Symposium

Peripheral and Central Regulation of Energy and

Fluid Tonicity Homeostasis

UNIVERSITY OF SÃO PAULO

RIBEIRÃO PRETO MEDICAL SCHOOL

October 04 - 06, 2017

Ribeirão Preto, SP, Brazil

Symposium
Peripheral and Central Regulation of Energy and Fluid Tonicity Homeostasis

Ribeirão Preto, SP, Brazil, October 04-06, 2017

UNIVERSITY OF SÃO PAULO
President: Marco Antonio Zago

RIBEIRÃO PRETO MEDICAL SCHOOL
Dean: Margaret de Castro

DEPARTMENT OF PHYSIOLOGY
Chair: Rubens Fazan Junior

GRADUATE PROGRAM IN PHYSIOLOGY
Coordinator: Luiz Carlos Carvalho Navegantes

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SYMPOSIUM
PERIPHERAL AND CENTRAL REGULATION OF ENERGY AND FLUID TONICITY HOMEOSTASIS

Purpose: This symposium is focused on the integrated autonomic, neuroendocrine and behavioral mechanisms involved in the control of energy and fluid tonicity homeostasis.

Target Audience: Undergraduates, PhD and master students, and post-doctoral fellows in the biomedical sciences.

Invited speakers: Scientists from Brazil, Argentina, Canada, Germany and the United States.

Argentina – (Univ. of Cordoba and La Plata)
Laura M. Vivas

Brazil (USP – Ribeirão Preto, USP-SP, UNICAMP and UNESP)
André de Souza Mecawi (UFRRJ)
Adriana Torsoni (UNICAMP).
Beatriz Borges (FMRP – USP)
José Donato Junior. (ICB-USP)
Jose Vanderlei Menani. (UNESP – Araraquara)
Laurival A. De Luca Jr. (UNESP – Araraquara)
Lício A Velloso (UNICAMP)
Lucas Debarba: (FMRP – USP)
Mariana Kiomy Osako (FMRP-USP).
Melina Pires. (FMRP-USP)
Renata Frazao. (ICB-USP)
Rodrigo Rorato (FMRP-USP)
Silvia G. Ruginsk (UNIFAL – MG)

Canada (McGill University Health Center)
C.W. Bourque – (Montreal General Hospital).

Mexico
Limei Zhang, PhD. (Univ. Nacional Autónoma of Mexico)

Germany
Sophie M. Steculorum. (Cologne, Germany).

United States of America
Malcolm J. Low. (University of Michigan).
Wolfgang Liedtke – (Duke University Medical Center).
Carol F Elias – (Universty of Michigan).
Carey Lumeng – (University of Michigan).
Darleen Sandoval – (University of Michigan).
David Garcia-Galiano – (University of Michigan)
Nicole Bellefontaine. (University of Michigan).
Willis K Samson – (University of St. Louis).

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SYMPOSIUM: " PERIPHERAL AND CENTRAL REGULATION OF ENERGY AND FLUID TONICITY HOMEOSTASIS"
(October 04-06, 2017)

Day October 04th - Anfiteatro Renato H. Migliorini.	
16h 18h	**Onsite registration** **Opening session** Margaret de Castro – Dean FMRP Rubens Fazan – Chairman od Dept. of Physiology J. Antunes- Rodrigues and Carol Elias – Organizing Committee **Plenary lecture:** Chair: Carol Elias. University of Michigan. **Malcolm Low.** University of Michigan. Hypothalamic integration of external and internal environments in the control of energy homeostasis
Day October 05th– Anfiteatro Pedreira de Freitas.	
Symposium 1: 8:00h -10:00h **Central regulation of energy homeostasis**	Chair: Lucila L K Elias(FMRP-USP) **Malcolm J. Low.** University of Michigan. New insights on the role of POMC neurons in energy homeostasis **Rodrigo C. Rorato.** FMRP-USP**.** DREADDs reveal arcuate AgRP/NPY neurons control both fasting-induced suppression of the HPT axis and activation of hepatic TH metabolism. **Sophie M. Steculorum**. Department of Neuronal Control of Metabolism, Max Planck Institute for Metabolism Research, Germany. Novel regulator of the central control of feeding and systemic insulin sensitivity. **Lucas DeBarba.** FMRP-USP. Non-Neuronal Cells and Nutritional Programming
Coffee break – 10:00h 10:15h	
Symposium 2: 10:15h -12:15h **Physiology of osmoregulation**	Chair: Charles W. Bourque (McGill University Health Center, Montreal General Hospital) **LA De Luca Jr.; JV Menani** – UNESP – Araraquara: Sodium loading induces similar effects on Fos immunoreactivity in the hindbrain associated with opposite effects on sodium intake. **Wolfgang Liedtke** - Departments of Neurology, Anesthesiology and Neurobiology. Duke University Medical Center - USA. Central regulation of fluid and tonicity by micro-RNAs.

	Wagner Luis Reis. FMRP – USP. Central apelin effects on drinking behavior and neuroendocrine hormones release: involvement of CO and NO functions
Lunch – 12:15h – 13:45h	**Poster session**

Symposium 3: 14:00h -16:00h **Peripheral regulation of energy homeostasis**	Chair: Márcio Torsoni (UNICAMP) **Carey Lumeng** (University of Michigan) - Dynamic of adipose tissue leukocytes contributes to tissue inflammation and metabolic disease. **Darleen Sandoval** (University of Michigan) – Using Genetics and Pharmacology to Understand GLP-1 Physiology. **Mariana Kiomy Osako** (FMRP-USP). RANKL system in adipose tissue **Lício A Velloso** (UNICAMP) -Nutritional factors impact on hypothalamic control of whole body energy homeostasis.
Coffee break – 16:00h 16:15h	
16:30 – 17:30h	**Oral session** **Cafarchio EM, Vale B, Silva DS, Silva LA, Sato MA.** Extracellular and intracellular dehydration affect urinary bladder reactivity to vasopressin and neurotransmitters of the autonomic nervous system in anesthetized female Wistar rats. Dept. Morphology and Physiology, Faculdade de Medicina do ABC, Santo Andre, SP. **Cruz-Machado SS, Pereira EP, Oliveira AP, Trevisan I, Silva-Sousa E, Markus RP**. Short-term high-fat diet feeding (HFD) induces inflammation and decreases nocturnal synthesis of melatonin. Laboratory of Chronopharmacology, Department of Physiology, Institute of Biosciences, USP. **Matsuo F S, Queiroz MS, Mota RF, Araújo PHC, Ferreira KCOS, Metzner RJ M, Osako MK.** Study of RANKL/RANK/OPG signaling pathway in beige adipose tissue differentiation. Department of Molecular and Cell Biology, FMRP-USP. **Ramos-Lobo AM, Teixeira PDS, Furigo IC, Lima AM, Donato Jr J.** Leptin absence in early life causes long-term disturbances in energy balance that cannot be completely restored by early intervention. Dept. Physiology, Institute of Biomedical Sciences, USP.
17:45h **Plenary lecture**	Chair: José Antunes Rodrigues (FMRP-USP) **C.W. Bourque.** Montreal General Hospital. Clock-driven vasopressin neurotransmission mediates anticipatory thirst prior to sleep

