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Neuropeptide Galanin Increase ROS and IL-1 β Production by Blood Cells from Patients with Multiple Sclerosis

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Abstract

Objective: Galanin (GAL), a neuropeptide produced in the central and peripheral nervous system, has been involved in the modulation of the inflammatory response. Our present study aimed to evaluate the effect of GAL on the production of reactive oxygen species (ROS) by granulocytes and the secretion of IL-1β by peripheral blood mononuclear cells (PBMNC) from patients with Multiple Sclerosis (MS) and healthy control cells, comparatively.

Methods: Granulocytes and PBMNC from patients with MS and healthy controls were purified simultaneously by the Ficoll-Hypaque gradient method. The effect of GAL on ROS generation by granulocytes was performed in a luminol-dependent chemiluminescence assay. The quantification of IL-1β in the supernatant of cultured-PBMNC was determined using immunoassay (ELISA).

Results: Our results showed that ROS production in the presence of GAL was significantly higher ($p<0.05$) in granulocytes of MS patients than that observed in healthy control cells. GAL activated IL-1β secretion similarly in both PBMNC of MS patients and healthy controls ($p>0.05$).

Conclusion: GAL modulates the production of ROS and activates inflammasome evaluated by the increase of IL-1β secretion and it may have consequences in the inflammatory process observed in MS.

Keywords: Multiple sclerosis; Galanin; Reactive oxygen species; IL-1β; Inflammasome; Inflammation; Innate immunity; Granulocytes; Mononuclear cells

Introduction

Multiple sclerosis (MS) is a neurodegenerative disease that affects the central (CNS) and peripheral (PNS) nervous system. Neuronal demyelination in the brain and spinal neurons is observed in MS leading to the total or partial interruption of nervous influx [1-3]. It is reported that the neuropeptide Galalin (GAL) is secreted mainly by oligodendrocytes, astrocytes and gastrointestinal apparatus protecting against demyelination and promoting myelination of the neuron [4,5]. Galanin (GAL) is a neuropeptide-containing a 29/30 amino acid and its biological action occur through interaction with three different receptors, GALR1, GALR2 and GALR3 [6-8] with main distribution in the CNS, PNS, and intestine [6,9]. Galanin is an immunomodulatory neuropeptide and act regulating several physiological processes, [6,10-14]. ROS and pro-inflammatory cytokines promote the migration of inflammatory cells (neutrophils, macrophages, lymphocytes) to the brain due to the increase of permeability of the blood-brain barrier (BBB) [2,3]. In pathological conditions such as MS, the increase in ROS production exceeds the physiological threshold, generating oxidative stress, an important factor associated with the development of demyelination [15-21]. Among proinflammatory cytokines, IL-1β plays a pivotal role in increasing the permeability of the BBB [22,23]. Its production depends on the inflammasome activation [24], a multiprotein complex of intracellular signalling, sensitive to oxidative stress. Inflammasome induces the maturation and secretion of IL-1β and IL-18 through the activation of caspase-1 besides inducing pyroptosis, a type of inflammatory cell death [25]. This study aimed to evaluate the role of galanin in the modulation of the production of ROS and IL-1β secretion by granulocytes and PBMNC, respectively, from MS patients.

Material and Methods

Ethical approval

The Ethical Committee from Santa Casa Hospital of Belo Horizonte-Brazil approved this study, and the informed consent was obtained from all participants included in the study.

Study population

Patients diagnosed with multiple sclerosis and healthy control, were selected by Dr. Paulo Pereira Christo, at the Neurology service of Santa Casa Hospital (Belo Horizonte, Minas Gerais, Brazil). Volunteers were within the age range of 18 and 65 years. Subjects presenting dementia, inflammation, infection or cancer were excluded from the study, as were pregnant women and individuals with alcohol or tobacco dependency.

Reagents

Human galanin compound was purchased from Merck (Merck KGaA, Darmstadt, Germany). For experiments, the concentration (2

μg/100 μL) was used according to previous studies performed by Agasse et al. [26].

Cell separation

Granulocytes and PBMNC were obtained from peripheral blood, according to Bicalho et al. [27], with slight modifications. Briefly heparinized peripheral venous blood samples (10 mL) were subjected to double-gradient density (1.08 and 1.12). The volume proportion among discontinuous gradient and blood were 4:3:3, respectively, from the bottom to the top using siliconized glass or Falcon tubes. After centrifugation during 30-40 min, layers at the top and middle interfaces were collected to yield fractions rich in PBMNC and granulocytes, respectively. The cells were identified and counted based on morphology, granulation and size using a stereoscopic microscope with 400X magnification. Cellular viability was evaluated by the Trypan Blue exclusion test.

