Intracerebroventricular Injection of Lipopolysaccharide Increases Gene Expression of Connexin32 Gap Junction in Rat Hippocampus

Mohammad Abbasian[1*], Mohammad Sayyah[2], Vahab Babapour[3], Reza Mahdian[4]

1. Department of Physiology, Faculty of Veterinary Medicine, Science and Research Branch, Islamic Azad University, Tehran, Iran.
2. Department of Physiology and Pharmacology, Pasteur Institute of Iran, Tehran, Iran.
3. Department of Physiology, Faculty of Veterinary Medicine, Tehran University, Tehran, Iran.
4 Biotechnology Research Center, Dept. of Molecular Medicine, Pasteur Institute of Iran.

A B S T R A C T

Introduction: Gap junctions are intercellular membrane channels that provide direct cytoplasmic continuity between adjacent cells. This communication can be affected by changes in expression of gap junctional subunits called Connexins (Cx). Changes in the expression and function of connexins are associated with number of brain neurodegenerative diseases. Neuroinflammation is a hallmark of various central nervous system (CNS) diseases, like multiple sclerosis, Alzheimer's disease and epilepsy. Neuroinflammation causes change in Connexins expression. Hippocampus, one of the main brain regions with a wide network of Gap junctions between different neural cell types, has particular vulnerability to damage and consequent inflammation. Cx32 – among Connexins– is expressed in hippocampal Olygodandrocytes and some neural subpopulations. Although multiple lines of evidence indicate that there is an association between neuroinflammation and the expression of connexin, the direct effect of neuroinflammation on the expression of connexins has not been well studied. In the present study, the effect of neuroinflammation induced by the Lipopolysaccharide (LPS) on Cx32 gene and protein expressions in rat hippocampus is evaluated.

Methods: LPS (2.5μg/rat) was infused into the rat cerebral ventricles for 14 days. Cx32 mRNA and protein levels were measured by Real Time PCR and Western Blot after 1st, 7th and 14th injection of LPS in the hippocampus.

Results: Significant increase in Cx32 mRNA expression was observed after 7th injection of LPS (P<0.001). However, no significant change was observed in Cx32 protein level.

Conclusion: LPS seems to modify Cx32 GJ communication in the hippocampus at transcription level but not at translation or post-translation level. In order to have a full view concerning modification of Cx32 GJ communication, effect of LPS on Cx32 channel gating should also be determined.

1. Introduction

Gap junctions are specialized cell-cell contacts between eukaryotic cells, composed of aggregates of transmembrane channels, which directly connect the cytoplasm of adjacent cells, allowing intercellular movement of small molecular weight molecules (up to 1 KDa) including ions, metabolites and second messengers (Condorelli et al., 2003; Sohl et al., 2005). Each channel consists of two hemi-

* **Corresponding Author:**
Mohammad Abbasian, PhD.
Department of Physiology, Faculty of Veterinary Medicine, Science and Research Branch, Islamic Azad University, Tehran, Iran.
Tel/Fax: +98 914 4410284
E-mail: abbasian.phd@gmail.com

channels (termed connexons), each of which is composed of six subunit proteins called connexin (Cx) (Sohl et al., 2005). A total of 21 Cx family members have been identified in the mammalian genome (Kielian, 2008). Gap junctions facilitate ionic homeostasis and synchronization of action potential in the nervous system (Sohl et al., 2000). Alteration in the expression and the function of connexins are associated with several of brain pathologies and neurodegenerative diseases, suggesting that they could contribute to the expansion of brain damages (Rouach et al., 2002). Some studies have provided evidence that gap-junctional communication is associated with the spread of cell death signals, while others have equally demonstrated neuroprotective effects (Cronin et al., 2008; Froger et al., 2009, 2010). Enhanced gap junctional coupling is proposed as a possible mechanism underlying neuronal synchronization (Li et al., 2001). Gap junction coupling can be regulated at several levels including alteration in Cx transcription, translation, stability, post translational processing and channel gating (Saez et al., 2003; Garg et al., 2005).

