PRÊMIO JOSÉ RIBEIRO DO VALLE – 2007

O prêmio José Ribeiro do Valle, oferecido a cada ano pela SBFTE, visa identificar a cada ano os melhores trabalhos científicos desenvolvidos por jovens investigadores na área da Farmacologia. Entre os trabalhos inscritos para esta décima edição do prêmio, foram selecionados cinco finalistas, que fizeram apresentações de seus respectivos trabalhos perante comissão julgadora, em sessão pública durante o 39º Congresso Brasileiro de Farmacologia e Terapêutica Experimental, em Ribeirão Preto, SP. Os cinco finalistas escolhidos para a edição de 2007 foram:

Primeiro Lugar

Expression and function of kinin B_2 receptors in injured dorsal root ganglion neurons during neuropathy induced by spinal nerve ligation in rats. Werner, M. F. P.[1]; Franco, C. R. C.[2]; Trevisani, M.[3]; Campi, B.[3]; Andre, E.[3]; Geppetti, P.[3]; Rae, G. A.[1] - [1]UFSC - Farmacologia; [2]UFPR - Biologia Celular; [3]University of Ferrara, Italy - Exp. Clin. Med. **Introduction:** Spinal nerve ligation (SNL) injury of L5 and L6 nerves in rats enhances kinin B_2 receptor expression in ipsilateral L4-L6 nerves and induces neuropathic thermal (cold/heat) and mechanical hypernociception sensitive to partial reversal by B_2 receptor blockade (Werner et al., *Neuropharmacology*, in press). Here we have investigated the localization of kinin B_2 receptors in the L4 and L5L6 rat dorsal root ganglia (DRG) and the functional responses of cultured L4 and L5L6 DRG neurons to bradykinin (BK). **Methods:** To induce SNL neuropathy, two tight 6-0 silk sutures were placed unilaterally around L5 and L6 spinal nerves. No ties were placed in Sham-operated rats. On day 12 after surgery, when SNL rats display hypernociception, L4 (intact) and L5L6 (injured) DRG were removed and processed for immunohistochemistry or were cultured for 48 h in order to measure variations in $[Ca^{2+}]_i$ induced by BK through Fura-2AM fluorescence (ratio 340/380 nm). Ratio changes were expressed as percentages of the peak response to ionomycin (5 mM). **Results:** Confocal fluorescence microscopy revealed that immunoreactive B_2 receptors were co-labeled with non-peptidergic (IB4-positive) C fibers and with myelinated (NF-200 positive) A fibers in the L4 and L5L6 DRG of Sham-rats and on the intact L4 DRG of SNL-rats. In L5L6 injured DRG of SNL-rats, B_2 receptor expression in IB4-positive C fibers was markedly decreased, whereas B_2 receptors were expressed mainly in NF-200 immunoreactive neurons. No B_2 receptor immunoreactivity was observed in GFAP-immunoreactive DRG glial cells from any of the groups studied. BK (10 and 100 nM) induced concentration-dependent increases in $[Ca^{2+}]_i$ in cultured L5L6 DRG neurons from both Sham- and SNL-rats. Increases in $[Ca^{2+}]_i$ induced by BK (100 nM) in SNL-injured L5L6 DRG neurons (34±8 %, n = 59) were 2-fold greater than those recorded in Sham (17±2 %, n = 27) and all other cultures studied. This response to BK was reduced by previous incubation (10 min) with selective B_2 receptor antagonist HOE 140 (1 mM) to 3±2 % (n = 26), but was not changed by the selective B_1 receptor antagonist R-715 (1 mM, 24±3 % n = 36). Importantly, indomethacin (5 mM) reduced the increases in $[Ca^{2+}]_i$ induced by BK in L5L6 DRG neurons from SNL-rats to 14±2 % (n= 53), but not from Sham-rats (BK + Vehicle: 16±3 %, n= 31 and BK + Indomethacin: 17±3 %, n= 43). In addition, the increases in $[Ca^{2+}]_i$ induced by BK in injured L5L6 DRG neurons were insensitive to inhibition by the TRP channel blocker ruthenium red (1 mM, n = 33), the TRPV1 antagonist capsazepine (10 mM, n = 13) or the non-selective TRPA1 antagonist camphor (1 mM, n = 31). **Discussion:** SNL modifies the expression and functionality of B_2 receptors in L5L6 injured DRG neurons, so that they are expressed to a greater extent on A-fibers and are more responsive to BK, through a mechanism which involves selective sensitization by prostaglandins, but does not require the activation of B_1 receptors or members of TRP receptor family. This novel mechanism may underlie the pronociceptive B_2 receptor-operated mechanisms that sustain the hypernociceptive state associated with SNL-induced neuropathy. Apoio Financeiro: **Supported by**: CAPES, CNPq and PRONEX.

