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Expression and Distribution of Kinin B_2 Receptor in Medulla and Spinal Cord Tissues of Rats Treated with Capsaicin

Expresión y Distribución del Receptor B_2 de Cininas en la Médula y Tejido Medular Espinal de Ratones Tratados con Capsaicina

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DE FRANÇA, C. E.; GUARDABASSI, C. & SMITH, R. L. Expression and distribution of kinin B_2 receptor in medulla and spinal cord tissues of rats treated with capsaicin. *Int. J. Morphol., 34(4)*:1465-1471, 2016.

SUMMARY: Kinins are vasoactive peptides that promote pain and inflammation, yet centrally believed to participate to cardiovascular defensive reflexes produced by noxious stimuli. These peptides signal through the activation of two transmembrane G-protein-coupled receptors named B_1 and B_2 receptors (B_1R and B_2R). The B_2R is constitutive in healthy tissues and animals. The aim of the study was to measure the gene and protein expression of B_2R kinin receptors in central and peripheral tissues isolated from control rats and rats were pre-treated with capsaicin on the second day of life (50 mg/kg, s.c.) or two weeks prior to sacrifice (125 mg/kg over three days, s.c.). The same treatment with saline was made in control animals. Levels of mRNA for B_2R were measured by quantitative RT-PCR and Qualitative while receptor binding sites were measured on tissue sections with the radioligands ^{125}I-HPP-Hoe 140 (B_2R). B_2R was expressed in all studied tissues (hypothalamus, paratrigeminal nucleus, nucleus of solitary tract, spinal cord, aorta and liver) and treatment capsaicin neonates when compared to controls, did not affect its level of expression. Capsaicin had no significant effect on the expression of B_2R in some tissues on binding sites. The synthesis of B2R kinin receptor is not associated with sensory C-fibre and tissues showed no significant difference indicating that B_2R was regulated by distinct mechanisms.

KEY WORDS: Medulla; Spinal Cord; Rats; Kinin receptor B_2; Capsaicin.

INTRODUCTION

Bradykinin, a potent neuropeptide and a vasoactive peptide, has a wide spectrum of biological activities (Couture & Lindsey, 2000; Leeb-Lundberg *et al*., 2005). Kinins are biologically active autacoids implicated in the response of the organism to trauma and tissue injury. Under physiological conditions, kinin activity is mediated by the interaction of the B_2 kinin receptors (Leeb-Lundberg *et al*.). These are present in organ and arterial smooth muscle cells and in rat and human brain nuclei, such as the paratrigeminal nucleus and the nucleus of the solitary tract (Lindsey *et al*., 1997; de Sousa Buck *et al*., 2002). Stimulation of arterial B_2 kinin receptor causes resistance to vessel vasodilatation and hypotension (Sharma *et al*., 2006). On the other hand, stimulation of central kinin receptors leads to blood pressure increases (Lindsey *et al*., 1997), mediated by central sympathetic mechanisms (Caous *et al*., 2004). Thus, among other impor-tant functions, such as blood pressure regulation, kinins are characterized as mediators of pain and inflammatory responses, both in the central and in the peripheral nervous systems (Leeb-Lundberg *et al*.); they are also important mediators in the cascade of the febrile response (Pessini *et al*., 2006). Additionally, bradykinin apparently plays a role in the rat reproductive tis-sues via the kinin B_2 receptor (França *et al*., 2010). As vascular agents they promote vascular permeability and vasodilatation (Leeb-Lundberg *et al*.), which are important mechanisms related to the anti-hypertensive action of kinase II inhibitors (Lindsey *et al*., 1987). In particular, bradykinin and kallidin are powerful algesic and vasoactive peptides generated locally following the proteolytic cleavage of their precursors, high- and low-molecular-weight cultures and in various regions of the brain including the medulla oblongata, the pons, the cerebral cortex and the hippocampus (Damasceno *et al*., 2015).

Two receptor types, both of the protein G receptor family, are known to mediate kinin effects in different tissues throughout the body (Leeb-Lundberg *et al*.). The widely