Inflammation is a hallmark of various CNS diseases such as bacterial and viral infections and cerebral ischemia (Garg et al., 2005). Brain injuries as well as neurodegenerative diseases, are associated with neuroinflammation (Froger et al., 2009). Alterations in Cx expression have been associated with neuroinflammation (Garg et al., 2005). However, the direct effects of neuroinflammation on the gene and protein regulation and expression of connexins as building blocks of gap junctions has not been fully characterized.

One of the main brain regions with a wide network of Gap junctions between different neural cell types is hippocampus, which has particular vulnerability to damage due to hypoglycemia, ischemia/hypoxia, trauma and subsequent Neuroinflammation (Sohl et al., 2000; Zeinieh et al., 2010; Karpuk et al., 2011). Among Cxs, Cx32 – which is generally expressed in Olygodandrocytes and some neural subpopulations – is well represented throughout the CNS (Sohl et al., 2000; Bennett et al., 2004). Cx32 in hippocampus is predominantly expressed in oligodendrocytes (oligodendrocyte/ oligodendrocyte or oligodendrocyte/astrocyte GJs) and parvalbumin-positive inhibitory interneurons of CA1 subfield (Rouach et al., 2001; Rash et al., 2002). There is no report regarding changes of this Cx during neuroinflammation.

The bacterial Endotoxin Lipopolysaccharide (LPS) is a stimulator of microglia and is used extensively as a model of neuroinflammation (Turrin et al., 2001; Kovacs et al., 2006).

The present study was undertaken to examine the changes of Cx32 mRNA and protein expression in rat hippocampus consequent to acute and chronic intracerebroventricular (i.c.v) injection of LPS.

2. Methods

2.1. Animals

Male Wistar rats (280–320 g, Institute Pasteur of Iran) were used in this study. The animals were housed in standard Plexiglas cages with free access to food (standard laboratory rodent's chow) and water. The animal house temperature was maintained at 23 ± 1.0 °C with a 12-h light/dark cycle (light on from 6.00 a.m.). All animal experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) in such a way to minimize the number of animals used and their suffering. Each animal was tested once.

2.2. Materials

Ketamine (Rotex Medica, Germany), Xylazine (Chanelle, Ireland), LPS (Escherichia coli serotype 026:B6, Sigma, UK), RNX-Plus Reagent, Agarose, Acrylamide (CinnaGene, Iran) Quantitect Reverse Transcriptase kit (Qiagen, Germany), Power Syber Green PCR Master Mix, Rox and dNTP mix (Warrnington, UK) Page Ruler™ Prestained Protein Ladder and Protein Loading Buffer Pack (Fermentas, Lithuania), Protease Inhibitor Cocktail (Roche, Germany), Enhanced Chemiluminescence (ECL) Advance Western Blotting Detection Kit (Amersham, UK). Monoclonal anti-connexin 32, Monoclonal anti-α-tubulin and anti-mouse IgG peroxidase conjugates (Sigma-Aldrich, USA) were used in this study. LPS was dissolved in phosphate buffer solution (PBS) and was prepared freshly on the day of use.

2.3. Stereotaxic Surgery and LPS Injection

The rats were stereotaxically implanted with a cannula in the left lateral ventricle (Paxinos & Watson, 2007). The animals were given 7 days recovery after surgery, before the injection protocol was started. LPS at the dose of 2.5 μg/rat was infused once daily i.c.v. for 14 days. For each experimental group, a sham (cannula-implanted non-injected) and a control (cannula-implanted phosphate buffer (PBS)-injected) groups were considered.

