NÖRO PSİKİYATRİ ARŞİVİ
ARCHIVES OF NEUROPSYCHIATRY

RESEARCH ARTICLE

Arch Neuropsychiatry 2024;61:24–29
https://doi.org/10.29399/npa.28377

Effects of Oxytocin on Glutamate Mediated Neurotoxicity in Neuroblastoma Cell Culture

Börte GÜRBÜZ ÖZGÜR[1], Kamil VURAL[2], Mehmet İbrahim TUĞLU[3]

[1]Aydın Adnan Menderes University Faculty of Medicine, Department of Child and Adolescent Psychiatry, Aydın, Turkey
[2]Manisa Celal Bayar University Faculty of Medicine, Department of Pharmacology, Manisa, Turkey
[3]Manisa Celal Bayar University Faculty of Medicine, Department of Histology and Embryology, Manisa, Turkey

ABSTRACT

Introduction: We aimed to investigate the effects of oxytocin on neurite growth, cell viability, cell proliferation and apoptosis to demonstrate its neuroprotective effect on glutamate induced neurotoxicity in human neuroblastoma SH-SY5Y cell culture.

Method: The effect of oxytocin on the toxic effects of glutamate in human neuroblastoma SH-SY5Y cell line with the Neurotoxicity Screening Test (NTT), apoptotic effects by Terminal Transferase dUTP Nick End Labeling (TUNEL) method and cell viability test by 3-(4.5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide (MTT) method. In the NTT test; Neurotoxicity was induced by adding glutamate at a concentration of 32 µM to the cell culture. Oxytocin was added at 1, 3, 10, 30 and 100 µM concentrations and its effect on neurite elongation was investigated. It was demonstrated by TUNEL method that application of glutamate caused apoptosis. Afterwards, when glutamate and different doses of oxytocin were given, antiapoptotic effect was evaluated with the apoptotic index.

Results: Glutamate was found to have a dose-dependent neurotoxic effect and reduced neurite elongation by 50% at a concentration of 32 µM. It was shown that the inhibition of neurite elongation caused by glutamate decreased in a dose-dependent manner by applying oxytocin. Especially oxytocin was found to significantly reduce neurite inhibition and show a neuroprotective effect starting from 10 µM concentrations. The concentration at which glutamate reduces cell proliferation by 50% was determined as 54 µM in MTT. Subsequently, it was observed that the adverse effect of glutamate on cell proliferation significantly decreased with oxytocin administration, depending on the dose.

Conclusion: It was found that different concentrations of glutamate have a significant toxic effect on cell proliferation and viability, glutamate inhibits neurite elongation in a dose-dependent manner; oxytocin reduces neurite inhibition caused by glutamate, has a neuroprotective effect, increases cell viability and has antiapoptotic effects.

Keywords: In vitro, neuroprotective, neurotoxicity, oxytocin, SY5Y

Cite this article as: Gürbüz Özgür B, Vural K, Tuğlu Mİ. Effects of Oxytocin on Glutamate Mediated Neurotoxicity in Neuroblastoma Cell Culture. Arch Neuropsychiatry 2024;61:24–29.

INTRODUCTION

Glutamate is one of the most important excitatory neurotransmitters in the central nervous system. It not only plays a crucial role in neurotransmission, but also in neural development, synaptic plasticity, memory, and learning ability (1). Glutamate is also a significant neurotoxin. Overstimulation of glutamate receptors is associated with hypoxic damage, hypoglycemia, stroke, epilepsy, depression, and Parkinson's disease (2–5). Under normal conditions, glutamate is responsible for the survival, migration, and differentiation of cells during brain development, while excessive glutamate can cause neuronal cell death through oxidative stress or excitotoxicity (6). One of the neurochemical abnormalities associated with the pathophysiology of autism is the suppression of gamma-aminobutyric acid (GABA) inhibition (7) and it has been suggested that this suppression may be due to a decrease in the expression of glutamic acid decarboxylase, which synthesizes GABA (8). There are studies that show an increase in the expression of many genes that contain glutamate excitatory amino acid carriers and glutamate receptors associated with the glutamatergic pathway in the brain tissues of individuals with autism (9,10), as well as studies that show evidence of autism being a hypoglutamatergic disorder (11,12). Because of its neurotoxic effect, glutamate is used in neuronal culture studies to induce neurotoxicity (13–15).

Highlights

- **It was detected that oxytocin significantly reduces the negative effect of glutamate on cell proliferation,**
- **Oxytocin has a significant effect on glutamate toxicity at different doses on cell proliferation,**
- **Oxytocin administration prevents cell death due to glutamate toxicity,**
- **Oxytocin had an antiapoptotic effect at different doses in the presence of glutamate.**

Oxytocin, a neuropeptide hormone, has regulatory effects on complex social behaviors including bonding, social recognition, and aggression. Additionally, it plays an important role in the neuroendocrine regulation of birth and lactation (16). There are studies that demonstrate oxytocin's site-protective effects through its anti-inflammatory, anti-apoptotic, and antioxidant properties (17,18). When examining the relationship between

Correspondence Address: Börte Gürbüz Özgür, Aydın Adnan Menderes Üniversitesi Tıp Fakültesi, Çocuk ve Ergen Ruh Sağlığı ve Hastalıkları Anabilim Dalı, Kepez Mevki, 09100, Aydın, Türkiye • **E-mail:** drborte@hotmail.com
Received: 09.12.2022, **Accepted:** 31.01.2023, **Available Online Date:** 21.02.2024

glutamate and oxytocin, it has been shown that oxytocin decreases glutamate levels by binding to receptors in the spinal cord (19). When its effects on cell viability are studied, oxytocin has been found to have positive effects on cell viability and neurite growth (20).