Day : October 06th - Anfiteatro Renato H. Migliorini.	
Symposium 4: 8:00h -10:00h **New Techniques to interrogate physiology** Round table	Chair: Carol F. Elias - University of Michigan - and Beatriz C. Borges. FMRP – USP. **Beatriz Borges –** FMRP – USP. Leptin effects on hippocampus: a behavior, molecular and morpho-functional study **.** **André de Souza Mecawi (**UFRRJ) Angiotensin II at the subfornical organ: new evidences on metabolic control. **Nicole Bellefontaine**. University of Michigan. Calcium imaging for the study of leptin signaling in the reproductive neuroendocrine axis. **José Donato Junior**. ICB-USP.– When the name does not tell you everything: central regulation of energy and glucose homeostasis by growth hormone
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Symposium 5 10:15h-12:15h **Metabolic Control of Reproduction and Growth**	Chair: Carol F Elias – Universty of Michigan **David Garcia-Galiano**. University of Michigan. PI3K signaling in reproduction and growth. **Renata Frazao**. ICB-USP. Somatotropic axis and reproductive function
12:15h -14h	Lunch **Meet the Professor**
Symposium 6 14:00h – 16:00h **Vasopressin Peptides: Osmoregulation and Beyond**	Chair: WK Samson (Saint Louis University School of Medicine) **Limei Zhang**. National Autonomous University of Mexico. Hypothalamic vasopressinergic and orexinergic pathways converge with midbrain aminergic inputs on LHb intrinsic GABAergic system regulated by neurosteroid signalling: implications for contingency-dependent motivated behavior. **WK Samson.** Saint Louis University School of Medicine**.** Novel mechanisms regulating vasopressin secretion. **Melina Pires**. FMRP-USP. Nitrergic Modulation of Magnocellular Neurons from Supraoptic Nucleus. **Silvia Ruginsk.** UFAL**:** Astrocytes as targets for the central control of body fluid homeostasis.
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Symposium 7 16:15h – 17:45 **Metabolic and Cardiovascular Programming**	Chair: Andre Mecawi (UFRRJ) **Laura Vivas**. CONICET- Cordoba, Argentina. Sex Chromosome Complement and gonadal steroid influences in the sexually dimorphic vasopressinergic system. **Adriana Torsoni** (UNICAMP). Maternal obesity and microRNAs associated with fatty liver disease.
18:00h Concluding Remarks and Closing	All participants

POSTER SESSION

POSTER # 1

ESTROGEN EFFECT IN THE BROWNING OF WHITE ADIPOSE TISSUE

Queiroz, M. S[1].; Carvalho, A. J. R.[1]; Matsuo, F. S.[1]; Ferreira, K. C. O. S.[1]; Mota, R. F.[1]; Araújo, P. H. C[1]; Metzner, R. J. M.[1]; Osako, M. K[1].

[1] Department of Molecular and Cell Biology, Ribeirao Preto Medical School, University of São Paulo

POSTER # 2

LEPTIN ABSENCE IN EARLY LIFE CAUSES LONG-TERM DISTURBANCES IN ENERGY BALANCE THAT CANNOT BE COMPLETELY RESTORED BY EARLY INTERVENTION

Ramos-Lobo, AM, Teixeira, PDS, Furigo, IC, Lima, AM, Donato Jr, J.

Dept. Physiology, Institute of Biomedical Sciences, University of São Paulo, Brazil.

POSTER # 3

INTERACTION OF GLUCOSE CONCENTRATION AND ANGIOTENSIN II IN SUBFORNICAL ORGAN.

[1]Paes-Leme, B, [2]Ferguson, AV, [1]Mecawi, AS

1Dept of Physiological Sciences, Federal Rural University of Rio de Janeiro, Seropédica, Brazil; 2Centre for Neuroscience, Queen's University, Kingston, Canada;

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[1]Souza, C.F. *; [1]Stopa, L.R.S.; [1]Santos.G.F.; [1]Takasumi, L.C.N.; [1]Martins, A.B. ; [1]Garnica-Siqueira, M.C.; [2]Zaia, D.A.M.; [1]Zaia, C.T.B.V.; [1]Uchôa, E.T.

[1]Department of Physiological Sciences, State University of Londrina, Londrina, PR, Brazil. [2]Department of Chemistry, State University of Londrina, Londrina, PR, Brazil

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Guillen, C.D.; De Luca Jr., L.A.; Menani, J.V. Andrade, CAF. Department of Physiology and Pathology, School of Dentistry, São Paulo State University (Unesp), Araraquara, SP, Brazil.

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EXTRACELLULAR AND INTRACELLULAR DEHYDRATION AFFECT URINARY BLADDER REACTIVITY TO VASOPRESSIN AND NEUROTRANSMITTERS OF THE AUTONOMIC NERVOUS SYSTEM IN ANESTHETIZED FEMALE WISTAR RATS.

[1]Cafarchio, EM, [1]Vale, B, [1]Silva, DS; [1]Silva, LA, [1]Sato, MA

[1]Dept. Morphology and Physiology, Faculdade de Medicina do ABC, Santo Andre, SP, Brazil.