2.4. Tissue Collection

To measure changes in Cxs mRNA expression, the hippocampi were dissected 24 h after 1st, 7th and 14th in-
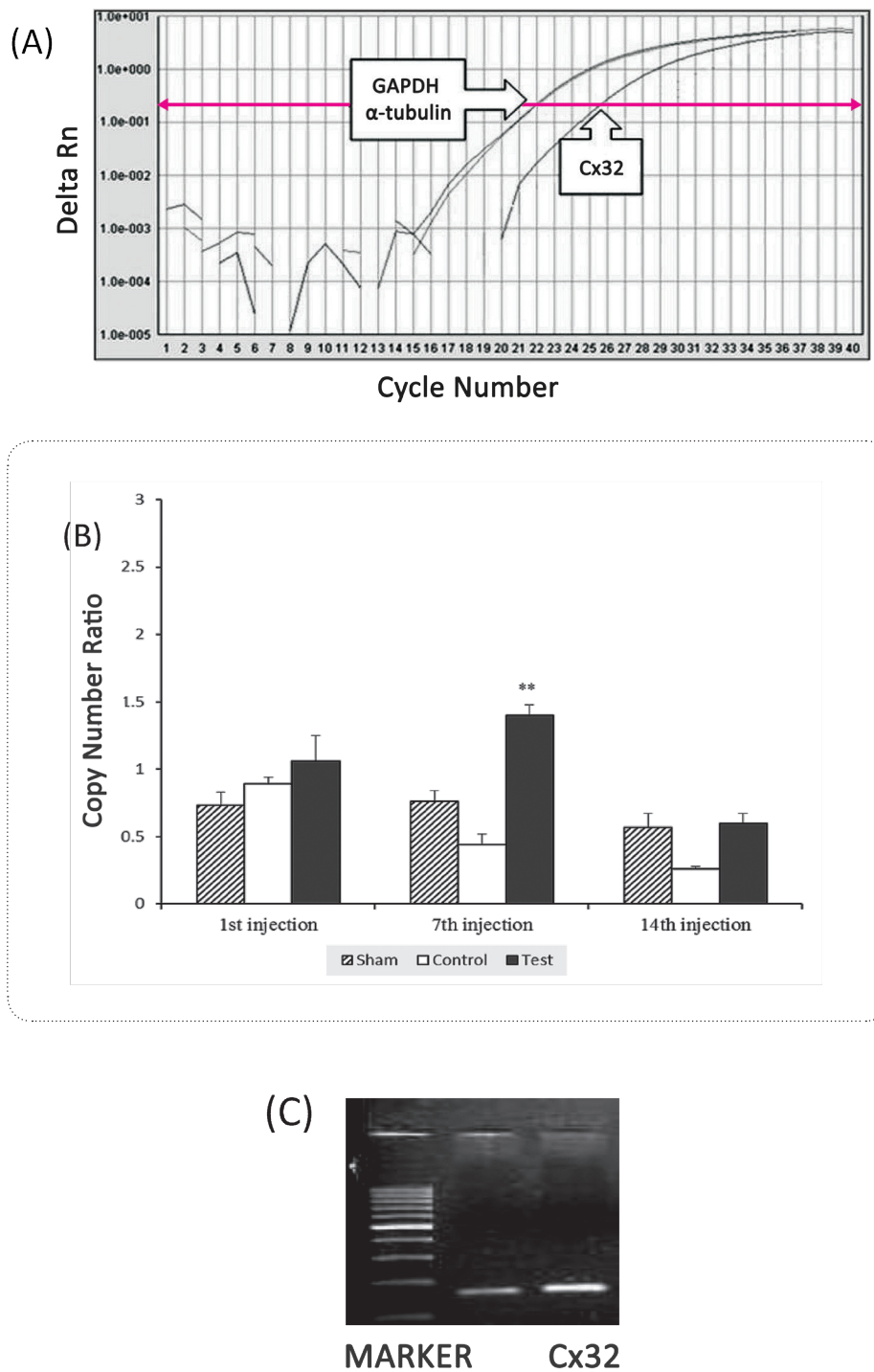
Figure 1 (A). Amplification plots of the target and reference genes (Cx32, α-Tubulin, GAPDH) in the Real-time PCR assay. The amplification curves of the both reference genes have crossed the threshold line at the same point. (mCt) Mean threshold cycle. (mCt GAPDH and α-Tubulin=21.95, mCt Cx32=25.61). (B) Cx32 mRNA level in the hippocampus of the rats after daily intracerebroventricular injection of LPS. Connexin mRNA level was normalized to α-tubulin and GAPDH mRNA level. Data are expressed as means ± S.E.M (n=5). ** $p<0.001$ compared to respective control group. (C) Denaturing agarose gel electrophoresis to evaluate samples for other DNA contamination during RT-PCR reaction and also proves the integrity of the samples in RNA extraction process.