The aim of this study is to investigate the effects of oxytocin on neurotoxicity induced by glutamate in human neuroblastoma cell line.

METHODS

The hypothesis of the study is that "oxytocin reduces neurotoxic effects in a glutamate-mediated neurotoxicity model in human neuroblastoma cell culture." Ethical approval was obtained from the Health Sciences Ethics Committee of Manisa Celal Bayar University Faculty of Medicine on September 13, 2017 with the decision number 20.478.486. The study was supported by the Scientific Research Projects Unit of Manisa Celal Bayar University with the project number 2018–209.

Data Collection Tools

The used cell line was the human neuroblastoma cell line SH-SY5Y (American Type Culture Collection, USA) (ATCC CRL-2266). Reagents such as glutamate (Sigma-Aldrich, St. Louis, MO, USA), oxytocin (Sigma-Aldrich, St. Louis, MO, USA), Dulbecco's Modified Eagle's Medium (DMEM), cell culture medium, fetal bovine serum, penicillin/streptomycin, and trypsin were obtained from Gibco (Invitrogen Inc., Grand Island, New York, USA).

Method of Data Collection

Neurotoxicity Screening Method

Cell Culture: SH-SY5Y neuroblastoma cells were proliferated in a culture medium containing 10% fetal calf serum and 1% penicillin/streptomycin solution (10000 U/10 mg) in Dulbecco's Modified Eagle's Medium (DMEM): F12, in an incubator providing 37°C and 5% CO_2 conditions (21).

Differentiation medium: A high-glucose DMEM was completed with 100 mL of 0%.1 penicillin/streptomycin solution+0.5 mM d-cAMP+gentamicin solution.

Oxytocin Neurotoxicity Screening Test

On the first day, 15.000 cells of SH-SY5Y neuroblastoma were placed in each well (500 µL volume) in the proliferation medium. After 24 hours, the first three wells had the proliferation medium (negative control) and the remaining wells had the medium removed and replaced with serum-free DMEM containing dibutyril cAMP (1 mM) differentiation medium. No substances were added to three wells (positive control). Oxytocin was placed in the remaining wells in concentrations of 0.1, 1, 3, 10, 30, and 100 µM in three wells. After 24 hours, cells were fixed in 4% formaldehyde (PBS) for 10 minutes and then stained with Coomassie Blue (0.6%) for 3 minutes. The stained samples were then washed with PBS. The lengths of neurites in 100 cells randomly selected from 10 different fields were measured using the image analysis system. The measured neurite lengths were evaluated by % inhibition (22).

Glutamate Neurotoxicity Screening Test

Before glutamate application, 16.000 cells of SH-SY5Y were placed in each well (500 µL volume) in the proliferation medium to determine the dose to be added to the cell culture. After 24 hours, the first three wells had the proliferation medium (negative control), and the remaining wells had the medium removed and replaced with serum-free DMEM containing dibutyril cAMP (1 mM) differentiation medium and glutamic acid in 20 mM/L, 40 mM/L, 60 mM/L, and 80 mM/L concentrations. After 24 hours, cells were fixed in 4% formaldehyde (PBS) for 10 minutes and then stained with Coomassie Blue (0.6%) for 3 minutes. The stained samples were then washed with PBS. The lengths of neurites in 100 cells randomly selected from 10 different fields were measured using the image analysis system. The measured neurite lengths were evaluated by % inhibition.

The Effects of Oxytocin on Neurotoxicity Induced by Glutamate

In this study, the aim was to determine the dose of glutamate to be added to the cell culture prior to application by placing 16.000 SH-SY5Y cells in each well of a proliferation medium, with a volume of 500 µL, in all wells. After 24 hours, neurotoxicity was induced by adding cAMP (1 mM) differentiation medium without serum and glutamate at a concentration of 32 µM to all wells except the first three, which were used as negative controls and contained only the proliferation medium. Oxytocin was added at concentrations of 1, 3, 10, 30, and 100 µM to all three wells. No substances were added to the three wells in the culture dish (positive control). After 24 hours, the cells were fixed for 10 minutes in 4% formaldehyde (PBS), then stained for 3 minutes with Coomassie Blue (0.6%). The stained samples were then washed with PBS. The lengths of 100 nerves from 10 different randomly selected fields were measured under a light microscope using an image analysis system. The measured nerve length values were evaluated by percent inhibition.