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distributed B_2 receptor is constitutive and mediates the effects of BK and KD, while the B_1 receptor, absent or under-expressed in healthy animals, mediates the effects of des-Arg9-BK and des- Arg10-KD (Marceau *et al*., 1998; Regoli *et al*., 2001). The B_1 receptor, normally not present in healthy tissues, has its expression promoted by inflammatory responses caused by injury, infection, or endogenous and exogenous pyrogens (Lungu *et al*., 2007). Spinal cord, B_2 receptor-binding sites were seen preferentially localized to the superficial laminae of the dorsal horn, the site of first synapse for terminals of primary sensory fibers (Lopes *et al*., 1995). Most, if not all, studies have restricted their work on brain, at a large macroscopic level, without particular focus on a more cellular localization such as neurons, glial cells and/or vascular tissue (capillary) and neurotoxin for small-diameter primary afferent fibers (C and A delta). There is considerable evidence showing that bradykinin is a major mediator of inflammation following peripheral issue injury, and can stimulate or sensitize peripheral nociceptive afferent terminals to elicit pain and hyperalgesia via the activation of the B_2 kinin receptor (Lindsey *et al*., 1997). Within the brain and the spinal cord, kinins have been extensively studied for their implications in the transmission of nociceptive information (Muto *et al*., 2012) the control of blood pressure (Scheuren *et al*., 2016). For a better understanding of the localization rat kinin B_2 receptor to gain insight, we analyzed mature RNA and binding sites in neonate normotensive rat strains using of bulbar nucleus, spinal cord and aorta were respectively analyzed for a clearer understanding of this receptor as originates from C and A delta sensory primary fibers.

MATERIAL AND METHOD

Chemicals and materials. HPP-Hoe 140 (3-(4 hydroxyphenyl) propionyl-D-Arg0[Hyp3, Thi5, D-Tic7 Oic8]-BK) was developed from the selective B_2 receptor antagonist Hoe 140 (Hock *et al*., 1991) and it was synthesized by Dr. Witold Neugebauer (Institute of Pharmacology, University of Sherbrooke, Sherbrooke, Quebec, Canada). Autoradiographic [^{125}I]-microscales and Kodak Scientific BIOMAXTM MS (Amersham Pharmacia Biotech Canada). Bacitracin, bovine serum albumin (protease free), captopril, dithiothreitol, magnesium chloride, piperazine- N, N0-bis [2-ethanesulfonic-acid] (PIPES), and 1,10-phenanthroline (Sigma- Aldrich, Canada). Hoe 140 a selective B_2 receptor antagonist (Hock *et al*.) (DArg0[Hyp3,Thi5,D-Tic7,Oic8]-BK, Peninsula Laboratories, San Carlos, CA, USA) and des-Arg9-BK and BK (Bachem Bioscience Inc., King of Prussia, PA, USA). All other chemicals were obtained from standard commercial sources.

Animals. Male Wistar newborn (2 days) and adult rats (weighting 200–250g) (n=4 per group, 8 groups – control and capsaicin treated) were purchased from Charles River (St-Constant Quebec, Canada) and CEDEME Bioterio from Federal University of São Paulo, Brazil. The care of animals and research protocols conformed to the guiding principles for animal experimentation as enunciated by the Canadian Council on Animal Care and approved by the CEP – Research Ethics Committee of our university.

Tissue preparation. Quantitative Autoradiography and PCR real time: After killing under CO_2 inhalation, rats were decapitated and the brains and spinal cords were immediately removed, frozen in 2-methyl butane cooled at 45 to 55 °C in liquid nitrogen, and then stored at 80 °C until use. Matched whole brains and thoracic spinal cords (T8–T12) were mounted together (2 brains or 4 spinal cords per gelatin bloc) and serially cut into 20-µm thick sections on a cryostat with temperature varying between 10 to 12 °C. Sections were thaw-mounted on 0.2 % gelatin/0.033 % chromium potassium sulfate-coated slides and stored at 80 °C. Three thousand total slides were used for the autoradiography analysis. Quantitative autoradiography Iodination of HPP-Hoe 140 and B_2 receptor autoradiography were performed according to the method of chloramine T described by Ongali *et al*. (2003). Sections were thawed at room temperature and preincubated for 30 s in 25 mM PIPES buffer (pH 7.4, 48 °C). Thereafter, slides were incubated for 90 min at room temperature in 25 mM PIPES buffer containing 1 mM 1,10-phenanthroline, 1 mM dithiothreitol, 0.014 % bacitracin, 0.1 mM captopril, 0.2 % bovine serum albumin(protease free), and 7.5 mM magnesium chloride in the presence of 200 pM [^{125}I]HPP-Hoe 140 (specific activity:1212 Ci/mmol). The nonspecific binding was determined in the presence of 1 µM of unlabeled ligand. At the end of the incubation period, slides were transferred sequentially through four rinses of 4 min each in 25 mM PIPES (pH 7.4, 48 °C) and dipped for 15 s in distilled water (48 °C) to remove the excess of salts, and air-dried. Kodak Scientific BIOMAXTM MS1 were juxtaposed onto the slides in the presence of [^{125}I]-microscales and exposed at room temperature for 2 days. The methodology employed was similar to that described previously by Lopes *et al*., with some modifications. Competition binding assays were carried out under the same assay conditions described above, using 200 pM [^{125}I] HPP-Hoe 140 as radioligand in the presence of increasing concentrations (ranging from 10-16 to 10-5 M) of non-radiolabeled selective agonist (BK) and antagonists (HPP Hoe 140, Hoe 140 and LF16-0687) for B_2 receptor.