jections of LPS. All the animals and their corresponding controls were decapitated under deep Ether anesthesia and their brain were removed immediately. The brains were incubated in chilled artificial cerebrospinal fluid (ACSF) with pH 7.3 consisted of the following composition (in mM): 124 NaCl, 4.4 KCl, 2 CaCl2, 2 MgCl2, 1.2 KH2PO4, 25 NaHCO3 and 10 Glucose. The hippocampus of the brains were removed and frozen immediately in liquid nitrogen and stored at -80°C. The rest of the brains were placed in 10% formalin for at least 3 days at room temperature and they were then processed, cut into 10μm thick slices and qualitatively examined for cannula position using a stereoscopic microscope (Olympus, Japan). The data of the animals, in which the cannula was in the false place, were not included in the results.

2.5. Gene Expression Assay

Tissue Preparation: The frozen hippocampus samples were pulverized completely and mixed with 200 μl of chilled phosphate buffered saline (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na2HPO4.7H2O, and 1.4 mM KH2PO4), vortexed for 30 sec then spinned and aliquoted in two micro tubes equally. One of so prepared samples was used for gene expression study and the second part for immunoblotting. An appropriate volume of a protease inhibitor cocktail according to manufacturer's proposal was added to samples, which were allocated for immunoblotting.

RNA Extraction: Total cellular RNA was isolated from the hippocampus by a modification of the guanidine Isothiocyanate Phenol-Chloroform method (Ausubel et al., 2002) using RNX-PLUS reagent. The RNA was treated with 10U RNase free DNase I (Roche, Germany) to avoid any DNA contamination (Fig 1C). The concentrations and purity of the RNAs were determined by spectrophotometry (NanoDrop ND-1000, NanoDrop Technologies, Wilmington, DE, USA). The mean absorbance ratio at 260/280 nm and 260/230 nm were 1.94 ± 0.0 and 1.98 ± 0.1, respectively.

cDNA Synthesis: The reverse transcription reaction was performed with first strand cDNA synthesis kit (Roche, Germany) using Oligo-dT primer, AMV reverse transcriptase and 1μg total RNA as template, according to the manufacturer's instructions. The concentration of synthetic cDNA was measured using NanoDrop ND-1000 Spectrophotometer at 260 and 280 nm. DNA samples with the A260∕A280 ratios higher than 1.5 were selected for quantitative analysis.

Real Time PCR and Comparative Threshold Cycle Method: Cx32 was chosen as target gene and α-tubulin and GAPDH were used as internal reference genes. All primers (Table 1) were designed using primer express software v.3.0 (Applied Biosystems, Foster City, CA, USA). The specificity of the primers for their target sequences was checked on NCBI website (www.ncbi.nlm.nih.gov/blast). SYBR Green I real time PCR assay was carried out in final reaction volumes of 25 μL with 12.5 μL of SYBR Green I Master mix (Applied Biosystems, Warrington, UK), 100nM of forward and reverse primers and 300ng of cDNA. Thermal cycling was performed on the ABI 7300 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) using the following cycling conditions: 10 min at 95ºC as first denaturation step, followed by 40 cycles at 95ºC for 15 sec and 60ºC for 1 min. Each complete amplification stage was followed by a dissociation stage at 95 ºC for 15 sec, 60 ºC for 30 sec and 5 ºC for 15 sec. The extent of gene expression was calculated using comparative threshold cycle. The mean threshold cycle (mCT) was obtained from duplicate amplifications during the exponential phase of amplification. Then, mCT of reference genes were subtracted from mCT value of the target genes to obtain ΔCT. ΔΔCT values of each sample was calculated from corresponding CT values where ΔΔCT = [mCT target (control sample) - mCT reference (control sample)] - [mCT target (test sample) - mCT reference (test sample)]. The calculated ΔΔCT was converted to ratio using the ratio formula (Ratio = 2_ΔΔCT) (Livak & Schmittgen, 2001). Dissociation curve analysis was performed for each amplification reaction to detect any possible primer dimmers or non-specific PCR product (Ruiz-Ponte et al., 2006). Before using comparative threshold cycle method, amplification efficiency of each gene was determined from the standard curve drawn by plotting the logarithmic input amount of template DNA versus the corresponding CT values. The corresponding real time PCR efficiencies were calculated according to the slope of the standard curve and the following equation: Efficiency = [10(-1∕Slope)] - 1 (Vaerman et al., 2004). Data evaluation was carried out using the ABI PRISM 7300 Sequence Detection System and the SDS software v.1.2.3 (Applied Biosystems, UK).