Cell Viability Test (MTT) Metabolism

The MTT assay is a method used to measure cell proliferation and viability by reducing 3-(4.5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide to its formazan product. On the first day, SH-SY5Y neuroblastoma cells were incubated for 24 hours in proliferation medium with a volume of 300 µL, with 16.000 cells per well. Starting from the first well and in sequence, oxytocin was added in concentrations of negative control, 1, 3, 10, 30, and 100 µM. The same experiment was repeated after inducing toxicity with glutamate for 24 hours, adding oxytocin in concentrations of negative control, 1, 3, 10, 30, and 100 µM. On the second day, cells were incubated in the proliferation medium. On the third day, the oxytocin-containing medium was removed and 100 µL of fresh medium was added to each well, followed by the addition of 10 µL of 12 mM MTT stock solution. The cells were incubated at 37°C for 4 hours. After incubation, the MTT-containing medium was removed by pipetting. Fifty µL of DMSO (DMSO, A3672, Darmstadt, Germany) was added to the cells, and they were left for 10 minutes at room temperature. Absorbance was detected at 570 nm using an ELx800UV microplate reader spectrophotometer (BioTek) with a wavelength selector system. The experiment was repeated three times. The absorbances measured in comparison to the control group were used to obtain information about the protective effect of oxytocin (22).

Terminal Transferase dUTP Nick End Labeling (TUNEL) Assay

The Terminal Deoxynucleotidyl Transferase-Mediated dUTP Nick-End Labeling (TUNEL) staining method was used to determine apoptotic cell death. Glutamate and non-glutamate cells were fixed with a 4% paraformaldehyde solution (pH 7.4) in PBS, and washed three times with PBS for 5 minutes each. Then, 20-µg/ml proteinase K was applied in a 1/500 dilution of PBS for 15 minutes. After being washed three times with PBS for 5 minutes each, the samples were treated with hydrogen peroxide for 5 minutes, and then washed three times with PBS at room temperature for 5 minutes each. The samples were left in equilibration buffer at room temperature for 5 minutes, and then were left for 60 minutes at 37°C in a moist atmospheric environment using TdT-enzyme, with plastic slides closed to cut sections. Afterwards, the samples were left in stop wash buffer for 10 minutes, and then treated with anti-dioxigenin peroxidase conjugate for 30 minutes. The samples were then washed three times with PBS for 5 minutes each, followed by staining with Diaminobenzidine (DAB), and washed several times with distilled water. Mayer's hematoxylin was used for counterstaining. TUNEL-positive cells were determined by blind method. In immunohistochemical evaluation, a semi-quantitative score was given based on the intensity and distribution of the stain. The apoptotic index was calculated by taking photos of tissue sections and counting the total number of cells and the number of TUNEL-positive cells in 10 different areas, according to the formula (23).

Statistical Analysis

Statistical analysis was performed using the GraphPad software (GraphPad Software, Inc., San Diego, CA). Descriptive data were indicated by mean±standard error. One-way analysis of variance (ANOVA) was applied to compare different dose levels of oxytocin, the control group with glutamate, and the control group with oxytocin and glutamate groups. The Tukey test was used as a post hoc test to determine which group was significant. A statistically significant p-value was considered to be less than 0.05.

RESULTS

Oxytocin Neurotoxicity Screening Test

The results of the neurotoxicity screening test for oxytocin showed that the effect of oxytocin on neurite extension at different doses was not different from that of the control group (p>0.05).

Glutamate Neurotoxicity Screening Test

The results of the study indicate that glutamate has a dose-dependent moderate neurotoxic effect on SH-SY5Y cultures (Figure 1). It was found that glutamate reduced neurite outgrowth by 50% at a concentration of 32 µM. The neurotoxicity caused by glutamate in SH-SY5Y cells was found to be reduced in a dose-dependent manner by the application of oxytocin. It was particularly observed that oxytocin significantly reduced the inhibition of neurite growth, showing neuroprotective effects, especially at concentrations of 10 µM and above (p<0.05) (Figure 2).

Cell Viability Test Metabolism Results

The results showed that the application of oxytocin increased cell proliferation significantly (p>0.001) (Figure 3 and 4). Glutamate was found to have a significant toxic effect on cell proliferation and viability at different concentrations (p<0.05). The concentration (IC50) at which glutamate reduced cell proliferation by 50% was determined to be 54 µM. Oxytocin was found to have a significant effect on cell proliferation and viability against the neurotoxic effect of glutamate at its IC50 dose (p<0.05) (Figure 5).

Terminal Transferase dUTP Nick End Labeling (TUNEL) Assay Results

The study found that when 54 µM glutamate was applied to cell cultures, the application of oxitocin at 10, 30, and 100 µM doses resulted in a decrease in apoptotic effects. The study found that the number of apoptotic cells significantly decreased compared to the group treated with glutamat alone and the groups treated with oxitocin (p<0.05) (Figure 6). There was no significant difference in the number of apoptotic cells between the group treated with 100 µM oxitocin and glutamat and the control group (p>0.05).

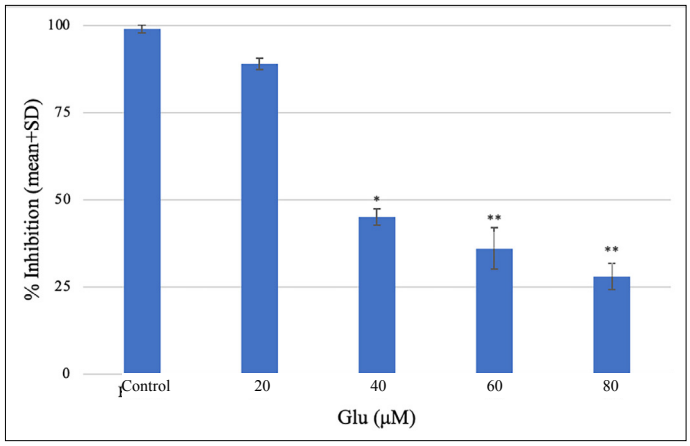


Figure 1. Inhibition of neurite extension in cells treated with glutamate (compared to the control group, *p<0.01, **p<0.001).