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[1] Department of Physiology and Pathology, School of Dentistry, São Paulo State University - UNESP, Araraquara, São Paulo, Brazil
[2] Department of Physiological Sciences, Biological Sciences Institute, Federal University of Goiás, Goiânia, Goiás, Brazil

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Pereira Jr, E.D.; Dantas, R.M.; Andrade-Franzé, G.M.F.; De Luca Jr., L.A.; Menani, J.V. Andrade, CAF. Department of Physiology and Pathology, School of Dentistry, São Paulo State University (Unesp), Araraquara, SP, Brazil.

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[1] Department of Molecular and Cell Biology, Ribeirao Preto Medical School, University of Sao Paulo

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Couto L Gisele, Donato J,
Department of Physiology and Biophysics USP

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[1]Delfino, H.B.P.; [1]Garófalo, M.A.R., [1]Zanon, N.M.; [1,2]Kettelhut, I.C.; [1]Navegantes, L.C.C.
Departments of [1]Physiology and [2]Biochemistry/Immunology. Ribeirao Preto Medical School, University of Sao Paulo. Ribeirao Preto, SP, Brazil.

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[1] Furigo, IC, [1] Teixeira, PDS, [1] Souza, GO, [1] Ramos-Lobo, A , [2] List, E, [2] Kopchick, J, [1] Donato Jr, J.
1 Department of Physiology and Biophysics, University of São Paulo, São Paulo, Brazil; 2 Edison Biotechnology Institute, Ohio University, Ohio, United States.

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Department of Physiology and Pathology, Dentistry School, São Paulo State University, UNESP, Araraquara, SP, Brazil, Department of Physiology and Biophysics, Institute of Biomedical Science, University of Sao Paulo, USP, Sao Paulo, SP, Brazil.

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Araújo, P.H.C., Queiroz, M.S., Mota, R.F., Matsuo, F.S., Metzner, R.J.M., Ferreira, K.C.O.S., Osako, M.K.

Department of Cell and Molecular Biology

Ribeirao Preto Medical School, University of Sao Paulo

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Teixeira, PDS[1]; Furigo, IC[1]; Donato Jr, J [1]

[1]Department of Physiology and Biophysics, ICB/USP, Sao Paulo – Brazil

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Mota, R.F., Queiroz, M.S, Araújo, P.H.C, Metzner, R.J.M, Matsuo,F.S, Ferreira, K.C.O.S, Osako, M.K. Department of Cell and Molecular Biology, Medical School of Ribeirao Preto – University of Sao Paulo

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SHORT-TERM HIGH-FAT DIET FEEDING (HFD) INDUCES INFLAMMATION AND DECREASES NOCTURNAL SYNTHESIS OF MELATONIN

Sanseray da Silveira Cruz-Machado, Eliana P Pereira, Aldeídia Pereira de Oliveira, Isabela Trevisan, Ewerton Silva-Sousa, Regina P Markus

Laboratory of Chronopharmacology, Department of Physiology, Institute of Biosciences, University of Sao Paulo

POSTER # 1

ESTROGEN EFFECT IN THE BROWNING OF WHITE ADIPOSE TISSUE

Queiroz, M. S[1].; Carvalho, A. J. R.[1]; Matsuo, F. S.[1]; Ferreira, K. C. O. S.[1]; Mota, R. F.[1]; Araújo, P. H. C[1]; Metzner, R. J. M.[1]; Osako, M. K[1].
[1] Department of Molecular and Cell Biology, Ribeirao Preto Medical School, University of São Paulo

Sexually dimorphic deposition of fat is associated with the development of obesity-associated pathologies. While the accumulation of visceral white adipose tissue in males is detrimental to metabolic health, the accumulation of subcutaneous white adipose tissue in females seems protective. Menopause is associated with adiposity increase and a higher risk of metabolic diseases, and our preliminary data showed an increase in beige adipose tissue in young female compared to age-matched male. Therefore, the current project has the objective of analyzing the effects of estrogen in the adipose tissue browning. Our histological images showed that female mice have accentuated browning of white adipose tissue compared to males, and it was correlated with gene expression of beige adipocyte markers. Ovariectomized mice showed no beige adipose tissue and increased adiposity. Body weight of these ovariectomized mice was also elevated. Beyond that, gene expression analysis showed decreased expression of PPARϒ, PRDM16 and PGC1α, genes related to thermogenesis. In vitro analysis using 3T3-L1 murine cells showed increased expression of PPARϒ under estrogen stimulation, as well as a rise of PRDM16 and PGC1α. ISince the thermogenic signaling pathway could lead to the activation of ERK and p38 MAPK pathways, we found that estrogen may increase p-ERK but not p-p38. We also investigated the possible receptors activated by estrogen through ERα and ERβ agonists, and higher gene expression of PPARϒ was observed with ERβ agonist stimulation. Together these data suggest an effect of estrogen in the browning of white adipose tissue, making for a possible therapeutic target for future treatments in obesity related diseases.

POSTER #2

LEPTIN ABSENCE IN EARLY LIFE CAUSES LONG-TERM DISTURBANCES IN ENERGY BALANCE THAT CANNOT BE COMPLETELY RESTORED BY EARLY INTERVENTION

Ramos-Lobo, AM, Teixeira, PDS, Furigo, IC, Lima, AM, Donato Jr, J.
Dept. Physiology, Institute of Biomedical Sciences, University of São Paulo, Brazil.

The adipocyte-derived hormone leptin is essential for the regulation of energy balance. However, studies have suggested that in early life leptin functions are associated with neuronal development and dependent of a critical neonatal period.
To determine the effects in adulthood of early absence of leptin signaling a LoxP-flanked transcription-blocking cassette was inserted in the Lepr gene to generate mice null for the leptin receptor. They were bred with animals expressing Cre-ERT2 fusion protein under the human ubiquitin C promoter sequence. Consequently, tamoxifen injections can induce Cre Recombinase activity and restore Lepr gene expression in adult or young Ubi-LepRNull mice.
To validate the model we assessed the central response to an acute leptin injection. LepRNull mice exhibited no leptin responsive cells, the neural projections from the arcuate nucleus (ARH) to the paraventricular nucleus were disrupted and were both restored in Ubi-LepRNull mice, as was their response to leptin.
Adult LepRNull and Ubi-LepRNull mice were morbidly obese and hyperphagic. Six weeks after tamoxifen treatment, Ubi-LepRNull mice restored normal food intake and partially restored body weight, adiposity, energy expenditure and locomotor activity. They were more glucose tolerant because of higher insulinemia. Their brain mass was partially recovered compared to Ubi mice.
When reactivated at weaning, Ubi-LepRNull mice showed normal body weight, food intake and glycemic homeostasis, but had lower energy expenditure, locomotor activity and lighter brain mass than Ubi mice.
These results suggest that absence of leptin signaling in early life causes long-term changes in energy balance that depend on a critical period.