Quantification of ROS production

A luminol-based chemiluminescence method was employed to assess the oxidative responses of granulocytes. An aliquot (200 μL) of of luminol (10^{-4} M) was mixed with a 100 μL of granulocytes suspension (1×10^6/mL) in phosphate buffered saline (PBS). The chemiluminescence assay was performed on the Turner BioSystems model 20/20 n luminometer (Promega, Sunnyvale, CA, USA) for 20 min (control without stimulation), following which 100 μL of GAL (2000 nM) was added to the reaction mixture and chemiluminescence was performed for an additional 25 min.

Quantification of IL-1β in supernatant of cultured PBMNC

PBMNC (1×10^5/100 μL) from MS patients and healthy controls in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) were incubated in the presence or absence of GAL (2 μM) for 72 h at 37°C under 5% CO_2. The final volume was adjusted to 300 μL in DMEM supplemented with 10% FBS. After incubation, the cells were centrifuged (200 g for 15 min) and the supernatant was collected. The interleukin-1β (human IL-1β-BioLegend, Inc., California, USA, cat. #437006) concentrations were determined through enzyme-linked immunosorbent assay (ELISA) according to the manufacturer instructions.

Statistical analysis

The Kolmogorov-Smirnov test was used to assess the normal distribution of the continuous variables; values were expressed as mean ± standard error. The Kolmogorov-Smirnov test was used to evaluate sample normality. Comparisons between groups were performed using unpaired Student t or the χ^2 test. All analyses were considered significant at values <0.05 using GraphPad Prism 5 (GraphPad Software, Inc).

Results

ROS production by granulocytes from MS patients increases in the presence of galanin

ROS production by granulocytes of MS patients and healthy controls in the presence or the absence of galanin are shown in Figures 1 and 2. The basal ROS production (absence of GAL) by granulocytes from MS patients and control group were similar (p>0.05). In the presence of GAL was observed an inhibition (26%) of ROS generation in cells from healthy control and activation (32%) in granulocytes from MS patients. The comparison was significant at p<0.05.

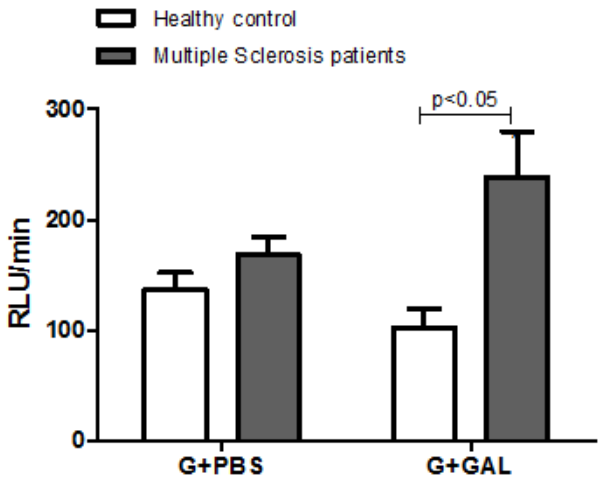

Figure 1: Production of reactive oxygen species (ROS) in galanin-stimulated granulocytes of patients with multiple sclerosis and healthy control. Values expressed as mean ± standard error; analysis determined by student's "t" test. RLU=Relative light unit; G=Granulocytes; PBS=Hosphate-saline buffer. n=20 for each group.

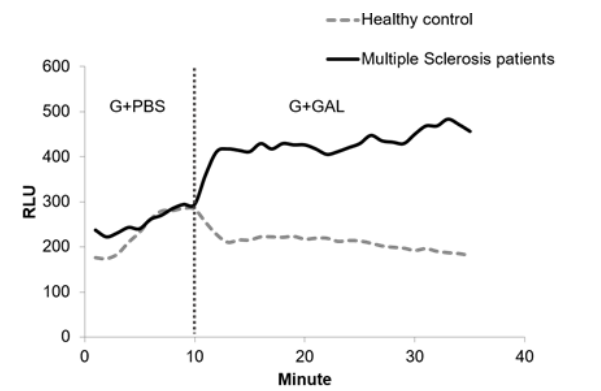

Figure 2: Typical curve of kinetic study of GAL-induced reactive oxygen species (ROS) production by granulocytes from patients with multiple sclerosis and healthy control. RLU=Relative light unit; G=Granulocytes; PBS=phosphate-saline buffer; GAL=Galanin