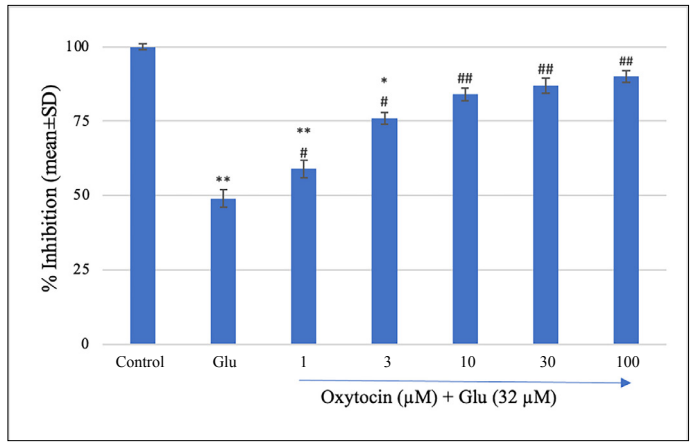
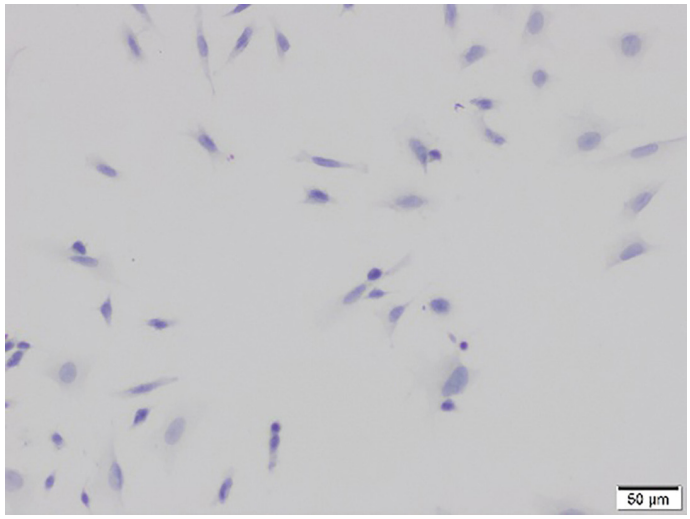


Figure 2. Effect of different concentrations of oxytocin on the inhibition of neurite caused by 32 µM glutamate (compared to the control group, *p<0.01, **p<0.001; compared to the 32 µM glutamate group, #p<0.05, ##p<0.001).



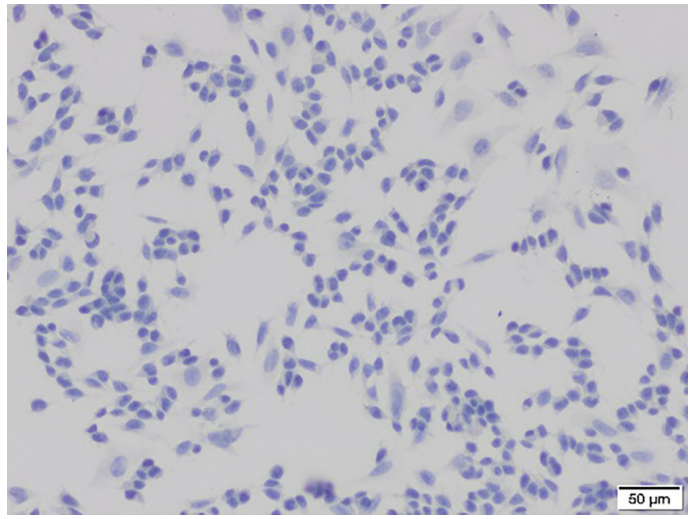


Figure 3. Phase contrast images of SH-SY5Y cells adhering and proliferating in the presence of oxytocin.

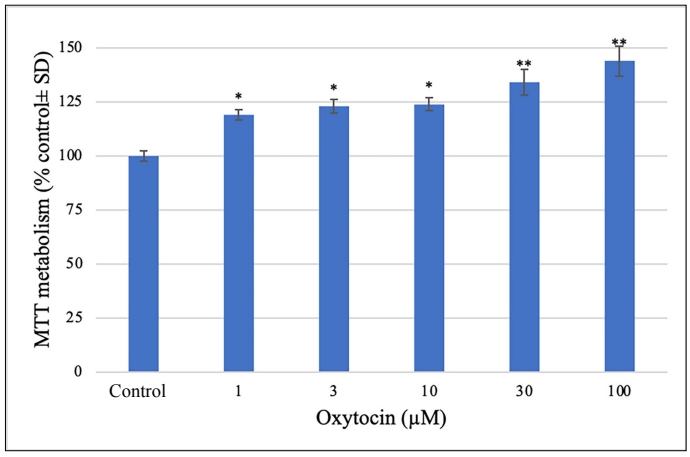


Figure 4. Effects of different concentrations of oxytocin on cell viability test (MTT) metabolism (compared to the control group, **p<0.01, ***p<0.001).