2.6. Immunoblotting

The second part of the homogenized hippocampus tissues was removed from−80 °C and centrifuged at 12,000 g, 4 °C for 10 min. The supernatant was collected and total protein concentration was determined using Bio-Rad DC protein assay reagents. Samples were dissolved in protein loading buffer and denatured for 5 min at 95

(A)

(B)
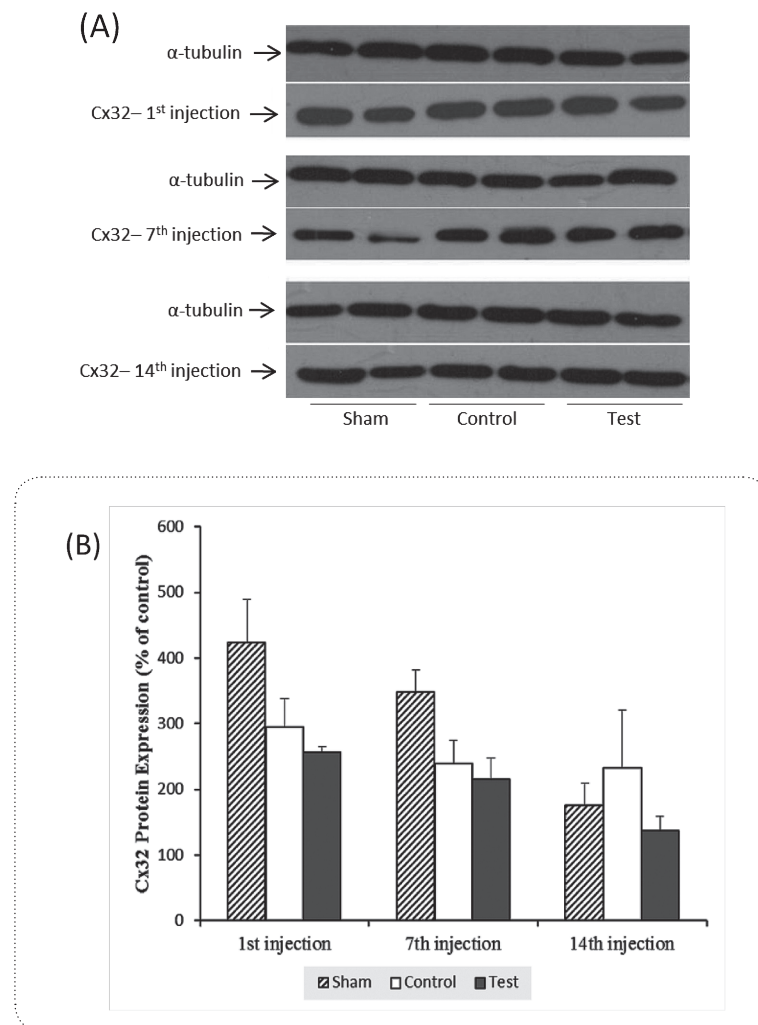
NEUR❖SCIENCE

Figure 2 (A). Immunoblots of Cx32 (32KDa) and α-tubulin (50KDa) for prepared samples. Each immunoblotting was performed in duplicate to increase the reliability of the measurements. (B) Cx32 protein level in the hippocampus of the rats after daily intracerebroventricular injection of LPS. Connexin protein level was normalized to α-tubulin protein level. Data are expressed as means ± S.E.M (n=5) compared to respective control group.

