The effect of salmon calcitonin against glutamate-induced cytotoxicity in the C6 cell line and the roles the inflammatory and nitric oxide pathways play

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Abstract

Recent evidence has shown that salmon calcitonin (sCT) has positive effects on the nervous system. However, its effect and mechanisms on glutamate-induced cytotoxicity are still unclear. The current experiment was designed to examine the effect of sCT on glutamate-induced cytotoxicity in C6 cells, involving the inflammatory and nitric oxide stress pathways. The study used the C6 glioma cell line. Four cell groups were prepared to evaluate the effect of sCT on glutamate-induced cytotoxicity. The control group was without any treatment. Cells in the glutamate group were treated with 10 mM glutamate for 24 h. Cells in the sCT group were treated with various concentrations (3, 6, 12, 25, and 50 µg/mL) of sCT for 24 h. Cells in the sCT + glutamate group were pre-treated with various concentrations of sCT for 1 h and then exposed to glutamate for 24 h. The cell viability was evaluated with an XTT assay. Nuclear factor kappa b (NF-kB), tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), neuronal nitric oxide synthase (nNOS), nitric oxide (NO), cyclic guanosine monophosphate (cGMP), caspase-3, and caspase-9 levels in the cells were measured by ELISA kits. Apoptosis was detected by flow cytometry method. sCT at all concentrations significantly improved the cell viability in C6 cells after glutamate-induced cytotoxicity (p<0.001). Moreover, sCT significantly reduced the levels of NF-kB (p<0.001), TNF-α, and IL-6 levels (p<0.001). sCT also decreased nNOS, NO, and cGMP levels (P<0.001). Furthermore, it decreased the apoptosis rate and increased the live-cell rate in the flow cytometry (P<0.001). In conclusion, sCT has protective effects on glutamate-induced cytotoxicity in C6 glial cells by inhibiting inflammatory and nitric oxide pathways. sCT could be a useful supportive agent for people with neurodegenerative symptoms.

Keywords  Salmon calcitonin · Glutamate · Inflammation · Nitric oxide · C6 Rat Glioma

Introduction

Glutamate, an excitatory neurotransmitter, is most commonly found in the central nervous system. Although it is found in many areas in the central nervous system, the most common areas are the cerebral cortex and hippocampus (Danbolt 2001). It acts as a primary neurotransmitter in some specific regions in the cerebellum such as granule cells. It has two groups of receptors: ionotropic and metabotropic. The ionotropic receptor family consists of three groups: N-methyl-D-aspartate (NMDA) receptors, alpha amino-3-hydroxy-5-methyl-4 isoxazolepropionic acid (AMPA) receptors, and kainate receptors (Mayer 2005). Glutamate, which plays an important role in synaptic plasticity, learning and memory in physiological conditions, is involved in the pathogenesis of some diseases such as Alzheimer’s disease, ischemic or hemorrhagic strokes, autism, and amyotrophic lateral sclerosis (Lau and Tymianski 2010). The basic mechanism in the pathogenesis of these diseases is excitotoxicity caused by excessive glutamate stimulation, which leads to excessive calcium ion influx into the cell (Matute et al. 2006). Calcium entrance to the cell triggers mitochondrial dysfunction, and increases intracellular nitric oxide levels, which induces apoptosis mechanisms in the cell (Arundine and Tymianski 2003).
Calcitonin is a single-chain peptide hormone containing 32 amino acids with a molecular weight of approximately 3500 Da. There are different types of calcitonin in the varied species such as human calcitonin and salmon calcitonin (sCT). Calcitonin is secreted by C cells, which are found in the thyroid gland in mammals, and in the ultimo branchial structure in fish, amphibians, reptiles and birds (Ostrovskaya et al. 2017). The main role of calcitonin is the regulation of mineral metabolism and to help eliminate the increase in calcium levels called 'calcium stress'. It also controls the movements of magnesium and phosphate ions as part of its function of maintaining the ionic balance (Masi and Brandi 2007). There are four types of calcitonin used in the clinic, derived human, pig, eel, and sCT. Calcitonin from fish (sCT and eel calcitonin) have approximately 30–40 times greater biological activity than mammalian calcitonin (human calcitonin and pig calcitonin) (Braga 1994). sCT, due to its more stable structure and high bioactivity, has been applied as one of many treatments in recent years, especially in diseases that cause bone destruction (Chesnut et al. 2008). There are some commercial types of sCT such as intravenous injection and nasal spray. These are used to treat osteoporosis in postmenopausal women in clinic. sCT commercial injection is also used to treat Paget’s disease of bone and to quickly reduce calcium levels in the blood when needed (Kawalski et al. 1999; Sullivan et al. 2018). Moreover, some studies have reported that different types of calcitonin have positive effects on the central nervous system. Furthermore, evidence has been suggested that sCT is closely related to the glutamatergic system, such as the NMDA and AMPA receptors, and glutamate releasing (Kilinc et al. 2018; Taskıran et al. 2020). However, its effect on glutamate-induced cytotoxicity and underlying mechanisms are still unclear. In the present study, the effect of sCT against glutamate-induced cytotoxicity in C6 glial cells involving in the inflammatory and nitric oxide pathways were examined.