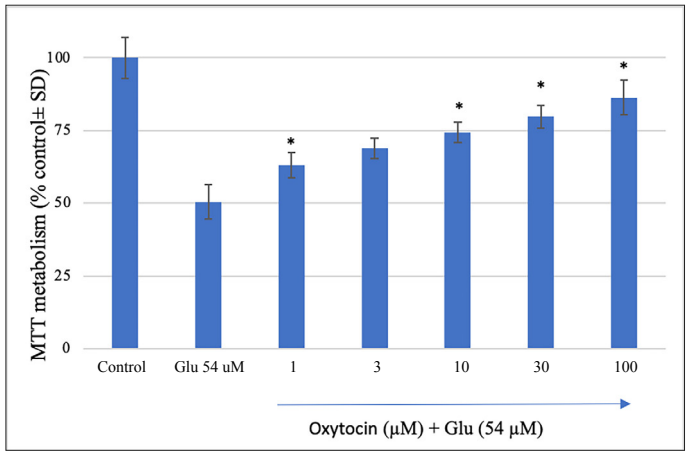


Figure 5. Effects of different concentrations of oxytocin on cell viability test (MTT) metabolism in the protection against glutamate toxicity (compared to the 54 µM glutamate group, *p<0.05).

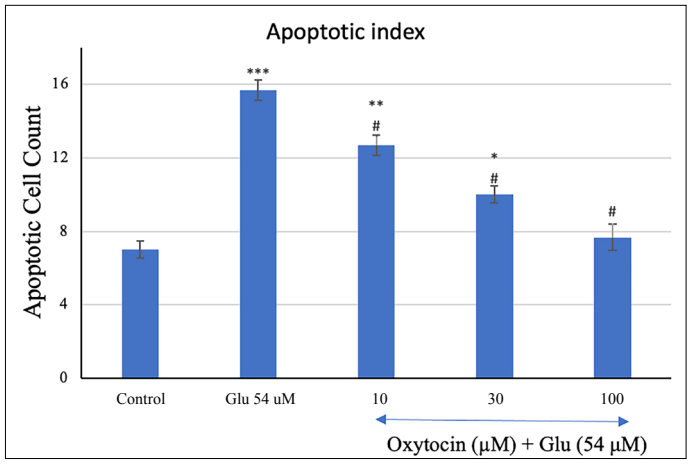


Figure 6. Effect of the presence of oxytocin on the apoptotic cell count in glutamate toxicity (compared to the control group, *p<0.05, **p<0.01, ***p<0.001; compared to the 54 µM glutamate group, #p<0.05).

DISCUSSION

This study investigated the effects of oxytocin on neuroblastoma SH-SY5Y cell culture neurotoxicity induced by glutamate. The determination of apoptotic cell death was done using TUNEL staining, cell viability and proliferation were determined by MTT test, and the possible neurotoxic effect of oxytocin and glutamate was determined by NTT test. The results showed that oxytocin reduced the inhibition of neurite growth caused by glutamate in a dose-dependent manner, demonstrated a neuroprotective effect that was dependent on the dose, and had anti-apoptotic effects.

The presence of oxytocin receptors in glial and neuroblastoma cell lines has been shown (24). Oxytocin receptors interact with G protein-coupled second messengers. Their activation can increase cytoplasmic calcium, leading to changes in the cell skeleton mediated by protein kinases regulated by signals (25). Structural changes in neuronal cells are related to the effects of a variety of neuropeptides. However, studies have suggested that various neuropeptides can modulate neurite growth through G protein-coupled receptors (26,27). The cell skeleton plays an important role in neurite growth. The interaction and dynamic changes between microtubules and microfilaments are required for neurite growth and neuronal differentiation (28). Oxytocin was found to be effective in SH-SY5Y, SK-N-SH, and U87-MG cell lines, but the most sensitive cell line to show oxytocin's effects was SH-SY5Y (20). Therefore, we used the SH-SY5Y cell line in our study.

In the first stage of our study, we attempted to determine the neurotoxic effects of glutamate and oxytocin and calculated the neurite lengths using the NTT test. Oxytocin did not show any neurotoxic effect by itself. However, glutamate showed a significant moderate neurotoxic effect in a dose-dependent manner. It was found that the application of oxytocin to glutamate's neurite inhibition had a dose-dependent protective effect. In a recent study, the effects of vazopressin and oxytocin on neurite growth in SH-SY5Y cells were also compared, and it was shown that oxytocin significantly increased neurite growth more (29). In another study, the effect of oxytocin on neurite growth and synapse formation in Magel2 knockout mice was shown (30). Bakos et al. reported that oxytocin increased cell proliferation and viability in the neuroblastoma cell line, but not with BDNF or NGF (20). Oxytocin has been shown to be effective in neurite growth and synapse formation processes (30,31), but the specific molecular mechanisms in these processes have not yet been fully illuminated.