Segundo Lugar

Expression of glucocorticoid receptors in the rat epididymis: impact of endogenous and synthetic glucocorticoids and co-localization with microtubule associated protein 1B. Silva, E. J. R.; Queiroz, D. B. C.; Honda, L.; Avellar, M. C. W. - UNIFESP-EPM – Farmacologia

Introduction: Glucocorticoids (GCs) are stress-induced steroid hormones that regulate several physiological functions in vertebrates such as reproduction. Clinically, natural and synthetic GCs are widely used for treatment of immune and inflammatory diseases. Curiously, the role of GCs in the epididymis, an androgen dependent organ that plays a vital role on sperm maturation, is still poorly understood. Previously, we have demonstrated that adrenalectomy (ADX) changes the dynamic of the expression and cellular distribution of glucocorticoid (GR) and androgen (AR) receptors in the rat epididymis. In the this study, we have evaluated the effect of ADX and dexamethasone (Dex) on the expression of GR and AR in the rat epididymis. The expression of two known glucocorticoid-dependent genes (IκBa and IL-1β) was also analysed. Co-localization of GR with a neuronal cytoskeleton marker (Microtubule-associated protein 1B, MAP 1B) in the rat epididymis was also evaluated. Methods: Wistar rats (90 days) were sham-operated (S) or submitted to bilateral ADX (1, 2, 7, 15 days). Rats were also submitted to ADX for 7 days and immediately treated with Dex (Acute - AcDex, 7 mg/kg, i.p., 6 h or

Chronic - CrDex, 5 µg/kg, i.p., 7 days). Plasma corticosterone levels were monitored by RIA. Caput (CP) and cauda (CD) epididymis from all groups were used in semi-quantitative RT-PCR assays using GR, AR, IκBa and IL1-b specific primers. Primers against the housekeeping gene GAPDH were used as internal control. Western blot (total protein extracts), immunohistochemistry and immunofluorescence (cryosections, 8mm) assays with samples from S, ADX 7 days, AcDex and CrDex rats were performed with antibodies against GR, AR and MAP 1B (negative controls with specific blocking peptides). Results were analyzed by ANOVA followed by Newman-Keuls test ($p<0.05$). Results and Discussion: RIA confirmed the significant reduction on corticosterone plasma levels in all ADX groups. Densitometric data of RT-PCR assays indicated that GR, AR, IκBa and IL1-b mRNA levels were not altered by ADX in CP and CD. However, AcDex and CrDex treatments caused a down-regulation on GR, AR and IL1-b and an up-regulation on IκBa mRNA levels in both CP and CD when compared to S and ADX 7 day groups. Western blot assays showed no difference when GR and AR protein levels from ADX 7 days, AcDex and CrDex groups were compared. On the other hand, immunohistochemical studies revealed that the loss of GR staining in the nuclei of epithelial cells from CP and CD caused by ADX 7 days was reversed in AcDex and CrDex tissues. AR immunostaining pattern was similar among all tested groups. Curiously, GR-positive interstitial fibers were observed mainly in the CD of all tested groups. Co-localization studies revealed that all GR-positive fibers were also immunostained by MAP 1B antibody, confirming the expression of GR on epididymal nerve fibers. In conclusion, our results suggest that endogenous and synthetic GCs can differently regulate GR and AR genes at transcriptional and post-transcriptional levels in the rat epididymis. The presence of GR in nerve fibers also suggests that GCs might have a role in the neuronal modulation of epididymal fuctions. Apoio Financeiro: Supported by: FAPESP, CAPES, CNPq, Fogarty International Center

Outros trabalhos apresentados

Effect of melatonin on nitric oxide production induced by lipopolysaccharide (*LPS*) *in cultured endothelial cells*. Tamura, E. K.[1]; Silva, C. L. M.[2]; Markus, R. P.[1] - [1]IB-USP - Fisiologia; [2]UFRJ - Farmacologia Básica e Clínica - ICB

Avaliação do mecanismo de ação antidepressivo da Riparina I (*RIPI*) *em camundongos*. Melo, R. A.[1]; Sousa, F. C. F.[1]; Vasconcelos, P. F.[1]; Melo, T. V.[1]; Silva, M. I. G.[1]; Barbosa-Filho, J. M.[2]; Luz, P. B.[1]; Araujo, F. L. O.[1] - [1]UFC - Fisiologia e Farmacologia; [2]UFPB - Tecnologia Farmacêutica

Angiotensin II AT1 receptor blockade worsen sepsis in the rat. Pacheco, L. K.; Fernandes, D.; Sordi, R.; Heckert, B. T.; Assreuy, J. - UFSC - Farmacologia

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