Image analysis. Densitometric readings were performed using an MCIDTM image analysis system (Imaging Research, St Catharines, Ontario, Canada). The number of

Table I. Oligonucleotides sentences.

Transcript	Gene symbol	Forward primer	Reverse primer
Gliceraldeide-3-phosphate desidrogenase	Gapdh	5_-GGAGATTGTTGCCATCAACGACC-3_	5_-GGTCATGAGCCCTTCCACAATGC-3_
Receptor B_2 de bradicinina	B_2R	5_-ACTCGGGTTTCTGTCGGTGCA-3_	5_-CCACTCGGTGTCTGGGCAGT-3_

replicates analyzed for each brain and spinal cord region was a minimum of 3 per animal. The nomenclature of anatomical structures and their abbreviations are adapted from the atlas of Paxinos & Watson (2013).

Tissue preparation for B_2R binding and mRNA expression. Spinal cord segments (T8–T11), brains, thoracic aorta were removed and immediately frozen in 2-methyl butane cooled at - 45 to - 55 °C with liquid nitrogen and kept at - 80 °C for autoradiography. The same tissues (Liver, Pa5, NTS and thoracic Spinal Cord) were removed and put in polyethylene microtubes containing TRizol reagent (Life Technologies, Burlington, Ont., Canada) and then frozen with liquid nitrogen and stored at -80 °C until extraction of total RNA and measurement by real-time polymerase chain reaction (real-time PCR). *In vitro* receptor autoradiography, used tissues of 4 rats per group were mounted together (2 brains or 4 spinal cords per gelatin bloc) and serially cut into 20-µm thick sections on a cryostat with temperature varying between -10 to – 12 °C. Sections were thaw-mounted on 0.2 % gelatin/0.033 % chromium potassium sulfate-coated slides and stored at - 80 °C. 3000 slides were used for the autoradiography analysis. The concentration of the radioligand [^{125}I]HPP-Hoe 140 chosen (200 pM) corresponds to maximal specific binding on the saturation curves (Bmax) in the all tissues analyzed in both treatment capsaicin and control rats on the 2nd day (Fig. 1). The nonspecific binding did not significantly differ between control and Capsaicin treatment.

Gene expression RNA were extracted according to manufactures protocol (Trizol and Qiagen). The SYBR Green System was used for quantification of gene expression. To this end, reactions were performed in a final volume of 20 µL containing 30–100 ng of cDNA or mtDNA, 10 µL of SYBR Green Universal PCR Master Mix 2x and 1 µL of each sense and antisense oligo (10 mM each). The cycling protocol was followed according to the determination of the unit standard 7500 from Applied Biosystems, including the dissociation curve. mRNA reactions were conducted with the following primers: (Table I).

Standard curves for each pair of oligo and cDNA sample group were made to establish the efficiency of these reactions. For the analysis of gene expression only reactions with high efficiency (> 95 %) were used. This allowed us to use the 2-DCt parameter for expressing arbitrary value of relative gene expression for each sample, using as endogenous control b-actin gene (mRNA).

Statistical analysis. The results were presented as the mean ± SEM of four animals per group. Statistical comparison of the data was performed with Graph-Pad Prism computer program using unpaired Student's t-test, or for multiple comparisons, one-way analysis of variance (ANOVA) followed by test T Tukey $P < 0.05$ was considered to be statistically significant and all values are expressed as mean ± SEM.

RESULTS

B_2 receptor Quantitative autoradiograpy analyses (binding sites). Specific kinin B_2 receptor binding sites were measured in control and capsaisin-sensitive treatment rats using [^{125}I]HPP-Hoe 140 as radioligand. Representative autoradiograms reveal that B_2 receptor binding sites are distributed throughout all the structures of the gray matter of the spinal cord, medulla oblongata and thoracic aorta (Fig. 1). The specific ligation range of 0,1277±0,04 to 2,303±0,54 fmol/mg (fentomol by miligram of tissue).

B_2 receptor binding sites displayed a distribution profile similar to that observed in control animals (Fig. 1). However, specific B_2 receptor densities were slightly but significantly in thoracic aorta. Representative autoradiograms showing the distribution of B_2 receptor binding sites in the brain of control depicted. Using the B_2 iodinated antagonist [^{125}I]HPP-HOE140 as radioligand to quantify and localize kinin B_2 receptors in brains specific binding sites were verifed all over the tissue.