Galanin increases the secretion of IL-1β by PBMNC from MS patients and healthy controls

Figure 3 shows that Galanin activates IL-1β secretion similarly in both PBMNC from MS patients and healthy controls (p>0.05). The results, expressed as pg/mL (mean ± standard error), were 8.6 ± 2.0 and 9.7 ± 1.2 in the absence of GAL and 41.1 ± 13.0 and 39.9 ± 12.6 for healthy controls and MS patients, respectively. The results on IL-1β secretion in the absence and in the presence of GAL were significantly different at p<0.05.

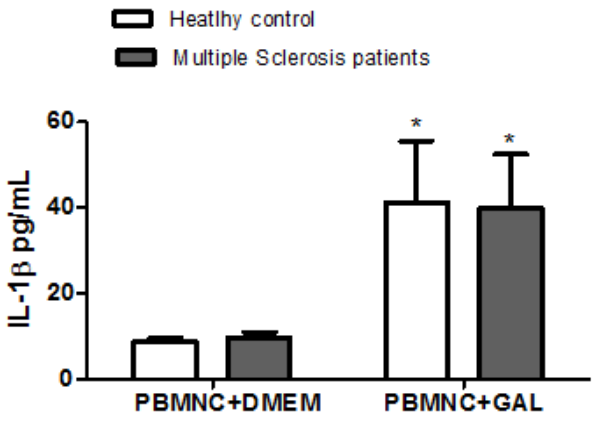

Figure 3: Galanin (GAL) activates the production of IL-1β in mononuclear cells (PBMNC) of patients with multiple sclerosis and healthy control. Values expressed as mean ± standard error; analysis determined by student's "t" test. *p<0.05 *vs.* PBMNC+DMEM. n=20 for each group.

Discussion

Galanin activated production of ROS and the secretion of IL-1β in cells from patients with multiple sclerosis (Figures 1-3). The action of GAL depends on the interaction with the respective receptors (GALR1-3) [7,12,28,29] which are associated with G-protein coupled receptor (GPCR) family. Thus, GALR1 linked to the subunits of G-protein, Gi/o, is responsible for the inhibition of adenyl cyclase and activation of small GTPase (Ras). GALR2 is associated with G12/13 G-protein subunits and activates phospholipase C (PLC) leading to the formation of diacylglycerol (DAG) and inositoltiphosphate (IP3). GALR3 inhibits adenyl cyclase, Rho, and Cdc42 by the Gi/o subunits. Several studies have shown that oxidative stress is an essential factor in the pathogenesis of demyelinating diseases [30-32]. Our results demonstrated that ROS production by granulocytes induced by galanin was significantly higher in cells from MS patients than that observed in healthy controls ($p<0.05$) (Figures 1 and 2). Sanadgol et al. [33] reported an active role for ROS in the pathogenesis of MS. Gruber et al. [34] demonstrated that increased ROS production interferes with myelin expression by oligodendrocytes. The ROS generation increases during the destruction of oligodendrocytes, astrocytes, and exacerbate the inflammatory process [33,35].

The present results may suggest that the action of galanin on granulocytes could involve mainly the activation of GALR2, PLC and consequently the signalling pathway DAG-PKC (protein kinase C)-NADPH-oxidase leading to the production of ROS. It is well known that inflammasome can be activated by ROS [36-39]. The production of pro-IL-1β by NF-κB in the presence of ROS promote inflammasomes activation and secretion of IL-1β [36,40,41]. GAL induced the increase of secretion of IL-1β in cell culture supernatant of PBMNC from MS patients and control group (Figure 3). Studies have identified IL-1β and caspase-1 on sclerotic plaques, and increased levels of caspase-1 and IL-18 in PBMNC of MS patients [42,43]. Inoue et al. [44,45] demonstrated that the activation of NLRP3 in experimental MS model promotes the migration of inflammatory cells to the CNS. The inflammatory process induced by GAL appears to be complicated, and a possible network of several signalling pathways could be involved in the pathogenesis of MS. The understanding of these inflammatory mechanisms and the exact role of GAL in MS may offer subsidies to the identification of new therapeutic strategies.

Conflict of Interest

The authors declare that they have no conflicts of interes.

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