Materials and methods

Cell culture

The C6 Glioma (CRL107) cell line was used in this study because of having appropriate glutamate-induced cytotoxicity (Kritis et al. 2015). C6 Glioma cell lines were obtained from the American Type Culture Collection. The cells were cultured in DMEM (Thermo Fisher Scientific, Altrincham, UK) containing 10% Fetal Bovine Serum (FBS) (Sigma-Aldrich Co., St Louis, MO, USA), and 1% L-glutamine (Sigma-Aldrich Co., St Louis, MO, USA) and 1% penicillin/streptomycin (Sigma-Aldrich Co., St Louis, MO, USA). The cells were maintained at 37°C within a 5% CO2 humidified atmosphere.

Drug administration

sCT (Biological Industries, Kibbutz Beit-Haemek, Israel) and glutamate (Sigma-Aldrich Co., St Louis, MO, USA) were dissolved in DMEM, and stock solutions were prepared before treatment.

Glutamate-induced cytotoxicity

Four-cell groups were prepared to evaluate the effect of sCT on glutamate-induced cytotoxicity. The control group was without any treatment. Cells in the glutamate group were treated with 10 mM glutamate for 24 h. The glutamate concentration (10 mM) causing the death of fifty percent of glial cells was selected according to the literature (Park et al. 2019). Cells in the sCT group were treated with various concentrations (3, 6, 12, 25, and 50 µg/mL) of sCT for 24 h. The rationale for dose selection of SCT was performed with respect to previous studies (Andreassen et al. 2014; Trier et al. 2015; Keum et al. 2020). Cells in the sCT+glutamate group were pre-treated with various concentrations (3, 6, 12, 25, and 50 µg/mL) of sCT for 1 h and then exposed to 10 mM glutamate for 24 h.

Cell viability assay

Cell viability was assessed using the XTT assay (Roche Diagnostic, MA, USA). C6 Glioma cells were seeded in 96-well plates at a density of 1 × 104 cells per well in 100-µL DMEM and grown overnight before sCT. The procedure of glutamate-induced cytotoxicity was performed as mentioned above. The following day, after 24 h of incubation, the medium was removed, and the wells were washed twice with phosphate-buffered saline (PBS). In the last step, 100 µL DMEM without phenol red and a mixture of 50 µL XTT labeling solution were added to all the wells, and then the plates were maintained at 37°C for 4 h. The plates were shaken, and the absorbance was detected using an ELISA microplate reader (Thermo Fisher Scientific, Altrincham, UK) at 450 nm. All the experiments were performed three times, and the cell viability was measured as a viable cell amount percent compared to the control, as untreated cells.

Preparation of cell homogenates

The cells for each group were collected by sterile tubes. They were centrifuged at 2000 rpm for approximately 10 min and the supernatants were removed. The component of cells in under the tubes were suspended by using PBS (pH: 7.4) to dilute cell suspension to the cell concentration of approximately 1 million/ml. The cells were damaged...
through repeated freeze–thaw cycles to let out the internal components. The freeze–thaw cycles were performed two times to avoid changing in the protein activity (Gagné 2014). They were centrifuged at 4000 rpm for 10 min at a temperature of 4 °C. Then, the supernatants were collected for biochemical analysis. The Bradford protein assay kit (Merck Millipore, Darmstadt, Germany) was used for determination of total protein levels in the samples.