Analysis of Gene Expression B_2 Receptor. For the analysis of gene expression of B_2R was performed RT-PCR (qRT-PCR). The qualitative RT-PCR was used only to confirm the gene expression of this receptor. Figure 2 shows the image of an agarose gel showing the amplified products of approximately 700 base pairs (bp) respectively. Amplification of the B_2 receptor gene encoding kinins, from qRT-PCR was obtained from different tissues of Central Nervous System regions (PA5, NTS, hypothalamus, spinal cord) and in peripheral tissues, as well as the thoracic aorta and liver. The liver tissue with intense vascularity was selected as a positive control for the amplification reaction.

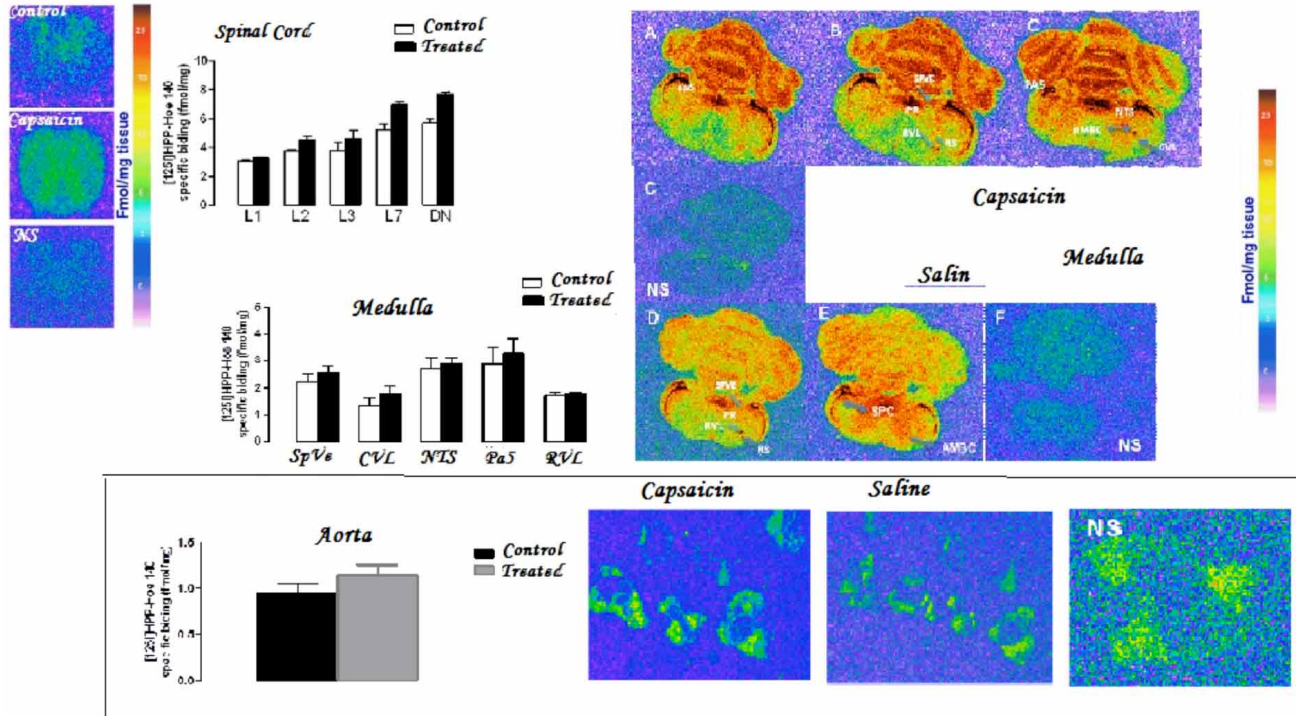

Fig. 1 - Spinal cord (left), Medulla (right) and AORTA (above): Colored pseudo images of radioautograms representing the anatomical distribution of B_2R in Wistar neonate rats. The nonspecific binding is represented by NS. (location based in Paxinos & Watson). Vertical bars in the graphics represent the mean ± standard error.