Financial support: FAPESP 2014/11752-6 and CAPES

POSTER # 3

INTERACTION OF GLUCOSE CONCENTRATION AND ANGIOTENSIN II IN SUBFORNICAL ORGAN.

[1]Paes-Leme, B, [2]Ferguson, AV, [1]Mecawi, AS
1Dept of Physiological Sciences, Federal Rural University of Rio de Janeiro, Seropédica, Brazil; 2Centre for Neuroscience, Queen's University, Kingston, Canada;

Angiotensin II (ANGII) is an octapeptide with marked actions in the subfornical organ (SFO). Although it's classically involved in hydroelectrolytic and in cardiovascular control, ANGII has recently been related to control of food ingestion. Proportion of responsive dissociated SFO neurons to ANGII in electrophysiological studies is increased higher the glucose concentration of incubation and recording solutions. We aimed to investigate if SFO cells incubation in different glucose concentrations could change the expression of AT1 receptors. Male Sprague-Dawley rats (100-175g) had SFO microdissected, dissociated and incubated at 1mM, 5mM and 10mM glucose concentration Neurobasal A solution for 1h, 6h and 24h. SFO cell cultures were then submitted to mRNA extraction and RT-qPCR for angiotensin receptor (AT1). Two-way ANOVA was used for statistical analysis (data shown as mean±SEM). No difference was observed between the three distinct glucose concentration in the same time group. Nonetheless, in all glucose concentration groups it was found significant decrease between at least the times of one and 24 hours [1mM: 1.533±0.186 vs 0.451±0.098 2ΔΔCT, p=0.0002 (n=5); 5mM: 1.088±0.208 vs 0.236±0.84 2ΔΔCT, p=0.0057 (n=5 and 4, respectively); 10mM: 1.448±0.358 vs 0.634±0.175 2ΔΔCT, p=0.0084 (n=5 and 4, respectively)]. It doesn't mean that there was a decrease in AT1 expression on the SFO cells after 24h, but it could be reflected on protein expression after the one-day period. Nevertheless, other technique could be assessed for quantifying expression of AT1¬. As glucose concentration doesn't change mRNA for AT1, mechanisms through how glucose may alter ANG response in SFO neurons are most probably acute.

Financial support: Cooredenação de aperfeiçoamento de pessoal de nível superior (Capes), Canadian Institutes of Health Research (CHIR)

POSTER # 4

ESTRADIOL PROTECTS AGAINST OVARIECTOMY-INDUCED SUSCEPTIBILITY TO THE ANABOLIC EFFECTS OF PROLONGED TREATMENT WITH CORTICOSTERONE IN RATS

[1]Souza, C.F. *; [1]Stopa, L.R.S.; [1]Santos.G.F.; [1]Takasumi, L.C.N.; [1]Martins, A.B. ; [1]Garnica-Siqueira, M.C.; [2]Zaia, D.A.M.; [1]Zaia, C.T.B.V.; [1]Uchôa, E.T.
[1]Department of Physiological Sciences, State University of Londrina, Londrina, PR, Brazil. [2]Department of Chemistry, State University of Londrina, Londrina, PR, Brazil

Both corticosterone and ovariectomy (OVX) increases appetite and body weight gain in rats, and estrogen replacement attenuates OVX-induced changes. This study aimed to evaluate the effects of ovariectomy and the protective role of estradiol on the responses induced by prolonged treatment with corticosterone on energy homeostasis. In water treated animals, there was an increase on body weight gain, mean daily food intake, LEE index, plasma cholesterol and triglycerides in OVX group compared to SHAM. In corticosterone treated animals, compared to SHAM animals, OVX group showed enhanced body weight gain, mean daily food intake, LEE index, retroperitoneal adipose tissue and plasma cholesterol. Estradiol reduced body weight gain, LEE index, area under curve of glucose tolerance test (AUC GTT), retroperitoneal and perirenal+perigonadal adipose tissues, while it increased mean daily fluid intake , plasma triglycerides, plasma free fatty acid and weight of uterus. Compared to water, corticosterone treatment increased only plasma triglycerides in SHAM group, however this glucocorticoid increased body weight gain, mean daily food intake, retroperitoneal adipose tissue, plasma triglycerides, mean daily fluid intake, with no effects on perirenal + perigonadal adipose tissues, LEE index, plasma free fatty acids (FFA) and triglycerides, as well as AUC GTT in OVX group. In the OVX+E only mean daily food intake was enhanced by corticosterone. These data demonstrate that protection against glucocorticoids-induced anabolic responses in females is eliminated by ovariectomy and estradiol can reverse part of these parameters, suggesting that estradiol has a protective role on the metabolic responses induced by corticosterone in ovariectomized rats.

POSTER #5

PARTICIPATION OF ALPHA$_2$-ADRENERGIC RECEPTORS OF THE LATERAL PARABRACHIAL NUCLEUS IN THE CONTROL OF SODIUM APPETITE IN SPONTANEOUSLY HYPERTENSIVE RATS

Guillen, C.D.; De Luca Jr., L.A.; Menani, J.V. Andrade, CAF. Department of Physiology and Pathology, School of Dentistry, São Paulo State University (Unesp), Araraquara, SP, Brazil.