In a comparison of a control group without oxytocin treatment and the SH-SY5Y cell line, the potential effects on cell proliferation were observed. It was found that oxytocin treatment did not exhibit toxicity in the MTT metabolism but rather increased cell proliferation. The study showed that oxytocin had a protective effect against the negative impact of glutamate on cell proliferation and viability, as increased concentrations of oxytocin were observed. The literature has investigated the impact of oxytocin on cell viability in response to various chemical agents that cause neurotoxicity. It has been reported that pre-treatment of SH-SY5Y cells with oxytocin did not affect the reduction in cell viability resulting from exposure to hydrogen peroxide, and also that 1 µM dose of oxytocin prevented the reduction in cell viability caused by the neurotoxin 6-OHDA (20). Furthermore, it has been demonstrated that unmatured hipocampal cultures incubated with oxytocin and exposed to oxygen-glucose deprivation (OGD) had significantly higher viability both immediately after OGD and after re-oxygenation (32).

Studies on the cytoprotective effects of oxytocin have been conducted in the literature. The said cytoprotective effect is argued to be provided through anti-oxidative, anti-apoptotic, and anti-inflammatory pathways (33–36). The neuroprotective effect of oxytocin has also been investigated in different experimental animal models of neuronal injury. Studies

that show the neuroprotective effect of oxytocin in models of ischemic neuronal injury have been reported (37,38). In a study conducted by Panaitescu et al., they reported that oxytocin treatment reduced both the seizure burden and hippocampal damage in a rat model of perinatal asphyxia-induced hippocampal damage, suggesting a potential neuroprotective role of oxytocin in perinatal asphyxia (39). Leuner et al. showed that oxytocin increased cell proliferation and neurogenesis in the hippocampus of rats subjected to glucocorticoid or cold water swimming stress tests (40).

In a study conducted on rats, it has been reported that the application of oxytocin has a cytoprotective effect by suppressing caspase-3, caspase-8, Bax, and increasing the expression of Bcl-2, thus reducing apoptosis in cell damage induced by rotenone in dopaminergic neurons (41). In another study conducted by Yuan et al., it was shown that oxytocin reduces excessive activation of microglial cells induced by lipopolysaccharide and inactivates the ERK/p38 MAPK signaling pathway, thus decreasing the expression of proinflammatory regulators and cytokines. Additionally, it was reported that the application of lipopolysaccharide statistically and significantly increased the expression of COX-2 and iNOS, however, prior application of oxytocin inhibited the protein expression of COX-2 and iNOS (42). In a study conducted by Akman et al., it was reported that oxytocin showed a protective effect against nephrotoxicity caused by cisplatin by increasing endogenous antioxidants, reducing lipid peroxidation and inflammation (43).

In our study, we determined that glutamate-induced apoptosis in cell culture causes cell death. Oxytocin was able to reduce the number of apoptotic cells by demonstrating an antiapoptotic effect when applied at 10, 30, and 100 µM. Reports indicate that oxytocin reduces apopotosis induced by methamphetamine and corticosterone in hippocampal neurons via oxytocin receptors (44,45).

It was found that the effects of intranasal administration of oxytocin mimic the behavioral effects observed after intraserebroventricular administration (46,47). It has also been reported that in vivo administration of oxytocin effectively improves symptoms similar to autism spectrum disorder by inhibiting ERK phosphorylation (48). Although it is predicted that oxytocin could be used for the treatment of such cases and other conditions causing neuronal damage, further experimental applications are needed to demonstrate this.

In our study, where the neuroprotective effect of oxytocin was determined, we anticipate that research will be conducted to examine the role of oxytocin in the treatment of diseases in which glutamate-mediated neurotoxicity plays a role, and in which the mechanisms underlying the neuroprotective effect are studied.

Explanation: This study was presented as an award-nominated oral presentation at the 58th National Psychiatry Congress held in Izmir on 19-23 October 2022.

Ethics Committee Approval: Ethical approval was obtained from the Health Sciences Ethics Committee of Manisa Celal Bayar University Faculty of Medicine on September 13, 2017 with the decision number 20.478.486.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept- BGÖ, KV; Design- BGÖ, KV, MİT; Supervision- KV, MİT; Resource- KV, MİT; Materials- KV, MİT; Data Collection and/or Processing- BGÖ, KV, MİT; Analysis and/or Interpretation- BGÖ, KV, MİT; Literature Search- BGÖ, KV; Writing- BGÖ, KV, MİT; Critical Reviews- KV, MİT.

Conflict of Interest: The authors declared that there is no conflict of interest.

Financial Disclosure: The study was supported by the Scientific Research Projects Unit of Manisa Celal Bayar University with the project number 2018–209.