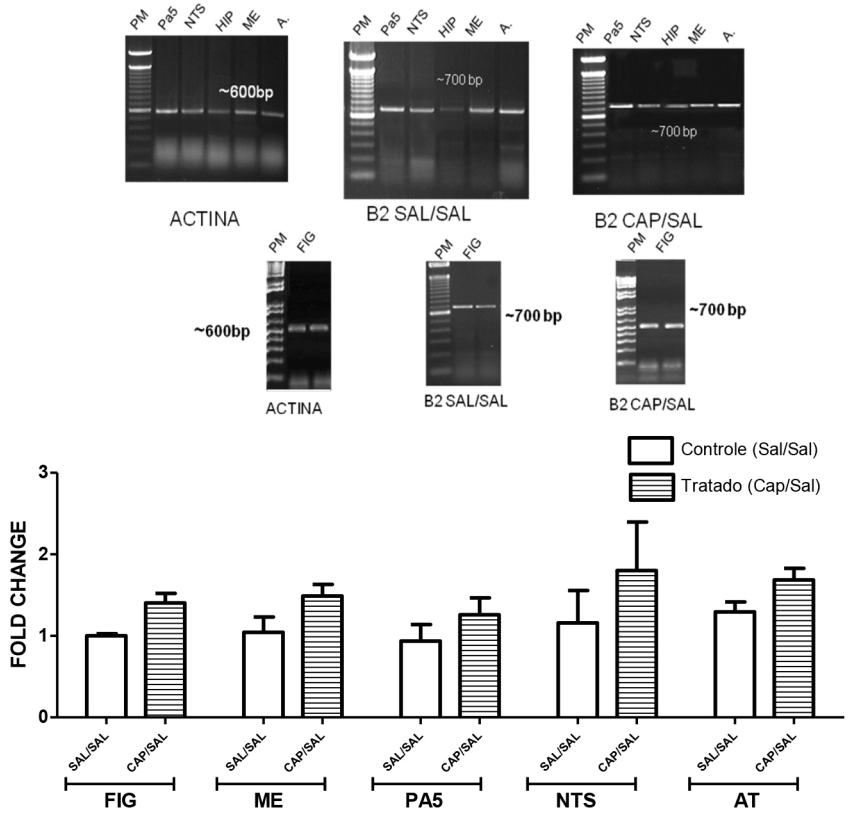

Fig. 2. Agarose gel showing the amplification of the kinin B_2 receptor. Product ~ 700pb B_2 receptor and β actin used as a positive control of reverse transcriptase reaction (RT) and amplification (PCR). (PM - molecular weight; B_2 salt / salt - saline control; B_2cap / salt - treated capsaicin). PA5 - paratrigeminal core, NTS - nucleus of the solitary tract, HIP - hypothalamus; ME – spinal cord, A –aorta. Above: Analysis of gene expression of kinin B_2 receptor after treatment with capsaicin and salt. FIG – liver, ME – spinal cord, PA5 – paratrigeminal core, NTS - nucleus of the solitary tract, AT – aorta. (T test non significant).

DISCUSSION

This study showed the distribution of the B_2 receptor binding sites of kinins in the spinal cord medulla cores and thoracic aorta, by quantitative autoradiography, as well as using real-time PCR to analyze gene expression of B_2 kinin receptor. The kallikrein-kinin system, of which bradykinin is the main representative, has raised interest, in view of the signaling pathways in which this peptide acts. B_2 bradykinin receptor has been found in many cell types such as vascular endothelium, primary sensory afferent neurons, vascular smooth muscle and non-vascular, epithelial cells, and perhaps in certain types of leukocytes, which gives bradykinin a wide physiological activities such as vasodilatation, increased vascular permeability, stimulation of sympathetic and sensory nerves connection (Coelho *et al*., 1997; Caous *et al*., 2001; Couture & Lindsey). The B2 kinin receptor has constitutive expression being relatively stable, regulated only by the transcription factor affinity for the promoter site of the gene (Pesquero et. al, 1996).

Studies have shown that treatment with capsaicin neonatal rats causes a permanent loss of A delta, C and small diameter fibers (Fitzgerald & Koltzenburg, 1986). To examine the relationship of these projections on the receivers, we used the autoradiography and molecular biology, to see if these losses could alter the expression and location of these receptors.

Treatment with capsaicin was determined to find out if the bradykinin receptor in paratrigeminal nucleus is located on the efferent core fibers. In other instances anatomical structures show that bradykinin also produces a pressor response when microinjected in PA5, demonstrating a functional glutaminergic receptor-mediated pathway in the nucleus, suggesting an excitatory action on the PA5 (Fior *et al*., 1993). Bilateral terminal fibers, connected to the paratrigeminal core was also observed in solitary tract nucleus, lateral reticular nucleus (LRT), ambiguous nucleus (Amb), rostroventrolateral reticular nucleus (RVL), beeing the vegetative actions explained by its projections (de Sousa Buck *et al*.; Caous *et al*., 2008). All binding sites of [^{125}I Tyr8] BK located in the medulla regions mainly of the solitary tract nucleus (NTS) and subnuclear structures project to the core of the spinal trigeminal tract of rats as indicated elsewhere (Privitera *et al*., 2003). The present results show that treatment of capsaicin does not alter the B_2 bradykinin receptor expression, suggesting that the response mediated kinin receptor is localized on sensory afferents affected by the neurotoxin. The change was not significant when compared with the control group, this observation can also be seen in the analysis of some medulla nuclei (PA5, NTS) and spinal cord using qPCR, RT and also in peripheral tissues such as the thoracic aorta and liver, in which the same result was found, with no significant difference between the groups. The distribution of binding sites was located in the spinal cord, aorta and medulla regions. Central pressor effect of bradykinin on the dorsolateral spinal cord was also demonstrated (Fior *et al*.). On the spinal cord of both control animals and animals treated, the specific connections are preferably in surface Rexed laminae I, II, III, VII and dorsal nucleus with B_2 iodinated [^{125}I] HPP-Hoe 140. Similarly the binding sites in the medulla are in the paratrigeminal nucleus, the solitary tract nucleus, spinal vestibular nucleus (SPVE), rostral ventrolateral nucleus (RVL), vestibular caudoventrolateral nucleus (CVL).

