The AT1R mediates the main physiological effects of Ang II. Chronic elevation of Ang II interferes negatively with the metabolic actions of insulin. Treatment with AT1R antagonists improves this condition. In contrast, little is know about the role of the AT2R on the modulation of insulin actions. The evidence obtained by the stimulation or antagonism of the AT2R, indicates that the AT2R is involved in the glucose homeostasis, but the information obtained so far using AT2RKO mice is not consistent.
Objective. To analyze the impact of the global elimination of the AT2R on glucose homeostasis. It is postulated that the elimination of the AT2R will generate a reduction of insulin sensitivity.
Methods. Male and female AT2KO mice (4 months), were provided by Dr. Pedro Miguel Geraldes, University of Sheerbroke, Montreal, Canada. We evaluated the effect of the absence of the AT2R on insulin sensitivity (insulin tolerance test), glucose tolerance (glucose tolerance test) and circulating metabolic parameters. The in vivo status of main components of the insulin pathway was analyzed by western blotting.
Results. Female AT2KO mice showed a reduction in insulin sensitivity while males did not present differences when compared to their respective controls. Female AT2KO mice displayed a reduction in the insulin-stimulated phosphorylation of both the insulin receptor and Akt at activating residues in white adipose tissue compared with respective controls.
Conclusions. Our findings show that female AT2RKO mice display decreased insulin sensitivity, associated with a reduction in the response to insulin at the phosphorylation of the insulin receptor and Akt in adipose tissue.

26. (199) **CROSS-TALK BETWEEN BMP AND WNT SIGNALING PATHWAYS IN HAIR FOLLICLE STEM CELLS DIFFERENTIATION. IMPLICATION IN ANDROGENETIC ALOPECIA.**

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Hair follicle (HF) cyclical growth is governed by interactions between dermal papilla cells (DPC) and epidermal HF stem cells (HFSC). During androgenetic alopecia (AGA) androgens deregulate these interactions and impair HFSC differentiation through inhibition of the Wnt/ B catenin signaling pathway. BMPs and WNTs act on DPC to maintain hair-inducing activity. We studied the role of BMPs on DPC spheres (DPC Sph)- induced HFSC differentiation. The activity of alkaline phosphatase, marker of hair inductivity, decreased in DPC Sph treated with DHT and the addition of BMP2 restored it. Conditioned media from DPC Sph induced HFSC hair-linage differentiation. When these media were conditioned in presence of DHT, HFSC-differentiation was impaired, however, when DPC Sph media were conditioned in presence of DHT and BMP-2, HFSC-differentiation was recovered, suggesting that BMPs can overcome the inhibitory effect of DHT on HFSC differentiation. To deepen in the mechanism by which BMPs could be exerting this pro- differentiating activity, we analyzed the beta-catenin nuclear translocation in HFSC. When BMP2 was added to the DPC Sph conditioned media, beta-catenin translocation was favored in differentiating HFSC compared with conditioned media alone or with DHT, implicating a cross-talk between BMPs and WNT signaling pathway in HFSC. We then analyzed, two WNT pathway inhibitors. CXXC5 mRNA was downregulated in differentiated HFSC independently of the presence of DHT or BMP2 in conditioned media. GSK-3 phosphorylation was not modified by BMP2, as it was in presence of LiCl, a known inhibitor of GSK-3. Even if further studies are necessary to elucidate at which level the cross-interactions of BMP and Wnt signaling may occur, BMPs contribute to DPC inductivity and HFSC differentiation. We conclude that BMPs are critical factors of the complex epithelial-mesenchymal interaction and their downregulation would favour AGA development.