°C prior to loading. Equal amounts of protein from each animal (5 μg per lane for α-tubulin, 10 μg per lane for Cx32) were resolved by denaturing SDS-Polyacrylamide gel electrophoresis (SDS-PAGE), 12% acrylamide and transferred to a PVDF membrane (Roche, Germany) by electroblotting (Mini trans blot electrophoretic transfer cell, Bio-Rad). The membrane was blocked in TBST buffer (100 mMTris base, 150 mMNaCl, and 0.2% Tween 20) containing 2% ECL Advance blocking agent at room temperature for 60 min, rinsed briefly with TBST buffer and then incubated for 60 min with the following primary antibodies: mouse monoclonal anti-connexin 32 diluted 1:200,000, and mouse monoclonal anti-α-tubulin diluted 1:200,000. The antibodies were diluted in blocking buffer. After washing with TBST buffer 4 times (1× for 15 min and 3× for 5 min), the membrane

was incubated with peroxidase conjugated goat anti-mouse IgG (diluted 1:100,000 and 1:2,000,000 for Cx32 and α-tubulin, respectively) for 1 h, then washed with TBST buffer 4 times (1× for 15 min and 3× for 5 min) and reacted with ECL Advance western blotting detection reagents, for 4 min. An X-ray film (Retina, USA) was used for 30s to 10min and then developed to visualize the antibody binding (Fig 2A). Bands were quantified by densitometry using Labworks analyzing software (Ultra Violet Products, U.K). The relative levels of Cx32 proteins were expressed as ratios (Cx32/α-tubulin×100).

2.7. Statistical Analysis

The data were analyzed by ANOVA with Tukey post hoc test and presented as mean ± S.E.M. In all experiments, $P<0.05$ was considered statistically significant.

Table 1. The characteristics of the primers used in the Real Time PCR assay

Gene	Sequence	GC%	Melting Temperature °c	Primer Length
Cx32-Forward	CGGCATCTGCATTATCCTCAAC	50%	60.4	22
Cx32-Reverse	CAGCAGCTTGTTGATCTCATTCTG	46%	60	24
α-Tubulin–Forward	CTGGAACCCACAGTTATTGATGAAG	45%	59.8	25
α-Tubulin-Reverse	GGCATAGTTATTGGCAGCATCCTTC	45.8%	60	24
GAPDH-Forward	AGTCAAGGCTGAGAATGGGAAG	50%	58.5	22
GAPDH-Reverse	CATACTCAGCACCAGCATCACC	54.6%	59.2	22

NEUR◌SCIENCE

3. Results

3.1. Elevation of Cx32 mRNA Levels in LPS-Treated Rats

Melting curve analysis for Cx32, GAPDH and α-tubulin gene fragments revealed unique PCR product in each reaction. Each peak represented a unique PCR product in each reaction. Melting temperature of 80.5 °C for GAPDH, 81.3 °C for α-tubulin and 79.0 °C for Cx32 were obtained. The amplification curves of the both reference genes (α-tubulin, GAPDH) have crossed the threshold line at the same point. Mean threshold cycle of 21.95 for GAPDH and α-tubulin and 25.61 for Cx32 were obtained. (Fig 1A)

mRNA level of Cx32 significantly increased after 7th injection of LPS relative to the control group (n=5, P<0.001). But no meaningful change was detected after 1st and 14th injection of LPS in Cx32 mRNA expression compared to control group (Fig 1B).

3.2. Effect of LPS on Cx32 Protein Expression

Data from immunoblotting of Cx proteins showed that, there were no significant changes in the levels of Cx32 protein during acute and chronic injection of LPS in rat Hippocampus. Although some decreases in Cx32 protein expression was observed after LPS injection that was not statistically significant compared to control (n=5, P>0.05) (Fig 2B).

4. Discussion

Our data indicate a significant increase in Cx32 mRNA expression after chronic injection of LPS. However, no significant changes were observed in Cx32 protein abundance during this period. To our knowledge, this is the first in vivo study on the effect of LPS on hippocampal expression of Cx32 GJs in experimental animals.

Alterations in Cx expression are well recognized following neuroinflammation conditions (Garg et al, 2005).