We also used the thoracic aorta, a well-vascularized area that possesses a lot of kinin B_2 receptor. As a constitutive receptor, it was important to consider whether there would be differences in the expression and quantification of this receptor after capsaicin (França *et al*.). This hypothesis was supported by the B_2 receptor involvement in nociception and antinociception (Calixto *et al*., 2001; 2004; Couture *et al*., 2001). The marking ion binding sites was observed in the fibers terminals associated with synaptic buttons in the nucleus ambiguus, and rostral ventrolateral nucleus (RVL) (Finley & Katz, 1992). Among the nuclei of the ventral medulla, for whom PA5 projections, the RVL is the most involved with the control of vasomotor tone (Caous *et al*., 2001). The spinal vestibular nucleus (SpVe), was analyzed by tracer and highlighted its connections to the spinal cord (Liang *et al*., 2015). In the spinal cord, the analysis of the binding sites present, suggests that cardiovascular regulation of bradykinin occurs (Ongali *et al*.). The occurrence of gene expression of B_2 kinin receptor mRNA was verified by both in peripheral tissues and the central (PA5, NTS, aorta, liver and spinal cord). In the organ and tissues examined, the mRNA levels encoding for the B_2 receptor kinin were analyzed using as internal control, the concentration of the β-actin mRNA. The high expression of RNA encoding for kinin receptors in the liver is due to the high organ irrigation (Couture *et al*., 2000).

In this study, we examine whether there would be differences in the expression and quantification of this receptor after treatment with capsaicin using qPCR, RT, which was obtained the same result, no significant difference between the groups, as occurred with autoradiography.

Among Central Nervous System structures, NTS, PA5 and spinal cord showed a greater amount of RNA encoding for the B_2 kinin receptor. It is possible that the cerebral vasculature contributes to the localization and expression of this receptor. The qPCR-RT methodology and autoradiography used to distinguish respectively the mRNA and B_2R kinin location in the medulla nuclei, spinal cord and peripheral tissues.

The results obtained with respect NTS, PA5, aorta and spinal cord have shown no significance with respect to control and treated animals. As noted in studies, the lesions C and A delta fibers do not prevent the actions of BK or alter isotopically-labeled radioligand (binding) the B_2 receptor PA5 (Lindsey *et al*., 1997; Couture & Lindsey). These data plus the results of the present study, suggest that this receptor is not present inafferents fibers sensitive to capsaicin.

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DE FRANÇA, C. E.; GUARDABASSI, C. & SMITH, R. L. Expresión y distribución del receptor B_2 de cininas en la médula y tejidos medular espinal de ratones tratados con capsaicina. *Int. J. Morphol., 34(4)*:1465-1471, 2016.

RESUMEN: Las cininas son péptidos vaso activos que participan de mecanismos de dolor e inflamación, con la promoción de reflejos cardiovasculares defensivos producidos por estímulos nocivos. Estos péptidos señalizan por medio de la activación de dos receptores transmembrana acoplados a proteína G llamados receptores B_1 y B_2 (B_1R y B_2R). El B_2R es constitutivo en tejidos saludables y en animales. El objetivo de este estudio consistió en tomar las medidas de la expresión del gen y la proteína del receptor B_2R de cinina en tejidos centrales y periféricos aislados de ratones control y ratones pre tratados con capsaicina en el segundo día de vida (50 mg/kg, s.c.) o con dos semanas antes del sacrificio (125 mg/kg por tres días, s.c.). El mismo tratamiento fue hecho con solución salina en los animales control. Fueron medidos niveles de mRNA para B_2R por RT-PCR en forma cuantitativa y cualitativa, en cuanto los sitios de unión del receptor fueron medidos en secciones de tejido con radioligante ^{125}I-HPP-Hoe 140 (B_2R). B_2R fue expresado en todos los tejidos estudiados (hipotálamo, núcleo paratrigeminal, núcleo del tracto solitario, médula espinal, aorta e hígado). El tratamiento con capsaicina en ratones neonatos comparados con controles no presentaron afectación del nivel de expresión. Por otro lado, la capsaicina no tuvo efecto significante en la expresión de los sitios de unión de B_2R en los tejidos. La síntesis del receptor B_2R de cininas no está asociado con fibras C sensoriales así como los tejidos no mostraron diferencias significativas indicando que B_2R es regulado por mecanismos distintos.