Excessive salt intake has been associated with the development or worsening of chronic diseases such as hypertension. Spontaneously hypertensive rats (SHR) have a typical increased sodium preference. The lateral parabrachial nucleus (LPBN) is an important inhibitory area for sodium intake control. In normotensive rats, α_2-adrenergic receptor activation of the NPBL increases 0.3 M NaCl intake induced by intra- and extracellular dehydration. However, it is still unknown whether α_2-adrenergic mechanisms of the NPBL influences sodium appetite in SHR. Here we used SHR (n = 11) and normotensive Holtzman rats (HTZ, n = 7) (290–310 g), with stainless steel guide cannulas implanted bilaterally in the LPBN. Rats with 24 h of water deprivation (WD) had access to only water for partial rehydration (PR), or WD-PR, before 2-h access to 0.3 M NaCl (sodium appetite test). Rats received bilateral LPBN moxonidine injections (α_2-adrenergic/imidazolinic receptor agonist, 0.5 nmol/0.2 µl) or vehicle 15 min before the beginning of the sodium appetite test. Moxonidine injected in SHR increased 0.3 M NaCl intake (15.8 ± 3.7, vs. vehicle: 5 ± 1.5 ml/120 min), without changing water intake (6.6 ± 1.5, vs. vehicle: 3.2 ± 1.2 ml/120 min). In HTZ moxonidine did not change 0.3 M NaCl intake (6.7 ± 4.3, vs. vehicle: 0.5 ± 0.3 ml/120 min) or water intake (5.4 ± 3.5, vs. vehicle: 3.7 ± 2 ml/120 min). The present results show that activation of α_2-adrenergic receptors in the LPBN increased the sodium appetite induced by WD-PR in SHR, suggesting that inhibitory mechanisms on LPBN contribute to limit sodium intake in spontaneously hypertensive rats.
Financial Support: CNPq, FAPESP

POSTER #6

EXTRACELLULAR AND INTRACELLULAR DEHYDRATION AFFECT URINARY BLADDER REACTIVITY TO VASOPRESSIN AND NEUROTRANSMITTERS OF THE AUTONOMIC NERVOUS SYSTEM IN ANESTHETIZED FEMALE WISTAR RATS.

[1]Cafarchio, EM, [1]Vale, B, [1]Silva, DS; [1]Silva, LA, [1]Sato, MA
[1]Dept. Morphology and Physiology, Faculdade de Medicina do ABC, Santo Andre, SP, Brazil.

Aim: Previous studies have shown that vasopressin increases urinary bladder contractility and intravesical pressure. Vasopressin is release during hypovolemic states. In this study we investigated if extracellular and intracellular dehydration affect urinary bladder reactivity to vasopressin and neurotransmitters of the autonomic nervous system in female Wistar rats.
Methods: Adult female Wistar rats (N=6/group) were submitted to 24-hours water deprivation or to S.C. injection of furosemide (50 mg/Kg) with low sodium diet and water or maintained normohydrated (control). Afterwards, rats anesthetized with 2% isoflurane in 100% O_2 underwent to cannulation of the femoral artery for mean arterial pressure and heart rate recordings. The urinary bladder was cannulated for intravesical pressure (IP) measurement. After baseline recordings of the physiological parameters for 15 min, drugs (vasopressin 1 ng/mL, or acetylcholine 2 ug/mL, or noradrenaline 2 ug/mL, or saline) were randomly administrated *in situ* (0.1 mL) on the urinary bladder and all the parameters were recorded for 15 min. Data are as mean±SE and were submitted to paired Student t-test ($p<0.001$).
Results: The water-deprivated group responses in IP to vasopressin (39.5±1.0% vs. 110.1±1.9% control), acetylcholine (125.9±12.4% vs. 488.8±24.9% control), and noradrenaline (-17.5±1.0% vs. -67.6±1.6% control) were attenuated. Similarly, the furosemide group responses in IP to vasopressin (42.8±2.8%), acetylcholine (145.0±5.0%), and noradrenaline (-28.8±1.0%) were reduced compared to control group. No difference was observed in the responses to saline.
Conclusion: Extracellular and intracellular dehydration decreased the urinary bladder reactivity to vasopressin and to the neurotransmitters released by the autonomic nervous system innervation on the urinary bladder.

Financial Support: FAPESP, CAPES and NEPAS.

POSTER #7

IMPORTANCE OF CAROTID BODY AND FOREBRAIN MECHANISMS FOR SYMPATHETIC AND VENTILATORY RESPONSES TO ACUTE SODIUM LOAD

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Hypertension is associated with dysfunction in the mechanism responsible for the salt-sensitive regulation of sympathetic outflow. In the present study, we investigated the relative contribution of the carotid bodies and forebrain for the autonomic and respiratory adjustments to acute NaCl load. Arterially perfused *in situ* preparations of male Holtzman rats (60-100 g, n = 5-16/group) were used. Intra-arterial saline infusions (0.17; 0.3; 0.7; 1.5 and 2 mol/L NaCl; 200 µL during 20 seconds each) or mannitol infusions (0.3, 0.5, 1, 2.7 and 3.8 mol/L) were performed in accumulative ascending order while thoracic sympathetic, phrenic nerve and carotid sinus nerve activities were recorded. The 2 mol/L NaCl load slightly increased phrenic burst frequency (5.8 ± 0.9 bpm, *vs.* Ringer: 0.4 ± 0.2 bpm, $p<0.05$) and marked enhanced sympathetic (63.3 ± 8.4%, *vs.* Ringer: -0.8 ± 1.9%, $p<0.05$) and carotid sinus nerve activities (105.1 ± 13.2%, *vs.* Ringer: -0.2 ± 1.3%, $p<0.05$), whereas mannitol load produced no change in these activities. Carotid body removal attenuated the sympathoexcitation (26.2 ± 4.9%, $p<0.05$), but not the tachypnea (3.6 ± 0.5 bpm) induced by NaCl load. The forebrain disconnection at the pre-collicular level completely abolished the sympathoexcitation (8.4 ± 3.7%, $p<0.05$) and the increase in phrenic burst frequency (1.2 ± 0.4 bpm, $p<0.05$) in response to NaCl load. The results suggest that carotid bodies are activated and in addition to forebrain mechanisms contribute for the autonomic responses to acute NaCl challenges, whereas the tachypnea is mostly mediated by forebrain mechanisms.
Support: Capes, CNPq, Fapesp

POSTER # 8

ESTROGEN INFLUENCE ON SODIUM PALATABILITY IN HYPERTENSIVE FEMALE RATS.