The GJ protein Cx32 is abundantly expressed in mammalian brain. Cx32-deficient mice display enhanced intrinsic excitability and dysfunction of inhibitory synaptic transmission in the neocortex (Suton et al., 2000). In the hippocampus, Cx32 is expressed predominantly in oligodendrocytes (Rash et al., 2001) and parvalbumin-positive inhibitory interneurons of CA1 subfield (Oguro et al., 2001). The expression of Cx32 GJ protein increases selectively in the CA1 GABAergic interneurons after global ischemia. Moreover, transgenic Cx32-null mice exhibit enhanced vulnerability to global ischemia-induced neuronal death, consistent with the role of Cx32 gap junctions in neuroprotection against ischemia-induced cell death (Oguro et al., 2001). These observations together with our findings in the present study – up-regulating of Cx32 mRNA expression after chronic seven days by LPS central injection – suggest that Cx32 gap junctions is overexpressed under brain damages as a part of adaptive processes in order to reduce damages and protect the hippocampal neurons. However, we could not detect any changes in hippocampal Cx32 protein expression during LPS injection period. It can be suggested that LPS has no effect on regulation of Cx32 expression at translational level. Our observation might be related to rapid turnover of Cxs protein (Kielian. 2008). Moreover, LPS might affect the stability of Cx proteins as reported by some researchers (Chanson et al., 2005). Indeed, the half-life of Cx32, the main Cx in the liver, is reduced during liver inflammation induced by LPS (De Maio et al., 2000). The lack of correlation between mRNA and protein level of Cxs observed in our study, is reported by other researchers as well. For instance, Oguro et al. (2001) found that global ischemia induces a marked reduction in Cx32 mRNA abundance and at the same time a marked increase in Cx32 protein level in mouse hippocampus.

Intercellular communication through GJs can be regulated at several levels including changes in Cx transcription, translation, stability, post translational processing, insertion/removal from the cell membrane and channel gating (Kielian. 2008). However, post-translational

processes are thought to be the major factor in regulating Cxs levels and functional coupling. Therefore, our results suggest no functional modulation of Cx32 GJ coupling by LPS. Nevertheless, effect of LPS on Cx32 channel gating should be determined to further elucidate effect of LPS on GJC.

In conclusion, the chronic central injection of LPS, upregulates Cx32 GJs in rat hippocampus at mRNA; however it does not affect the protein level. Evaluation of the expression changes of these Cxs in other models of neuroinflammation is required to clarify the role of Cx32 GJs in pathology of CNS inflammation and disease.

References

Ausubel, F. M., Brent, R., Kingston, R. E., et al. (2002). Short Protocols in Molecular Biology. (5th ed.). Wiley, New York.

Bennett, M.V.L. and Zukin, R.S. (2004). Electrical coupling and neuronal synchronization in the mammalian brain. Journal of Neuron. (41) 495-511.

Chanson, M., Derouette, J, P., Roth, I., Foglia, B., Scerri, I., Dudez, T., Kwak, B, R. (2005). Gap junctional communication in tissue inflammation and repair. Biochim Biophys Acta, (1711)197-207.

Condorelli, D, F., Trovato-salinaro, A., Mudo, G., Mirone, M. B., Belluardo, N. (2003). Cellular expression of connexins in the rat brain: neuronal localization, effects of kainite-induced seizures and expression in apoptotic neuronal cells. Euro Journal of Neurosci. (18) 1807-27.

Cronin, M., Anderson, P. N., Cook, J. E., Green, C. R., Becker, D. L. (2008). Blocking connexin43 expression reduces inflammation and improves functional recovery after spinal cord injury. Journal of Mole and Cell Neurosci. (39) 152-160.

De Maio, A., Gingalewski, C., Theodorakis, N, G,. Clemens, M, G. (2000). Interruption of hepatic gap junctional communication during acute inflammation. Shock, (14):53-59.Froger, N., Orellana, J. A., Calvo, C. F., Amigou, E., Kozoriz, M. G., Naus, C. C., et al. (2010). Inhibition of cytokine-induced connexin43 hemichannel activity in astrocytes is neuroprotective. Journal of Mole and Cell Neurosci. (45) 37-46.