27. (228) **MOLECULAR EFFECTORS AND MECHANISMS INVOLVED IN THE SUSTAINED CAMP RESPONSE MEDIATED BY CRHR1**

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Corticotropin-releasing hormone (CRH) plays a central role in the stress response and dysregulation of its action is linked to psychiatric disorders as anxiety and depression. CRH is a high affinity ligand for corticotropin-releasing hormone receptor type 1 (CRHR1) whose activation is associated to a sustained increase in cAMP levels and ERK1/2 activation in cellular models that recapitulate contexts of CRHR1 action. In a hippocampal neuronal model, CRHR1-mediated ERK1/2 activation triggered by CRH is biphasic, with an early phase B-Raf and PKA dependent, and a late phase of sustained ERK1/2 phosphorylation dependent on β-Arrestin2 and CRHR1 internalization. The aim of this work is to characterize the mechanisms implicated in the sustained cAMP response to CRH mediated by CRHR1. As a model, mouse hippocampal neuronal cell line HT22 stably expressing CRHR1 (HT22-CRHR1) was used together with the FRET-based biosensor Epac-S^{H187} to monitor cAMP levels in real time in living cells. By means of pharmacological and genetic tools, we revisited the role of PKA and β-Arrestin and explored PI3K/Akt signaling pathway in our system. In HT22-CRHR1 cells, expression of a β-arrestin dominant negative mutant altered Akt and CREB responses to CRH. Furthermore, Akt activation proved to be dependent on cAMP in HT22-CRHR1 cells as forskolin or 8-CPT-cAMP treatment promoted its phosphorylation. Pharmacological inhibition of PI3K, Akt and PKA triggered a further increase in cAMP levels in CRH-stimulated cells. Besides, PI3K/Akt inhibition led to a decrease in CRH-induced ERK1/2 and CREB activation, similar to what had already been reported when PKA activity was repressed. Moreover, our results showed that PKA inhibition is associated to increased Akt activation in response to CRH. These results suggest that PKA and PI3K/Akt may play a role in the mechanisms involved in CRH-elicited/CRHR1-mediated sustained cAMP response in a hippocampal context.
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28. (343) **THE CROSS-REGULATION BETWEEN H1 AND H2 HISTAMINE RECEPTORS MODULATES THE BEHAVIOR OF H2 RECEPTOR BLOCKERS**

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Histamine, modulates several biological processes, including allergy and gastric acid secretion, through H1 and H2 receptors. H2 blockers (H2B) are mainly used to treat gastrointestinal disorders, and much interest is focused on their repositioning for other pathologies. For it, deep understanding of their mechanisms of action is needed. We have previously described that H1 and H2 agonists (H1A and H2A) induce the receptor's co-internalization and cross-desensitization. We have also reported that H2B lead to desensitization and internalization of H2 receptor. Now, we hypothesize that H2B may also induce H1 receptor's cross-desensitization and co-internalization and that the cross-regulation induced by H1A will affect the behavior of H2B. In HEK293 cells transfected with both receptors (HEK-H1-H2), pretreatment with the H2B (cimetidine, ranitidine and famotidine) significantly reduced the activation of H1 receptor, evaluated through IL-6 promoter´s activity. Similar results were obtained for COX-2 and IL-8 gene expression, by qPCR in U937 cells. Also, we analyzed the impact of H2B in the antiproliferative/apoptotic response induced by H1A. Proliferation, cell cycle and annexin V assays showed that H2B reduced the antiproliferative/apoptotic response induced by H1A. Regarding the effect of H1 receptor activation on H2B response, H1A prevented the reduction of cAMP

levels induced by H2B, in U937 and HEK-H1-H2 cells. Additionally, saturation-binding assays showed that H2B lead to a decrease in H1 receptor binding sites. Finally, we analyzed the cross-regulation induced by H2B in presence of the receptor internalization inhibitor, dynasore. In this condition, none of the H2B used were able to modify the IL-6 promoter´s activity induced by H1A. This indicates that the co-internalization process is responsible of the cross-regulation induced by H2B. Our study provides new insights in the mechanisms of action of H1 and H2 receptors that explain the effect of antihistamines and opens up new venues for novel therapeutic applications.

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29. (93) **BORON NEUTRON CAPTURE THERAPY (BNCT) COMBINED WITH BCG IMMUNOTHERAPY IN AN ECTOPIC COLON CANCER MODEL, IMMUNOLOGICAL RESPONSE AND CYTOTOXIC EFFECT**

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BNCT combines selective tumor uptake of ^{10}B compounds and neutron irradiation. The abscopal effect refers to the inhibitory action of radiotherapy on tumor growth at a site distant from the area of irradiation. Bacillus Calmette-Guerin (BCG) is known to induce a potent cytotoxic immune response. Having demonstrated the capacity of BNCT alone to induce abscopal effect, the aim of the present study was to evaluate the local therapeutic efficacy of BNCT combined with BCG immunotherapy in the BDIX rat ectopic colon cancer model and assess the abscopal effect and the tumor specific cytotoxicity. BDIX rats were inoculated with syngeneic colon cancer cells in the right leg. Four weeks post-inoculation, tumor was locally irradiated at RA-3 employing borono-phenyl-alanine (BPA): BNCT-group and BNCT+BCG-group (three intratumoral applications of BCG). BCG-group: BCG only; Beam only-group (BO-group): irradiated without BPA; BO+BCG-group; Sham-group: untreated tumor-bearing animals (tumor in both legs); Naive: untreated tumor-bearing animals (tumor in left leg). To evaluate abscopal effect, two weeks post-BNCT, colon cancer cell were inoculated in left leg. Once weekly for 7 weeks post BNCT tumor volume was measured in both legs. A significant local therapeutic efficacy was observed in the BNCT-group and BNCT+BCG-group vs. Sham-group $p < 0.05$. An abscopal effect, defined as tumor volume ≤ 50 mm^3, was observed in the BNCT-group (22%), BCG-group (44%) and BNCT+BCG-group (38%) compared to Sham-group (3%). A specific cytotoxicity assay against colon tumor cells using splenocytes from different experimental groups was performed. The cytotoxicity median (range) were: Normal rats 67% (60-70); Naive 20% (8-36). The 20% of BNCT and 43% of BCG+/-BNCT rats restored the normal cytotoxicity. The present study demonstrates that the therapeutic efficacy of BNCT could be improved if it is combined with BCG immunotherapy. This combination increases immune tumor cytotoxicity inducing an abscopal response in a higher number of animals.