REFERENCES

1. McEntee WJ, Crook TH. Glutamate: its role in learning, memory, and the aging brain. Psychopharmacology (Berl). 1993;111:391–401. [Crossref]
2. Glushakov AV, Dennis DM, Sumners C, Seubert CN, Martynyuk AE. L-phenylalanine selectively depresses currents at glutamatergic excitatory synapses. J Neurosci Res. 2003;72:116–124. [Crossref]
3. Hynd MR, Scott HL, Dodd PR. Glutamate-mediated excitotoxicity and neurodegeneration in Alzheimer's disease. Neurochem Int. 2004;45:583–595. [Crossref]
4. Sapolsky R. Biology and human behavior: the neurological origins of individuality, 2nd ed. Springfield, VA: Teaching Co.; 1996.
5. Shinohe A, Hashimoto K, Nakamura K, Tsujii M, Iwata Y, Tsuchiya KJ, et al. Increased serum levels of glutamate in adult patients with autism. Prog Neuropsychopharmacol Biol Psychiatry. 2006;30:1472–1477. [Crossref]
6. Choi DW. Glutamate neurotoxicity and diseases of the nervous system. Neuron. 1988;1:623–634. [Crossref]
7. Hussman JP. Suppressed GABAergic inhibition as a common factor in suspected etiologies of autism. J Autism Dev Disord. 2001;31:247–248. [Crossref]
8. Fatemi SH, Halt AR, Stary JM, Kanodia R, Schulz SC, Realmuto GR. Glutamic acid decarboxylase 65 and 67 kDa proteins are reduced in autistic parietal and cerebellar cortices. Biol Psychiatry. 2002;52:805–810. [Crossref]
9. Purcell AE, Jeon OH, Zimmerman AW, Blue ME, Pevsner J. Postmortem brain abnormalities of the glutamate neurotransmitter system in autism. Neurology. 2001;57:1618–1628. [Crossref]
10. Rubenstein JL, Merzenich MM. Model of autism: increased ratio of excitation/inhibition in key neural systems. Genes Brain Behav. 2003;2:255–267. [Crossref]
11. Jamain S, Betancur C, Quach H, Philippe A, Fellous M, Giros B, et al. Linkage and association of the glutamate receptor 6 gene with autism. Mol Psychiatry. 2002;7:302–310. [Crossref]
12. Rojas DC. The role of glutamate and its receptors in autism and the use of glutamate receptor antagonists in treatment. J Neural Transm (Vienna). 2014;121:891–905. [Crossref]
13. Churchwell KB, Wright SH, Emma F, Rosenberg PA, Strange K. NMDA receptor activation inhibits neuronal volume regulation after swelling induced by veratridine-stimulated Na+ influx in rat cortical cultures. J Neurosci. 1996;16:7447–7457. [Crossref]
14. Dessi F, Charriaut-Marlangue C, Ben-Ari Y. Glutamate-induced neuronal death in cerebellar culture is mediated by two distinct components: a sodium-chloride component and a calcium component. Brain Res. 1994;650:49–55. [Crossref]
15. Lau A, Tymianski M. Glutamate receptors, neurotoxicity and neurodegeneration. Pflugers Arch. 2010;460:525–542. [Crossref]
16. Björklund A, Hökfelt T, editors. GABA and neuropeptides in the CNS. Amsterdam, New York: Elsevier; 1985.
17. Erkanli Senturk G, Erkanli K, Aydin U, Yucel D, Isiksacan N, Ercan F, et al. The protective effect of oxytocin on ischemia/reperfusion injury in rat urinary bladder. Peptides. 2013;40:82–88. [Crossref]
18. Faghihi M, Alizadeh AM, Khori V, Latifpour M, Khodayari S. The role of nitric oxide, reactive oxygen species, and protein kinase C in oxytocin-induced cardioprotection in ischemic rat heart. Peptides. 2012;37:314–319. [Crossref]
19. Breton J-D, Veinante P, Uhl-Bronner S, Vergnano AM, Freund-Mercier MJ, Schlichter R, et al. Oxytocin-induced antinociception in the spinal cord is mediated by a subpopulation of glutamatergic neurons in lamina I-II which amplify GABAergic inhibition. Mol Pain. 2008;4:19. [Crossref]
20. Bakos J, Strbak V, Ratulovska N, Bacova Z. Effect of oxytocin on neuroblastoma cell viability and growth. Cell Mol Neurobiol. 2012;32:891–896. [Crossref]
21. McLean WG, Holme AD, Janneh O, Southgate A, Howard CV, Reed MG. The effect of benomyl on neurite outgrowth in mouse NB2A and human SH-SY5Y neuroblastoma cells in vitro. Neurotoxicology. 1998;19:629–632. https://pubmed.ncbi.nlm.nih.gov/9745922/
22. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983;65:55–63. [Crossref]
23. Aydemir I, Kum S, Tuğlu MI. Histological investigations on thymus of male rats prenatally exposed to bisphenol A. Chemosphere. 2018;206:1–8. [Crossref]
24. Cassoni P, Sapino A, Stella A, Fortunati N, Bussolati G. Presence and significance of oxytocin receptors in human neuroblastomas and glial tumors. Int J Cancer. 1998;77:695–700. [Crossref]
25. Wang Y-F, Hatton GI. Interaction of extracellular signal-regulated protein kinase 1/2 with actin cytoskeleton in supraoptic oxytocin neurons and astrocytes: role in burst firing. J Neurosci. 2007;27:13822–13834. [Crossref]