Measurement of nuclear factor kappa B (NF-kB), tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), nitric oxide neuronal nitric oxide synthase (nNOS), nitric oxide (NO), cyclic guanosine monophosphate (cGMP), caspase-3, and caspase-9 levels in the cells

The levels of NF-kB, TNF-α, IL-6, nNOS, NO, and cGMP in the supernatants of the cells for each group were measured using ELISA commercial kits (BT Lab, Shanghai, China). The operation protocols were according to the manufacturer’s instructions. In brief, the standard and tissue samples were added into the plate and incubated for 60 min at 37 °C. After the washing step, the staining solutions were added and incubated for 15 min at 37 °C. The stop solution was added and read at 450 nm in the ELISA microplate reader (Thermo Fisher Scientific, Altrincham, UK). Standard curves were plotted to determine the value of samples. The coefficients of variation within and between plates were less than 10%.

Annexin V binding assay

Apoptosis was evaluated using a Muse Annexin V/Dead Cell (Merck Millipore, Darmstadt, Germany) assay. In summary, the cells were seeded into six-well plates and were allowed to attach overnight before treatment. The cells were then treated with sCT (3 and 50 µg/mL), glutamate (10 mM), or their combinations and incubated for 24 h. After incubation, the annexin V assay was performed according to the report in our previous study (Taskiran et al. 2020).

Statistical analysis

The results were expressed as a mean ± standard error of the mean (SEM). The data analyses were performed with SPSS Version 23.0 for Windows. The data were evaluated using a one-way analysis of variance (ANOVA) and the posthoc Tukey test was chosen to identify the differences between the experimental groups. A value of $p < 0.05$ was accepted as statistically significant.

Results

Effect of sCT on cell survival after glutamate-induced cytotoxicity in C6 cells

The protective effects of sCT against glutamate-induced cytotoxicity in C6 cells were evaluated using a XTT cell
proliferation assay. In this study, increasing doses of sCT (3–50 µg/mL) on cell survival were performed in both control and glutamate-treated C6 cells. Initially, the cells were pretreated with increasing doses (3–50 µg/mL) of sCT for 1 h and then incubated with or without 30 mM glutamate for the next 24 h. As shown in Fig. 1, pre-incubating the C6 cells with glutamate for 24 h significantly decreased cell survival compared with the untreated control cells (p < 0.001; Fig. 1). However, all doses of sCT increased cell survival in C6 cells as compared with glutamate-treated cells (p < 0.001; Fig. 1). Furthermore, sCT, in all doses, did not change C6 survival compared with the untreated control cells (p > 0.05; Fig. 1).

**Effect of sCT on NF-kB, TNF-α, and IL-6 after glutamate-induced cytotoxicity in C6 cells**

The effects of sCT on NF-kB, TNF-α, and IL-6 were measured via ELISA kits after glutamate-induced cytotoxicity in the C6 cells. The cells were pretreated with two doses of sCT (3 and 50 µg/mL) for 1 h and then incubated or not incubated with 10 mM glutamate for the next 24 h. Pre-incubating the C6 cells with glutamate during 24 h significantly increased NF-kB, TNF-α, and IL-6 levels as compared with the untreated control cells (p < 0.001; Fig. 2). However, the sCT (3 and 50 µg/mL) significantly decreased NF-kB, TNF-α, and IL-6 levels in C6 cells compared with the glutamate-treated cells (p < 0.001; Fig. 2).

**Effect of sCT on nNOS, NO, and cGMP after glutamate-induced cytotoxicity in C6 cells**

The ELISA measurements were performed to assess the effects of sCT on nNOS, NO, and cGMP after glutamate-induced cytotoxicity in the C6 cells. The cells were pretreated with two doses of sCT (3 and 50 µg/mL) for 1 h and then incubated or not incubated with 10 mM glutamate for the next 24 h. Pre-incubating the C6 cells with glutamate for 24 h significantly raised nNOS, NO, and cGMP levels compared with the untreated control cells (p < 0.001; Fig. 3). However, sCT (3 and 50 µg/mL) significantly reduced nNOS, NO, and cGMP levels in C6 cells compared with the glutamate-treated cells (p < 0.001; Fig. 3).
Effect of sCT on caspase-3 and caspase-9 after glutamate-induced cytotoxicity in C6 cells

The effects of sCT on caspase-3 and caspase-9 were measured by ELISA kits after glutamate-induced cytotoxicity in C6 cells. The cells were pretreated with two doses of sCT (3 and 50 µg/mL) for 1 h and then incubated or not incubated with 10 mM glutamate for the next 24 h. Pre-incubating the C6 cells with glutamate for 24 h significantly raised caspase-3 and caspase-9 levels compared with the untreated control cells (p < 0.001; Fig. 4). However, sCT (3 and 50 µg/mL) significantly reduced caspase-3 and caspase-9 levels in C6 cells compared with the glutamate-treated cells (p < 0.001; Fig. 4).