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Excessive salt intake has been associated with the development or worsening of chronic diseases such as hypertension. Spontaneously hypertensive rats (SHR) have a typical increased sodium preference. Estrogen influences arterial pressure and sodium appetite, but we do not know how much it influences sodium palatability, particularly in female SHR. Here we evaluated the influence of estrogen on sodium palatability of female SHR (12 months of age), which were treated with estrogen [ECP, estradiol cypionate (2 mg/ml), 0.1 ml i.m., Zoetis), (n = 12)] or oil vehicle (VEH; 0.1 ml i.m., n = 7) every 3 days, during 12 days. The efficacy of ECP treatment was confirmed by uterine index (ECP: 599.3 ± 60.1 vs. VEH: 315.5 ± 49.5 mg/100 g b. w.). Rats with 24 h of water deprivation (WD) had access to water only for partial rehydration (PR), or WD-PR protocol. Then, the animals had 1 h access to 0.3 M NaCl and water (sodium appetite test). We monitored sodium palatability by videotaping hedonic and aversive orofacial motor responses to minute intra-oral infusions of 0.3 M NaCl (IO-NaCl) immediately prior and after to the sodium appetite test. Prior to sodium access, ECP decreased hedonic responses (162 ± 22, vs. VEH: 243 ± 15/min) and increased aversive responses (16.8 ± 3.4, vs. VEH: 3.8 ± 2.3/min) to IO-NaCl. ECP decreased sodium intake (1.9 ± 0.4 vs. vehicle: 3.3 ± 0.4 ml/60 min/100 g b.w.), without changes in hedonic (148 ± 34 vs. VEH: 195 ± 32/min) or aversive responses (7 ± 3, vs. VEH: 3 ± 2/min). The results suggest that estrogen has an inhibitory effect on sodium palatability thereby influencing sodium appetite.

Supported by: CNPq, FAPESP.

POSTER #9

EPILEPSY AND SECRETION OF HORMONE INVOLVED WITH HYDROELETROLITIC: WHAT IS THEIR RELATIONSHIP?

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Epilepsy is a syndrome that results in a diverse range of symptoms including seizures. These can affect several systems, but not all are well elucidated, among which is an interaction with hormones related to hydroelectrolyte balance. Thus, the objective of our study is to evaluate the relationship between mesencephalic and limbic seizures in WAR mice with secretion of the hormones involved in the hydroelectrolytic balance.
Male wistar rats and WAR (90 days) were divided into groups with or without audiogenic kindling. This consists in subjecting the animal to acoustic stimulation for 1 minute or until the animal presents a generalized tonic-clonic seizure. This is done twice a day for 10 days. After this protocol, behavioral evaluations were performed according to the Categorized Severity Index and according to the Severity Index of Limbic Crises according to Racine and then the animals were euthanized and blood was collected for hormonal dosing.
No significant result was found for any hormone tested, but in relation to ANP there is a trend of the curve to be smaller in WAR kindling group, as a higher concentration of OT and AVP in these group. Also we cannot find significant results in WAR mesencephalic and limbic groups.
In this study it was not possible to obtain any significant results of the hormones analyzed, but for the future one hopes to repeat the experiment by increasing the number of animals to verify if the trends of the graphs are confirmed.
Financial support: PIBIC

POSTER #10

STUDY OF RANKL/RANK/OPG SIGNALING PATHWAY IN BEIGE ADIPOSE TISSUE DIFFERENTIATION

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RANKL system is classically involved in bone metabolism, share common points of regulation in the thermogenic pathway, but its role in browning of white adipose tissue has not yet been explored. Thus, the purpose of this project is to clarify the molecular mechanism of RANKL system in beige adipose tissue differentiation. Histological analysis of 5-week-old OPG knockout (OPG-/-) mice showed increased browning of subcutaneous WAT compared to heterozygous and wild-type (WT) mice. Process of whitening was also observed in brown adipose tissue of WT mice group compared to OPG-/- mice. We confirmed the effect of RANKL by in injecting soluble recombinant RANKL by mini-osmotic pump in WT mice, and besides the increase in beige adipocytes, this group also showed increased insulin sensitivity compared to non-treated group. These data show a remarkable role of RANKL in beige adipocyte differentiation *in vivo*. To investigate the molecular mechanism, we differentiated 3T3L1 to mature adipocyte, and found induction of UCP1 and PGC1α gene expression after treatment with RANKL by real time PCR. We also confirmed higher expression of PGC1α in primary culture cells from stromal vascular fraction of adipose tissue from OPG-/- mice compared to those from WT mice. In the classical thermogenic pathway, p38 MAPK phosphorylates and activates Atf2 and PGC1α that induces the transcription of UCP1. We showed through western blot analysis the activation of the protein p38-MAPK by RANKL. So far our data show that RANKL is directly involved in browning of adipocytes, inducing UCP1 and PGC1αexpression, probably through p38-MAPK activation.
Keywords: Browning, Obesity, RANKL **Financial support:** CAPES/FAPESP

POSTER #11

THE IMPORTANCE OF GROWTH HORMONE'S ACTION ON NPY/AGRP NEURONS

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O hormônio do crescimento (GH) age sobre tecidos periféricos e está relacionado com várias funções do organismo como, o controle do metabolismo, crescimento somático e processos celulares. Existem evidências que o GH pode exercer efeitos sobre o Sistema Nervoso Central (SNC). Utilizamos animais deficientes do receptor de GH (GHR KO) em neurônios AgRP com o objetivo de verificar se a falta do GHR, pode impactar fatores metabólicos. Depois da validação do modelo observamos que os neurônios AgRP do nucleo arqueado do hipotalamo (ARH) são responsivos ao GH. Os machos GHR KO tendem a ser mais leves que os controles enquanto as fêmeas tendem a ser mais pesadas. Porém não observamos diferença na tolerância a glicose ou na sensibilidade a insulina. As fêmeas GHR KO apresentaram maior gasto energético que as controle. Quando desafiados a restrição alimentar as fêmeas GHR KO e controles tiveram a mesma resposta no consumo alimentar e na glicemia. Fizemos então o teste para verificar a resposta contra regulatória a hipoglicemia onde as GHR KO e as controle foi a mesma. Porém quando expostas a estresse por contenção, as fêmeas GHR KO e as controles não tiveram diferenças no inicio do teste, porém nas quarenta e oito horas as fêmeas controles contidas tiveram o consumo menor. Essa resposta foi perdida nas fêmeas GHR KO, o que sugere que o GHR é importante nessa resposta fisiológica ao estresse. Esses resultados sugerem que o GH tem um papel no SNC para a regulação de alguns aspectos metabólicos.