26. Cotta-Grand N, Rovere C, Guyon A, Cervantes A, Brau F, Nahon J-L. Melanin-concentrating hormone induces neurite outgrowth in human neuroblastoma SH-SY5Y cells through p53 and MAPKinase signaling pathways. Peptides. 2009;30:2014–2024. [Crossref]
27. Hobson S-A, Vanderplank PA, Pope RJ, Kerr NC, Wynick D. Galanin stimulates neurite outgrowth from sensory neurons by inhibition of Cdc42 and Rho GTPases and activation of cofilin. J Neurochem. 2013;127:199–208. [Crossref]
28. Stiess M, Maghelli N, Kapitein LC, Gomis-Ruth S, Wilsch-Brauninger M, Hoogenraad CC, et al. Axon extension occurs independently of centrosomal microtubule nucleation. Science. 2010;327:704–707. [Crossref]
29. Pierce M. Effects of arginine vasopressin and oxytocin on neurite outgrowth and cellular signaling in SH-SY5Y human neuroblastoma cell line. FASEB J. 2020;34:1. [Crossref]
30. Reichova A, Schaller F, Bukatova S, Bacova Z, Muscatelli F, Bakos J. The impact of oxytocin on neurite outgrowth and synaptic proteins in Magel2-deficient mice. Dev Neurobiol. 2021;81:366–388. [Crossref]
31. Bakos J, Srancikova A, Havranek T, Bacova Z. Molecular mechanisms of oxytocin signaling at the synaptic connection. Neural Plast. 2018;2018:4864107. [Crossref]
32. Ceanga M, Spataru A, Zagrean A-M. Oxytocin is neuroprotective against oxygen-glucose deprivation and reoxygenation in immature hippocampal cultures. Neurosci Lett. 2010;477:15–18. [Crossref]
33. Rashed LA, Hashem RM, Soliman HM. Oxytocin inhibits NADPH oxidase and P38 MAPK in cisplatin-induced nephrotoxicity. Biomed Pharmacother. 2011;65:474–480. [Crossref]
34. Authier S, Tanguay JF, Geoffroy P, Gauvin D, Bichot S, Ybarra N, et al. Cardiovascular effects of oxytocin infusion in a porcine model of myocardial infarct. J Cardiovasc Pharmacol. 2010;55:74–82. [Crossref]
35. Szeto A, Nation DA, Mendez AJ, Dominguez-Bendala J, Brooks LG, Schneiderman N, et al. Oxytocin attenuates NADPH-dependent superoxide activity and IL-6 secretion in macrophages and vascular cells. Am J Physiol Endocrinol Metab. 2008;295:E1495–E1501. [Crossref]
36. Petersson M, Lagumdzija A, Stark A, Bucht E. Oxytocin stimulates proliferation of human osteoblast-like cells. Peptides. 2002;23:1121–1126. [Crossref]
37. Kaneko Y, Pappas C, Tajiri N, Borlongan CV. Oxytocin modulates GABAAR subunits to confer neuroprotection in stroke in vitro. Sci Rep. 2016;6:35659. [Crossref]
38. Karelina K, Stuller KA, Jarrett B, Zhang N, Wells J, Norman GJ, et al. Oxytocin mediates social neuroprotection after cerebral ischemia. Stroke. 2011;42:3606–3611. [Crossref]
39. Panaitescu AM, Isac S, Pavel B, Ilie AS, Ceanga M, Totan A, et al. Oxytocin reduces seizure burden and hippocampal injury in a rat model of perinatal asphyxia. Acta Endocrinol (Buchar). 2018;14:315–319. [Crossref]
40. Leuner B, Caponiti JM, Gould E. Oxytocin stimulates adult neurogenesis even under conditions of stress and elevated glucocorticoids. Hippocampus. 2012;22:861–868. [Crossref]
41. Erbaş O, Oltulu F, Taşkiran D. Amelioration of rotenone-induced dopaminergic cell death in the striatum by oxytocin treatment. Peptides. 2012;38:312–317. [Crossref]
42. Yuan L, Liu S, Bai X, Gao Y, Liu G, Wang X, et al. Oxytocin inhibits lipopolysaccharide-induced inflammation in microglial cells and attenuates microglial activation in lipopolysaccharide-treated mice. J Neuroinflammation. 2016;13:77. [Crossref]
43. Akman T, Akman L, Erbas O, Terek MC, Taskiran D, Ozsaran A. The preventive effect of oxytocin to Cisplatin-induced neurotoxicity: an experimental rat model. Biomed Res Int. 2015;2015:167235. [Crossref]
44. Latt HM, Matsushita H, Morino M, Koga Y, Michiue H, Nishiki T, et al. Oxytocin Inhibits Corticosterone-induced Apoptosis in Primary Hippocampal Neurons. Neuroscience. 2018;379:383–389. [Crossref]
45. Li C, Wang H, Wang M, Chen C, Bai F, Ban M, et al. Oxytocin Attenuates Methamphetamine-Induced Apoptosis via Oxytocin Receptor in Rat Hippocampal Neurons. Front Pharmacol. 2021;12:639571. [Crossref]
46. Wang Z, Liu D, Wang F, Liu S, Zhao S, Ling E-A, et al. Saturated fatty acids activate microglia via Toll-like receptor 4/NF-kappaB signalling. Br J Nutr. 2012;107:229–241. [Crossref]
47. Lukas M, Neumann ID. Nasal application of neuropeptide S reduces anxiety and prolongs memory in rats: social versus non-social effects. Neuropharmacology. 2012;62:398–405. [Crossref]
48. Wang Y, Zhao S, Wu Z, Feng Y, Zhao C, Zhang C. Oxytocin in the regulation of social behaviours in medial amygdala-lesioned mice via the inhibition of the extracellular signal-regulated kinase signalling pathway. Clin Exp Pharmacol Physiol. 2015;42:465–474. [Crossref]