Effect of sCT on apoptosis after glutamate-induced cytotoxicity in C6 cells

The anti-apoptotic effects of sCT were evaluated by flow cytometry after glutamate-induced cytotoxicity in the C6 cells. As shown in Fig. 5, it is exhibited that 10 mM glutamate remarkably increased the proportion of apoptotic cells for 24 h compared with the untreated control cells (p < 0.001; Fig. 5). Moreover, an sCT pretreatment at doses of 3 and 50 µg/mL significantly reduced the apoptotic percentage of C6 cells after glutamate-induced cytotoxicity in the cells (p < 0.001; Fig. 5). However, pretreatment of sCT (3 and 50 µg/mL) alone did not demonstrate a significant apoptotic effect in the C6 cells (p > 0.05; Fig. 5).

Discussion

The present study is the first time that the effects of sCT against glutamate-induced cytotoxicity in C6 cells have been evaluated. sCT pretreatment increased C6 cell survival and reduced cell death after glutamate-induced cytotoxicity in the cells. Furthermore, pretreatment with sCT decreased inflammatory pathway proteins, NF-κB, TNF-α, and IL-6, levels in the C6 cells. Moreover, sCT suppressed the nNOS, NO, and cGMP levels in the C6 cells, and sCT inhibited apoptosis related factors, caspase-3 and caspase-9, levels after glutamate-induced cytotoxicity in the C6 cells.

Several in vivo and in vitro studies have demonstrated that sCT has positive effects on the nervous system. It has been reported that sCT stimulates neuritis elongation in the nerve cells of mollusks (Grimm-Jørgensen 1987). Moreover, it has
been claimed that sCT changes the neuronal excitability in different brain regions of rats and decreased intracellular calcium by modulating synaptosomes (Twery and Moss 1985). It has also been found that sCT reduces intracellular calcium levels in response to high potassium stimulation (Welch and Olson 1991). Calcium plays a vital role in glutamate-induced cytotoxicity (Pastukhov and Borisova 2018). The current study found that sCT raised the cell viability and inhibited apoptosis after glutamate-induced cytotoxicity in C6 cells.

Inflammation is closely related to the neurodegenerative process in the nervous system (Takeuchi 2013). The activation of NF-kB, the main modulator of inflammation in the organism, leads to releasing of proinflammatory cytokines, such as TNF-α and IL-6, and other chemokines (Bonomi et al. 2004; Ulivi et al. 2008). It has been shown that glutamate-induced cytotoxicity increases proinflammatory cytokines, which are associated with nervous system disorders (Chaparro-Huerta et al. 2005). The previous findings have found that sCT has an antiinflammatory effect by inhibiting proinflammatory cytokines production in the brain and blood tissues (Zhang et al. 2017; Taskiran et al. 2020). Furthermore, it has been claimed that other types of calcitonin, such as human calcitonin, decreases IL-1 stimulated chondrocytes activation by suppressing the p50-NF-κB pathway (Bai et al. 2019). In this study, sCT decreased NF-kB, TNF-α, and IL-6 levels after glutamate-induced cytotoxicity in the C6 cells, which is consist with previous studies.