PALABRAS CLAVE: Médula; Ratones; Receptor B_2 de Cinina; Capsaicina.

REFERENCES

Calixto, J. B.; Cabrini, D. A.; Ferreira, J. & Campos, M. M. Inflammatory pain: kinins and antagonists. *Curr. Opin. Anaesthesiol., 14(5)*:519-26, 2001.

Calixto, J. B.; Medeiros, R.; Fernandes, E. S.; Ferreira, J.; Cabrini, D. A. & Campos, M. M. Kinin B_1 receptors: key G-protein-coupled receptors and their role in inflammatory and painful processes. *Br. J. Pharmacol., 143(7)*:803-18, 2004.

Caous, C. A.; de Sousa Buck, H. & Lindsey, C. J. Neuronal connections of the paratrigeminal nucleus: a topographic analysis of neurons projecting to bulbar, pontine and thalamic nuclei related to cardiovascular, respiratory and sensory functions. *Auton. Neurosci., 94(1-2)*:14-24, 2001.

Caous, C. A.; Balan, A. & Lindsey, C. J. Bradykinin microinjection in the paratrigeminal nucleus triggers neuronal discharge in the rat rostroventrolateral reticular nucleus. *Can. J. Physiol. Pharmacol., 82(7)*:485-92, 2004.

Caous, C. A.; Koepp, J.; Couture, R.; Balan, A. C. & Lindsey, C. J. The role of the paratrigeminal nucleus in the pressor response to sciatic nerve stimulation in the rat. *Auton. Neurosci., 140(1-2)*:72-9, 2008.

Couture, R. & Lindsey, C. J. *Brain Kallikrein-Kinin System: From Receptors to Neuronal Pathways and Physiological Functions.* In: Quirion, R.; Björklund, A. & Hökfelt, T. (Eds.). Handbook of Chemical Neuroanatomy. Vol. 16: Peptides Receptors. Amsterdan, Elsevier, 2000.

Couture, R.; Toma, N. & Barbot, L. SR142801 behaves as a tachykinin NK-3 receptor agonist on a spinal nociceptive reflex in the rat. *Life Sci., 66(1)*:51-65, 2000.

Couture, R.; Harrison, M.; Vianna, R. M. & Cloutier, F. Kinin receptors in pain and inflammation. *Eur. J. Pharmacol., 429(1-3)*:161-76, 2001.

Coelho, M. M.; Oliveira, C. R.; Pajolla, G. P.; Calixto, J. B. & Pelá, I. R. Central involvement of kinin B_1 and B_2 receptors in the febrile response induced by endotoxin in rats. *Br. J. Pharmacol., 121(2)*:296-302, 1997.

Damasceno, I. Z.; Melo, K. R.; Nascimento, F. D.; Souza, D. S.; Araujo, M. S.; Souza, S. E.; Sampaio, M. U.; Nader, H. B.; Tersariol, I. L. & Motta, G. Bradykinin release avoids high molecular weight kininogen endocytosis. *PLoS One, 10(3)*:e0121721, 2015.

de Sousa Buck, H.; Ongali, B.; Thibault, G.; Lindsey, C. J. & Couture, R. Autoradiographic detection of kinin receptors in the human medulla of control, hypertensive, and diabetic donors. *Can. J. Physiol. Pharmacol., 80(4)*:249-57, 2002.

Finley J.C. & Katz D.M. The central organization of carotid body afferent projections to the brainstem of the rat. *Brain Res., 572(1-2)*: 108-16, 1992.

França, C. E.; Vicari, C. F.; Piza, A. M.; Geroldo, E. A.; Beçak, M. L.; Beçak, W.; Stocco, R. C. & Lindsey, C. J. The kinin B(2) receptor gene structure, product processing and expression in adult and fetal rats: evidence for gene evolution. *Genet. Mol. Res., 9(1)*:215-30, 2010.

Fior, D. R.; Martins, D. T. & Lindsey, C. J. Localization of central pressor action of bradykinin in medulla oblongata. *Am. J. Physiol., 265(3 pt. 2)*:H1000-6, 1993.