FAPESP

POSTER #12

NEUROENDOCRINE REGULATION OF HEPATIC GLUCONEOGENESIS IN RODENTS EXPOSED TO LOW TEMPERATURES

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Although it is well established that mammals increase hepatic glycogenolysis and gluconeogenesis to provide fuel for glucose-dependent tissues upon stress conditions, the molecular mechanisms and signaling pathways involved in the neural regulation of gluconeogenesis in the liver remain poorly understood. To address the role of hepatic sympathetic innervation in glucose production, we investigated the effect of pharmacological sympathetic denervation (6-OH-Dopamine; 100 $mg.kg^{-1}.day^{-1}$) in mice on gene expression and activity of key enzymes of gluconeogenesis, and CREB signaling during acute cold exposure (4^0C). The transcriptional activity of CREB in vivo was evaluated by an imaging system (IVIS) in transgenic animals that expresses the reporter for CRE-luciferase. Cold exposure for 3 and 6h in sham mice induced hyperglycemia, depletion of hepatic glycogen levels, and activation of gluconeogenesis as estimated by the higher gene expression and activity of the two key enzymes of this pathway, glucose-6-phosphatase and PEPCK. The sympathectomy did not affect the cold-induced glycogenolysis but abolished the increase in the hepatic content of norepinephrine and the transcriptional activity of CREB as well as induced hypoglycemia associated with decreased gene expression and activity of the glucose-6-phosphatase and PEPCK. Similar results were observed in 24h cold-exposed rats submitted to selective hepatic denervation with phenol. Both adrenalectomy and adrenodemedullation in mice did not alter the stimulatory effect of cold on the activity and gene expression of the gluconeogenetic enzymes. These results suggest that during cold stress, activation of the liver sympathetic innervations stimulates the gluconeogenesis through the CREB signaling pathways.

POSTER #13

GH CONTROLS METABOLIC ADAPTATIONS TO STARVATION BY ACTING IN LEPTIN RECEPTOR CELLS, PRECISELY IN AGRP NEURONS, AS WELL AS REGULATES THE COUNTER-REGULATORY RESPONSE TO HYPOGLYCEMIA VIA VMH.

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Growth hormone (GH) responsive neurons are extensively distributed in LepR-expressing neurons at different hypothalamic nuclei. In the present study, we generated mice lacking GH receptor (GHR) specifically in LepR-expressing cells (LepR GHR KO mice). Although LepR GHR KO mice exhibited a similar body weight, food intake, energy expenditure, glucose tolerance and leptin sensitivity compared to control mice, we observed a lower adiposity in mutant mice, as well as difficulty to recover from insulin-induced hypoglycemia and a blunted counterregulatory response evoked by 2-deoxyglucose (2DG) administration. Remarkably, while control mice adapted to a 60% food deprivation period by progressively saving energy, LepR GHR KO mice exhibited a blunted metabolic adaptation to starvation, which led to hypoglycemia and higher energy expenditure. In order to identify precisely the neuronal population(s) that controls these adaptations we generated 2 mice models, one lacking the GHR in AGRP neurons and another lacking GHR in steroidogenic factor-1 (SF1) cells. AGRP GHR KO mice presented the similar response to food deprivation, ledding to hypoglycemia and higher energy expenditure compared to control mice, eventhough did not present lower adiposity. Additionally, SF1 GHR KO mice exhibited a lower capacity to recover from insulin-induced hypoglycemia and a blunted counterregulatory response evoked by 2DG. In summary, GHR signaling in the AGRP neurons seems to regulate the metabolic adaptation to starvation, while GHR signaling in VMH controls the conter-regulatory responses to hypoglycemia, showing a coordination between GH and leptin to promote metabolic adaptations in order to ensure survival.

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POSTER #14

ARTERIAL PRESSURE AND SYMPATHETIC ACTIVITY IN HYPERTENSIVE RATS TREATED WITH CATALASE INHIBITOR.

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The 2-kidneys, 1-clip (2K1C) hypertension is characterized by increased renin-angiotensin system and sympathetic activity. In rats with 2K1C hypertension, chronic subcutaneous (sc) administration of the catalase inhibitor 3-amino-1,2,4-triazole (ATZ) reduces mean arterial pressure (MAP) and improves autonomic modulation. In the present study, the changes in renal and splanchnic sympathetic nerve activity (SNA) were recorded and the level of sympathetic activity was tested using ganglionic blockade with hexamethonium in 2K1C rats treated with sc injection of ATZ. Male Holtzman rats (initial weight 150-180 g, n=4-6/group) received a silver clip around the left renal artery to generate 2K1C hypertension. Six weeks after the surgery, ATZ (300 mg/kg of body weight) injected sc in urethane-anesthetized and artificially-ventilated 2K1C rats reduced MAP (160 ± 13 mmHg 1 h after ATZ, vs. control: 180 ± 13 mmHg pre-ATZ) and renal SNA (-52 ± 10% 1 h after ATZ), without changing splanchnic SNA (-8 ± 16%). In another group of conscious, freely moving 2K1C rats, the treatment with ATZ sc reduced the hypotension and bradycardia to hexamethonium (30 mg/kg of body weight) iv (-58 ± 5 mmHg and -25 ± 5 bpm, respectively) compared to the responses to iv hexamethonium in 2K1C rats treated with saline sc (-106 ± 7 mmHg and -55 ± 2 bpm, respectively). The results suggest that increasing the availability of endogenous H_2O_2 with the injection of ATZ produces anti-hypertensive effects associated with decrease in sympathetic nerve activity.
Supported by: FAPESP, CNPq and PROPE/FUNDUNESP.

