) van Lith et. al. J. Biol. Chem. 280: 1376 (2004), 3) van Lith et. al. MBC. 18: 2795 (2007), 4) Tokuhiro et. al. PNAS. 109: 3850 (2012).