30. (102) **BORON NEUTRON CAPTURE THERAPY MEDIATED BY BORIC ACID COMBINED WITH ELECTROPORATION IN THE HAMSTER CHEEK POUCH ORAL CANCER MODEL**

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BNCT is based on the selective incorporation of ^{10}B carriers in tumor followed by neutron irradiation. The biodistribution of boron carriers in tumor in terms of absolute and relative ^{10}B concentration, retention in tumor, targeting homogeneity and microdistribution conditions the therapeutic efficacy of BNCT. Given that electroporation (EP) can act as a non-specific system to administer anti-tumoral agents, the aim of this study was to evaluate if EP optimizes the delivery of the boron compound Boric Acid (BA), improving the therapeutic efficacy of BNCT in the hamster cheek pouch oral cancer model. Exophytic tumors (Squamous Cell Carcinoma) were induced in the pouch of Syrian hamsters by topical application of the carcinogen dimethyl-benzanthracene (DMBA) twice a week for 3 months. For boron biodistribution and BNCT studies we administered BA (50 mg ^{10}B/kg iv) followed by EP (1000 v/cm, 8 pulses of 100μs) 10 min. post-administration. Three hours after the administration of BA we sacrificed the animals for boron biodistribution studies or performed BNCT/BA studies. We observed a statistically significant increase ($p < 0.0001$) in boron uptake in tumors corresponding to the protocol BA+EP (47±10 ppm) versus control BA without EP (36±7 ppm) whilst no changes were observed in normal pouch and precancerous tissues. The ongoing studies of BNCT/BA+EP showed a high therapeutic efficacy (94%), similar to that observed with BNCT/BA (84%). In addition, these results are statistically significant ($p<0.0001$) compared to those of BNCT/GB-10 (48%), a boron compound with characteristics similar to BA, although we observed an increase in radiotoxicity. Biodistribution studies showed that EP induced an increase in mean gross boron concentration in tumor and would contribute to BNCT/BA induced tumor response.

31. (98) **OLIGO-FUCOIDAN ENHANCES THE THERAPEUTIC EFFICACY OF BORON NEUTRON CAPTURE THERAPY (BNCT) IN THE ORAL CANCER AND ECTOPIC COLON CANCER EXPERIMENTAL MODELS**

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Introduction BNCT combines selective tumor uptake of 10-B compounds and neutron irradiation. We demonstrated the therapeutic effect of BNCT in the hamster cheek pouch oral cancer and BDIX rat ectopic colon cancer models. We also studied the radiotoxic effects of BNCT, mucositis and dermatitis, respectively. Oligo-Fucoidan is extracted from seaweeds and has exhibited anticancer and anti-inflammatory properties. The aim of the present study is to evaluate the radioprotective and therapeutic effect of Oligo-Fucoidan+BNCT in both cancer models. Materials and Methods BDIX rats were injected subcutaneously in the right hind flank with DHD/K12/TRb syngeneic colon cancer cells. The tumor-bearing legs were treated locally with BNCT mediated by BPA (boronophenylalanine) at RA-3 Nuclear Reactor, with or without Oligo-Fucoidan (200mg/ml, oral administration during 16 days). Cancerized hamster cheek pouches (DMBA in mineral oil, applied twice a week for 8 weeks) were exposed to BPA-BNCT with or without Oligo-Fucoidan (200mg/kg, 16 days). Results Oligo-Fucoidan was nontoxic. It did not reduce the percentage of animals with severe dermatitis or mucositis, but it did enhance BNCT therapeutic effect on tumors. BDIX rats treated with BNCT+Oligo-Fucoidan exhibited a mean ratio tumor volume Post-BNCT/Pre-BNCT significantly lower than the BNCT group (0.35±0.31 vs 1.09±0.76 respectively, $p= 0.0002$). In the oral cancer model, BNCT+Oligo-Fucoidan group exhibited a higher Overall tumor response than the BNCT group and also enhanced tumor complete response (94% vs 67%; 71% vs 41% respectively). Conclusion Oligo-Fucoidan enhances the therapeutic efficacy of BNCT in the hamster oral cancer model and BDIX rat ectopic colon cancer model. Acknowledgments We gratefully acknowledge the provision of Oligo-Fucoidan by Hi-Q Marine Biotech International Ltd (Taiwan), and the efforts of Ming-Chen Hsiao to promote these studies.