Fitzgerald, M. & Koltzenburg, M. The functional development of descending inhibitory pathways in the dorsolateral funiculus of the newborn rat spinal cord. *Brain Res., 389(1-2)*:261-70, 1986.

Hock, F. J.; Wirth, K.; Albus, U.; Linz, W.; Gerhards, H. J.; Wiemer, G.; Henke, S.; Breipohl, G.; König, W.; Knolle, J. & Schölkens, B. A. Hoe 140 a new potent and long acting bradykinin-antagonist: in vitro studies. *Br. J. Pharmacol., 102(3)*:769-73, 1991.

Leeb-Lundberg, L. M.; Marceau, F.; Müller-Esterl, W.; Pettibone, D. J. & Zuraw, B. L. International union of pharmacology. XLV. Classification of the kinin receptor family: from molecular mechanisms to pathophysiological consequences. *Pharmacol. Rev., 57(1)*:27-77, 2005.

Liang, H.; Bácskai, T. & Paxinos, G. Termination of vestibulospinal fibers arising from the spinal vestibular nucleus in the mouse spinal cord. *Neuroscience, 294*:206-14, 2015.

Lindsey, C. J.; Bendhack, L. M. & Paiva, A. C. Effects of teprotide, captopril and enalaprilat on arterial wall kininase and angiotensin converting activity. *J. Hypertens. Suppl., 5(2)*:S71-6, 1987.

Lindsey, C. J.; Buck, H. S.; Fior-Chadi, D. R. & Lapa, R. C. Pressor effect mediated by bradykinin in the paratrigeminal nucleus of the rat. *J. Physiol., 502(Pt. 1)*:119-29, 1997.

Lopes, P.; Kar, S.; Chrétien, L.; Regoli, D.; Quirion, R. & Couture, R. Quantitative autoradiographic localization of [125I-TYR8]bradykinin receptor binding sites in the rat spinal cord: Effects of neonatal capsaicin, noradrenergic deafferentation, dorsal rhizotomy and peripheral axotomy. *Neuroscience, 68(3)*:867-81, 1995.

Lungu, C.; Dias, J. P.; França, C. E.; Ongali, B.; Regoli, D.; Moldovan, F. & Couture, R. Involvement of kinin B1 receptor and oxidative stress in sensory abnormalities and arterial hypertension in an experimental rat model of insulin resistance. *Neuropeptides, 41(6)*:375-87, 2007.

Marceau, F. & Bachvarov, D. R. Kinin receptors. *Clin. Rev. Allergy Immunol., 16(4)*:385-401, 1998.

Muto, Y.; Sakai, A.; Sakamoto, A. & Suzuki, H. Activation of NK_1 receptors in the locus coeruleus induces analgesia through noradrenergic-mediated descending inhibition in a rat model of neuropathic pain. *Br. J. Pharmacol., 166(3)*:1047-57, 2012.

Ongali, B.; Campos, M. M.; Bregola, G.; Rodi, D.; Regoli, D.; Thibault, G.; Simonato, M. & Couture, R. Autoradiographic analysis of rat brain kinin B1 and B2 receptors: normal distribution and alterations induced by epilepsy. *J. Comp. Neurol., 461(4)*:506-19, 2003.

Paxinos, G. & Watson, C. *The Rat Brain in Stereotaxic Coordinates.* 7th ed. San Diego, Elsevier Academic Press, 2013.

Pessini, A. C.; Santos, D. R.; Arantes, E. C. & Souza, G. E. Mediators involved in the febrile response induced by Tityus serrulatus scorpion venom in rats. *Toxicon, 48(5)*:556-66, 2006.

Pesquero, J. B.; Lindsey, C. J.; Paiva, A. C.; Ganten, D. & Bader, M. Transcriptional regulatory elements in the rat bradykinin B2 receptor gene. *Immunopharmacology, 33(1-3)*:36-41, 1996.

Privitera, P. J.; Beckstead, R. M.; Yates, P. & Walgren, R. Autoradiographic localization of [125I-Tyr0]bradykinin binding sites in brains of Wistar-Kyoto and spontaneously hypertensive rats. *Cell. Mol. Neurobiol., 23(4-5)*:805-15, 2003.

Regoli, D.; Rizzi, A.; Perron, S. I. & Gobeil, F. Jr. Classification of kinin receptors. *Biol. Chem., 382(1)*:31-5, 2001.

Scheuren, R.; Duschek, S.; Schulz, A.; Sütterlin, S. & Anton, F. Blood pressure and the perception of illusive pain. *Psychophysiology, 53(8)*:1282-91, 2016.

Sharma, S. K.; Khanna, N. & Bhandari, S. Should beta-blockers continue to open against hypertension? *Indian Heart J., 58(4)*:297-300, 2006.

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