NO is an essential neuromodulators of neurotransmitters in the central nervous system. It is synthesized from the oxidation of the L-arginine amino acid via three types of nitric oxide synthases, which are endothelial NOS (eNOS), inducible NOS (iNOS), and neuronal NOS (nNOS) (Bahremand et al. 2010). nNOS is commonly expressed in neurons (Zhou and Zhu 2009), and it has been reported that C6 cells express nNOS (Feinstein et al. 1994; Raso et al. 2002). The activation of nNOS leads to an increase in NO in the neurons, which initiates soluble guanylate cyclase (sGC) / guanosine monophosphate (cGMP). cGMP, a secondary messenger, gives rise to glutamate releasing in the neurons and causes excitation (Kiss 2001). Moreover, nNOS/NO/cGMP is also involved in glutamate-induced cytotoxicity (Kritis et al. 2015). A previous study has demonstrated that calcitonin protects chondrocytes from lipopolysaccharide-induced apoptosis by suppressing NO production (Zhang et al. 2017). In contrast to this study, it has been claimed that ecartin, a synthetic eel calcitonin, vasodilates retinal blood vessels in rats by increasing NO levels in endothelial cells (Mori et al. 2015). In this study, it has been found that the rise in nNOS/NO/cGMP levels in the cells after glutamate-induced cytotoxicity. Nevertheless, sCT decreased nNOS/NO/cGMP after glutamate-induced cytotoxicity in the C6 cells.

The other type of fish derived calcitonin using in the clinic is eel calcitonin. It has been shown that eel calcitonin has regulatory effects on the nervous system like sCT (Gudobono et al. 1991; Aoki et al. 2012). The mammalian derived calcitonins particularly human calcitonin also used in the clinic treatment. Moreover, it has been demonstrated that human calcitonin decreases neuronal calcium influx and modulates excitation-inhibition balance in the nervous system (Hagenacker et al. 2011; Aksoy et al. 2014). Based on these studies, it could speculate that other calcitonins types would be protective effects against after glutamate-induced cytotoxicity smiliar to sCT. However, More evidences are needed to point out this speculation.

According to our findings, sCT has a protective effect in glial cell survival after glutamate-induced cytotoxicity in C6 cells. Since glial cells are critical for neurodegenerative diseases, sCT could be a supportive therapeutic agent to the
Fig. 5  Effect of sCT on caspase-3 and caspase-9 levels after glutamate-induced cytotoxicity in C6 cells. The data are expressed as mean ± SEM. αααp < 0.001 as compared to untreated control group; ββββp < 0.001 compared to the glutamate-treated group.
treatment of neurodegeneration related diseases. However, this needs to be proven by further studies.

The study has potential limitation. It is necessary to examine the effect of sCT on the calcium fluxes associated with the protection from apoptosis after glutamate-induced cytotoxicity in C6 cells. However, sCT effects on the calcium fluxes is missing in the present study. This situation is a limitation of the present study.

Conclusion

The findings of this study showed that sCT reduced cell death after glutamate-induced cytotoxicity in C6 cells. These effects could be associated with inhibition of inflammation (NF-kB/ TNF-α/ IL-6) and nitric oxide (nNOS/NO/cGMP) pathways. Therefore, sCT could be a useful supportive therapeutic agent for glutamate-related neurodegenerative diseases. However, further investigation is required to answer the questions raised about the probable mechanisms involved.

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Authors’ contributions AST designed the study, interpreted the data, and had a major contribution in writing and revising the manuscript. ME performed the experiment, drafted the manuscript and analyzed data. All authors read and approved the final manuscript.

Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest The authors declare that they have no conflict of interest.

References

Aksoy D, Solmaz V, Erbas O (2014) Positive effect of calcitonin on the seizures induced by pentylenetetrazole in rats. Epilepsy Res 108:390–395. https://doi.org/10.1016/j.eplepsres.2014.01.012

Andreasons KV, Hjuler ST, Furness SG et al (2014) Prolonged calcitonin receptor signaling by salmon, but not human calcitonin, reveals ligand bias, PLoS ONE 9:e92042. https://doi.org/10.1371/journal.pone.0092042

Aoki M, Mori A, Nakahara T et al (2012) Effect of synthetic eel calcitonin, elicatin, on cold and mechanical allodynia induced by oxaliplatin and paclitaxel in rats. Eur J Pharmacol 696:62–69. https://doi.org/10.1016/j.ejphar.2012.09.007

Arundine M, Tymianski M (2003) Molecular mechanisms of calcium-dependent neurodegeneration in excitotoxicity. Cell Calcium 34:325–337. https://doi.org/10.1016/S0143-4160(03)00141-6

Bahreman A, Ziai P, Khodadad TK et al (2010) Agmatine enhances the anticonvulsant effect of lithium chloride on pentylenetetrazole-induced seizures in mice: involvement of L-arginine/nitric oxide pathway. Epilepsy Behav 18:186–192. https://doi.org/10.1016/j.yebeh.2010.04.014