POSTER # 15

UNIQUE MACROPHAGE PROFILE INDUCED BY RANK-RANKL ACTIVATION

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Introduction: RANKL system is composed by the receptor activator of NFκB (RANK), its ligand - RANKL and the decoy receptor osteoprotegerin (OPG). Recently, we described the role of the RANKL system in vascular calcification and anti-inflammatory responses in macrophages during cerebral ischemia in murine models. Bacterial components, such as liposaccharides (LPS), activate specially toll like receptor-4 in macrophages, polarizing them to M1 profiles; in contrast, cytokines such as IL-4 induce M2 profiles through MAPK-ERK axis which culminates in the expression of PPARγ and Arginase-1. However, macrophages responds to a combination of factors and can develop mixed phenotypes, once that M1 and/or M2 signatures do not necessarily exclude each other and often coexist. **Objective:** Elucidate the molecular mechanism of RANK-RANKL system in macrophage polarization. **Methodology and results:** The responses of THP-1 cells to RANKL stimulus were initially evaluated by the activation of MAPK-ERK axis by western blot at intervals up to 24 hours, in which pERK level was increased in response to RANKL. We also analyzed by RT-qPCR the expression of IL-1 and TNF- in cells treated with RANKL after priming with LPS, and their expression reduced compared to the group stimulated only with LPS. However, the expression of M2 macrophage markers, such as Arginase-1 and PPAR was not detected. **Conclusion:** These preliminary data suggest that RANKL reduce the inflammatory response of THP1 under LPS stimulus, but is not able to induce the typical M2 polarization in macrophages, demonstrating a macrophage profile that can be associated to a non-canonical anti-inflammatory profile.
Keywords: RANKL, Osteoprotegerin, Macrophage, Immunometabolism

POSTER #16

GHR ABLATION IN NEURONS OR LEPR CELLS CAUSE METABOLIC EFFECTS AND CHANGES IN GLYCEMIC CONTROL DURING PREGNANCY

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Pregnancy lead to extensive adaptations in the female's body, by changing production and responses to certain hormones. GH is required for fetal nutrition and growth during pregnancy and for mammary development and lactation, although the central roles is not completely clarified. The aim of this study was investigate the role of GH in the CNS in the face of physiological and metabolic changes during pregnancy. Females of two different genomic models of GH receptor (GHR) deletion: neuronal ($GHR^{flox/flox}$/$Nestin^{Cre}$) and in cells that expressing leptin receptor ($GHR^{flox/flox}$/$LepR^{Cre}$) were used. The females were mated and, when the first day of gestation (copulatory plug) was identified, they were individualized and evaluated. Pregnant GHR/Nestin females had higher food intake, weight gain and lower adiposity, higher insulin sensitivity (ITT), lower serum concentrations of insulin and leptin, whereas IGF-1 concentrations were higher during pregnancy, these females presented higher deposition of subcutaneous fat in the lactation period. The deletion of the GHR in leptin receptor cells resulted in higher food intake, without altering weight gain, lower adiposity in all evaluated periods, better glucose tolerance, higher insulin sensitivity and lower serum concentrations of this hormone, as well as of leptin. These results indicate that GH may play a role not previously described and important in metabolic control during gestation. Other experiments are being conducted to better clarify the mechanisms involved.

Keywords: energetic balance, gestation, growth hormone, neuronal signaling

Funding: CNPq and FAPESP

POSTER #17

RANKL SYSTEM IN TLR4 SIGNALING PATHWAY

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The inflammatory microenvironment surrounding adipocytes interferes on insulin signaling and cause insulin resistance in adipose tissue. In recent studies, our group demonstrated a role of RANKL as an anti-inflammatory molecule during brain ischemia through inhibition of M1 profiles. Furthermore, RANKL also induced LPS tolerance in mice. Thus, we hypothesized that RANKL polarizes M1 towards M2 through competition between molecules existent in RANK-RANKL and TLR4 pathway. We aim to elucidate the molecular mechanisms of the RANKL system upon TLR4's canonical pathway, regulating macrophage polarization. Output genes classically activated in response to TLR4's activation, such as IL-1 and TNF were downregulated in THP-1 cells stimulated with RANKL and when co-stimulated with RANKL+LPS. We performed Luciferase Assays using THP-1 cells transfected with a Luc-Plasmid containing NFkB's binding site (pNFkB) in the same conditions aforementioned. RANKL and the co-stimuli RANKL-LPS revealed a diminished NFkB activity when compared to LPS stimuli, corroborating with the prior data. In a phenotypic scale, we carried out Flow Cytometry analysis where CD68 was diminished in THP-1 cells stimulated with RANKL and RANKL-LPS. Based on unpublished data we constructed a plasmid to super express protein ''X'' which possibly cross-links RANK-RANKL and TLR4 pathways in THP-1. TLR4's downstream activation (NFkB activity) was rescued even when co-stimulated with RANKL+LPS. As a counter test, we super expressed a point-mutated RANK in these cells and as predicted TLR4's activation was not inhibited when co-stimulated. Therefore, RANKL possibly antagonizes TLR4 pathway and this may occur through competition with the protein ''X'' between both pathways.

Financial support: FAPESP, CAPES.

POSTER #18

SHORT-TERM HIGH-FAT DIET FEEDING (HFD) INDUCES INFLAMMATION AND DECREASES NOCTURNAL SYNTHESIS OF MELATONIN

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Melatonin, produced by the pineal gland at night, orchestrates rhythmic functions and has exquisite roles in regulating metabolism and immune responses (Markus et al., Int J Mol Sci, 2013). Acute HFD triggers neuroinflammation resulting in weight gain and adiposity (Thaler et al., Diabetes, 2013). Several lines of evidence indicate that disturbing the circadian system leads to metabolic dysregulation. Nevertheless, up till now there are no clear experimental data to what extent the disturbance of the rhythmicity of hormones in association with increased inflammation is important for regulation of weight. This study addressed whether circadian rhythms are changed by acute inflammation induced by HFD. Adult Wistar rats received *ad libitum* access to LFD/HFD (7 days) and where killed every 3h along light/dark cycles. Melatonergic regulation of changes in inflammation and metabolism was accessed by reposition of melatonin (25 ng/mL) or by blocking melatonin receptor (DH97, 170 ng/mL, antagonist of MT2) in the drinking water. HFD rapidly increased food intake, body weight and fat depots. Rhythmic amplitudes of leptin and insulin was increased, whilst melatonin levels and pineal activity was significantly reduced. Reduction of melatonin formation was mediated by microglia-derived TNF, as intracerebroventricular injections of mynocycline or SPD304, blockers of microglia or TNFR1 activation, respectively, reversed the effects of HFD. Nocturnal reposition of melatonin protected weight gain through MT2. Blocking MT2 increased hepatic steatosis, reduced IL-4 and IL12p70 cytokines, and induced hyperphagia and hyperglycemia. Altogether, our findings uncover novel roles for MT2 in energy homeostasis and suggest that melatonin rhythm is pivotal for circadian regulation of metabolism and immune responses. Financial Support: FAPESP, CNPq and CAPES.

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