Froger, N., Orellana, J. A., Cohen-Salmon, M., Ezan, P., Amigou, E., Saez, J. C. (2009). Cannabinoids prevent the opposite regulation of astroglial connexin43 hemichannels and gap junction channels induced by pro-inflammatory treatments. Journal of Neurochem. (111) 1383-1397.

Garg, S., Syed, M. M., Kielian, T. (2005). Staphylococcus aureus-derived peptidoglycan induces Cx43 expression and functional gap junction intercellular communication in microglia. Journal of Neurochem. (95) 475-483.

Karpuk, N., Burkovetskaya, M., Fritz, T., Angle, A., Kielian, T. (2011). Neuroinflammation Leads to Region-Dependent Alterations in Astrocyte Gap Junction Communication and Hemichannel Activity. Journal of Neuroscience. (31) 414-425.

Kielian, T. (2008). Glial connexins and gap junctions in CNS inflammation and disease. Journal of Neurochem. (106) 1000-1016.

Kovacs, Z., Kekesi, K. A., Szilagyl, N., et al. (2006). Facilitation of spike-wave discharge activity by lipopolysaccharides in Wistar Albino Glaxo/Rigswijk rats. Journal of Neurosci. (140) 731-742.

Livak, K. J., Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C (T)) Method. Methods. (25) 402–8.

Oguro, K., Jover, T., Tanaka, H., Lin, Y. Takashi, K., Oguro, N., et al. (2001). Global ischemia-induced increases in the gap junctional proteins connexin 32 (Cx32) and Cx36 in hippocampus and enhanced vulnerability of Cx32 knock-out mice. Journal of Neurosci. (21) 7534-7542

Paxinos, G., Watson C, R. The Rat Brain in Stereotaxic Coordinates. (2007). (6th ed.). Elsevier, San Diego.

Rash, J. E., Yasumura, T., Davidson, K. G., Furman, C. S.,Dudek, F. E, Nagy, J. I. (2001). Identification of cells expressing Cx43, Cx30, Cx26, Cx32, and Cx36 in gap junctions of rat brain and spinal cord. Journal of Cell Commun Adhes. (8) 315-320.

Rouach, N., Avignone, E., Meme, W., et al. (2002). Gap junctions and connexin expression in the normal and pathological central nervous system. Journal of Biol cell (94) 451-75.

Ruiz Ponte, C., Carracedo, A., Barros, F. (2006). Duplication and deletion analysis by Fluorescent real time PCR based genotyping. Journal of Clin Chem Acta. (363) 138-146.

Saez, J. C., Berthoud, V. M., Branes, M. C., Martinez, A. D., Beyer, E. C. (2003). Plasma membrane channels formed by connexins: their regulation and functions. Journal of Physiol. Rev. (83) 1359–1400.

Sohl, G., Guldenagel, M., Beck, H., et al. (2000). Expression of connexin genes in hippocampus of kainite-treated and kindled rats under conditions of experimental epilepsy. Journal of Mol Brain Res. (83) 44-51.

Sohl, G., Maxeiner, S., Willecke, K. (2005). Expression and functions of neuronal gap junctions. Journal of Nat Rev Neurosci. (6) 191-200.

Sutor, B., Schmolke, C., Teubner, B., Schirmer, B., Willecke, K. (2000). Myelination defects and neuronal hyperexcitability in the neocortex of connexin32-deficient mice. Journal of Cereb Cortex. (10) 684-697.

Turrin, N. P., Gayle, D., Ilyin, S. E., et al. (2001). Pro-inflammatory and anti-inflammatory cytokine mRNA induction in the periphery and brain following intraperitoneal administration of bacterial lipopolysaccharide. Brain Res. Bull. (54) 443-453.

Vaerman, J. L., Saussoy, P., Ingargiola, I. (2004). Evaluation of real-time PCR data. Journal of Biol Regul Homeost Agents. (18) 212–4.

Zeinieh, M., Talhouk, R., Marwan, E. (2010). Differential expression of hippocampal connexins after acute hypoxia in the developing brain. Journal of Brain and Development. (32) 810-817.