Bai X, Guo A, Li Y (2019) Protective effects of calcitonin on IL-1 stimulated chondrocytes by regulating MMPs/TIMP-1 ratio via suppression of p50-NF-κB pathway. Biosci Biotechnol Biochem 83:598–604. https://doi.org/10.1080/09164518.2018.1559718

Bonizzi G, Karin M (2004) The two NF-κB activation pathways and their role in innate and adaptive immunity. Trends Immunol 25:280–288. https://doi.org/10.1016/j.it.2004.03.008

Braga PC (1994) Calcitonin and its antiinflammatory activity: animal and human investigations 1975–1992. Agents Actions 41:121–131. https://doi.org/10.1007/BF02001904

Chaparro-Huerta V, Rivera-Cervantes MC, Flores-Soto ME et al (2005) Proinflammatory cytokines and apoptosis following glutamate-induced excitotoxicity mediated by p38 MAPK in the hippocampus of neonatal rats. J Neuroimmunol 165:53–62. https://doi.org/10.1016/j.neuroim.2005.04.025

Chesnut CH, Azria M, Silverman S et al (2008) Salmon calcitonin: a review of current and future therapeutic indications. Osteoporos Int 19:479–491. https://doi.org/10.1007/s00198-007-0490-1

Danbolt NC (2001) Glutamate uptake. Prog Neurobiol 65:1–105. https://doi.org/10.1016/S0301-0082(00)00067-5

Feinstein DL, Galea E, Roberts S et al (1994) Induction of nitric oxide synthase in Rat C6 Gliona cells. J Neurochem 62:315–321. https://doi.org/10.1046/j.1471-4149.1994.62010315.x

Gagné F (2014) Tissue preparation and subcellular fractionation techniques. In: Biochemical ecotoxicology: principles and methods. Elsevier, pp 21–31

Grimm-Jørgensen Y (1987) Somatostatin and calcitonin stimulate neurite regeneration of molluscan neurons in vitro. Brain Res 403:121–126. https://doi.org/10.1016/0006-8993(87)90130-2

Guidobono F, Netti C, Villani P et al (1991) Antiinflammatory activity of eel calcitonin, injected into the inflamed paw in rats. Neuropharmacology 30:1275–1278. https://doi.org/10.1016/0028-3908(91)90023-5

Hagenacker T, Ledwig D, Büsselberg D (2011) Additive inhibitory effects of calcitonin and capsaicin on voltage activated calcium channel currents in nociceptive neurons of rat. Brain Res Bull 85:75–80. https://doi.org/10.1016/j.brainresbull.2011.02.006

Kawalski H, Polanowicz U, Jonderko G et al (1999) Immunological parameters and respiratory functions in patients suffering from atopic bronchial asthma after intravenous treatment with salmon calcitonin. Immunol Lett 65:280–288. https://doi.org/10.1016/S0165-2478(99)00118-2

Keum T, Noh G, Seo JE et al (2020) In vitro and ex vivo evaluation of penetratin as a non-invasive permeation enhancer in the penetration of salmon calcitonin through tr146 buccal cells and porcine buccal tissues. Pharmaceuticals 13:1–13. https://doi.org/10.3390/ph13110408

Kilinc E, Dagistan Y, Kukner A et al (2018) Salmon calcitonin ameliorates migraine pain through modulation of CGRP release and dural mast cell degranulation in rats. Clin Exp Pharmacol Physiol 45:536–546. https://doi.org/10.1111/1440-1681.12915

Kiss J (2001) Nitric oxide: a novel link between synaptic and nonsynaptic transmission. Trends Neurosci 24:211–215. https://doi.org/10.1016/S0166-2236(00)01745-8

Kritis AA, Stamoula EG, Paniskaki KA, Vavilis TD (2015) Researching glutamate—induced cytotoxicity in different cell lines: a comparative/collective analysis/study. Front Cell Neurosci 9:91. https://doi.org/10.3389/fncel.2015.00091
Lau A, Tymianski M (2010) Glutamate receptors, neurotoxicity and neurodegeneration. Pflügers Arch Eur J Physiol 460:525–542. https://doi.org/10.1007/s00424-010-0809-1

Masi L, Brandi ML (2007) Calcitonin and calcitonin receptors. Clin Cases Miner Bone Metab 4:117–122

Matute C, Domercq M, Sánchez-Gómez M-V (2006) Glutamate-mediated glial injury: mechanisms and clinical importance. Glia 53:212–224. https://doi.org/10.1002/glia.20275

Mayer ML (2005) Glutamate receptor ion channels. Curr Opin Neurobiol 15:282–288. https://doi.org/10.1016/j.conb.2005.05.004

Mori A, Suzawa H, Sakamoto K et al (2015) Vasodilator effects of elcatonin, a synthetic eel calcitonin, on retinal blood vessels in rats. Biol Pharm Bull 38:1536–1541. https://doi.org/10.1248/bpb.b15-00303

Ostrovskaya A, Findlay DM, Sexton PM, Furness SGB (2017) Calcitonin ☆. In: Reference module in neuroscience and biobehavioral psychology. Elsevier

Park E, Kim J, Kim DK et al (2019) Protective effects of alpha-lipoic acid on glutamate-induced cytotoxicity in C6 Glioma cells. Biol Pharm Bull 42:94–102. https://doi.org/10.1248/bpb.b18-00603

Pastukhov A, Borisova T (2018) Levetiracetam-mediated improvement of decreased NMDA-induced glutamate release from nerve terminals during hypothermia. Brain Res 1699:69–78. https://doi.org/10.1016/j.brainres.2018.06.032

Raso GM, Meli R, Gualillo O et al (2002) Prolactin induction of nitric oxide synthase in Rat C6 Glioma cells. J Neurochem 73:2272–2277. https://doi.org/10.1046/j.1471-4159.1999.073227.x

Sullivan R, Abraham A, Simpson C et al (2018) Three-month randomized clinical trial of nasal calcitonin in adults with X-linked hypophosphatemia. Calcif Tissue Int 102:666–670. https://doi.org/10.1007/s00223-017-0382-0

Takeuchi H (2013) Roles of glial cells in neuroinflammation and neurodegeneration. Clin Exp Neuroimmunol 4:2–16. https://doi.org/10.1111/cen.12059

Taskiran AS, Ergul M, Gunes H et al (2020a) The effects of proton pump inhibitors (pantoprazole) on pentylenetetrazole-induced epileptic seizures in rats and neurotoxicity in the SH-SY5Y human neuroblastoma cell line. Cell Mol Neurobiol. https://doi.org/10.1007/s10571-020-00956-6

Taskiran AS, Ozdemir E, Gumus E, Ergul M (2020b) The effects of salmon calcitonin on epileptic seizures, epileptogenesis, and postseizure hippocampal neuronal damage in pentylenetetrazole-induced epilepsy model in rats. Epilepsy Behav 113:107501. https://doi.org/10.1016/j.yebeh.2020.107501

Trier S, Linderoth L, Bjerringgaard S et al (2015) Acylation of salmon calcitonin modulates in vitro intestinal peptide flux through membrane permeability enhancement. Eur J Pharm Biopharm 96:329–337. https://doi.org/10.1016/j.ejpb.2015.09.001

Twyer MJ, Moss RL (1985) Calcitonin and calcitonin gene-related peptide alter the excitability of neurons in rat forebrain. Peptides 6:373–378. https://doi.org/10.1016/0196-9781(85)90098-1

Ulivi V, Giannoni P, Gentili C et al (2008) p38/NF-κB-dependent expression of COX-2 during differentiation and inflammatory response of chondrocytes. J Cell Biochem 104:1393–1406. https://doi.org/10.1002/jcb.21717

Welch SP, Olson KG (1991) Salmon calcitonin-induced modulation of free intracellular calcium. Pharmacol Biochem Behav 39:641–648. https://doi.org/10.1016/0091-3057(91)90140-W

Zhang LB, Man ZT, Li W et al (2017) Calcitonin protects chondrocytes from lipopolysaccharide-induced apoptosis and inflammatory response through MAPK/Wnt/NF-κB pathways. Mol Immunol 87:249–257. https://doi.org/10.1016/j.molimm.2017.05.002

Zhou L, Zhu D-Y (2009) Neuronal nitric oxide synthase: Structure, subcellular localization, regulation, and clinical implications. Nitric Oxide 20:223–230. https://doi.org/10.1016/j.niox.2009.03.001

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