https://doi.org/10.52973/rcfcv-e361819 Revista Científica, FCV-LUZ / Vol. XXXVI

Demonstration of ovarian and brain tissue interactions as a result of stimulation of oogenesis in Migraine Modeled rats.

Demostración de las interacciones del tejido Ovárico y Cerebral como resultado de la estimulación de la Ovogénesis en ratas con Migraña modelada.

Berna Özdenoğlu-Kutlu*, Serpil Ünver-Saraydin

[1]Sivas Cumhuriyet University, Medical Faculty, Histology&Embriyology Department, Sivas, Türkiye

**Corresponding author: bernaozdenoglu@gmail.com*

ABSTRACT

Migraine, is a primary neurological disease. Although there is no gender difference in the prepubertal period, migraine is more common in adult women than in adult men. This situation decreases with menopause, but still its dominance in women continues. In our study, it is aimed to determine whether follitropin alfa could have a developmental benefit on the ovarian follicles of animals in migraine model after creating experimental migraine model with nitroglycerin on rats. In this study, the animals were divided into a total of four groups: a control group and three experimental groups. The first experimental group animals were injected with nitroglycerin, the other groups were injected with follitropin alfa and follitropin alfa together with nitroglycerin. At the end of 21 days, euthanasia was provided with pentothal sodium. Immunohistochemical methods were applied to post-mortem brain and ovarian tissues of rats. c-Fos was used as a migraine marker, Zona Pellucida 3 was used to show changes in the zona pellucida on the ovarian surface, MMP-9 and transient receptor potential vanilloid 1 were used to elucidate the pathogenesis of migraine. In our study, samples were evaluated by comparison with immunohistochemical staining. In animals, high c-Fos localization in the brain stem, high expression of Matrix metalloproteinase-9 in granular neurons, transient receptor potential vanilloid 1 in theca layer and Zona Pellucida 3 in the zona pellucida region were detected. The expression relationships of c-Fos, Matrix metalloproteinase-9, transient receptor potential vanilloid 1 and Zona Pellucida 3 in the brain stem, brain frontal cortex and ovary tissues where migraine and follitropin alfa were studied together have been shown for the first time in this study.

Key words: Brain; migraine; ovary; rat; c-Fos.

RESUMEN

La migraña es una enfermedad neurológica primaria. Si bien no existen diferencias de género en el período prepuberal, es más común en mujeres adultas que en hombres. Esta situación disminuye con la menopausia, pero persiste su prevalencia en mujeres. Nuestro estudio busca determinar si la folitropina alfa podría tener un efecto beneficioso sobre el desarrollo de los folículos ováricos de animales en un modelo de migraña, tras crear un modelo experimental de migraña con nitroglicerina en ratas. En este estudio, los animales se dividieron en un total de cuatro grupos: un grupo de control y tres grupos experimentales. Los animales del primer grupo experimental recibieron nitroglicerina; los demás grupos recibieron folitropina alfa y folitropina alfa junto con nitroglicerina. Al cabo de 21 días, se les practicó la eutanasia con pentotal sódico. Se aplicaron métodos inmunohistoquímicos a tejidos cerebrales y ováricos post mortem de ratas. Se utilizó c-Fos como marcador de migraña, zona pelúcida 3 para mostrar cambios en la zona pelúcida en la superficie ovárica, metaloproteinasa de matriz-9 y receptor de potencial transitorio vaniloide 1 para dilucidar la patogénesis de la migraña. En nuestro estudio, las muestras se evaluaron comparándolas con tinción inmunohistoquímica. En animales, se detectó una alta localización de c-Fos en el tronco encefálico, una alta expresión de metaloproteinasa de matriz-9 en neuronas granulares, receptor de potencial transitorio vaniloide 1 en la capa de la teca y zona pelúcida 3 en la región de la zona pelúcida. Las relaciones de expresión de c-Fos, metaloproteinasa de matriz-9, receptor de potencial transitorio vaniloide 1 y zona pelúcida 3 en el tronco encefálico, la corteza frontal cerebral y los tejidos ováricos donde se estudiaron conjuntamente la migraña y la folitropina alfa se han demostrado por primera vez en este estudio.

Palabras clave: Cerebro; migraña; ovario; rata; c-Fos

Recibido: 08/10/2025 Aceptado: 19/01/2026 Publicado: 11/02/2026

INTRODUCTION

Migraine is a disease that is triggered by various factors with not yet fully known pathology and progresses with different clinical pictures. Although there is no gender difference in the prepubertal period, migraine is more common in women than in adult men. This decreases with menopause, but its predominance in women continues [1].

Since the pathophysiology of migraine, which causes significant loss of labor force in the society, has not yet been fully explained, the drugs used for the treatment of migraine today are prophylactic and symptomatic and a treatment parameter that provides complete recovery has not been developed [2].

There are available many studies showing that nitric oxide plays a very important role in the pathophysiology of migraine disease. Systemic administration of nitroglycerin (NG), a nitric oxide donor, activates the brain nuclei involved in nociceptive transmission. NG infusion is an approved migraine model in humans [3].

However, the experimental migraine model induced by NG infusion in free and to the rats that roam awake is shown as the closest model to the human model of migraine accepted by everyone [4].

The c-Fos protein, one of the proto-oncogenes whose synthesis increasing with pain or a noxious stimulus, is a good marker for neuronal activation. Neuronal activation areas are widely used to show pain [5].

In this respect, an increase in c-Fos expression in the caudalis of the trigeminal nucleus is expected in the headache phase of migraine [6].

In this study, it was investigated whether follitropin alpha (FA), a follicle stimulating hormone, could have a developmental benefit on ovarian follicles in migraine modeled animals by inducing an experimental migraine model with NG in rats. FA belongs to the gonadotropins hormone family [7].

Gonadotropins are associated with reproduction and fertility. In adult women, Gonal-f is used to induce ovulation in women who are unable to ovulate and have not responded to treatment with clomiphene citrate, and to promote the development of multiple follicles in assisted reproductive technology (IVF) such as in vitro fertilization. Gonal-f contains a drug called 'follitropin alfa' [7].

Gonal-f was used in this study to determine whether migraine has any role in the relationship between migraine and fertility. c-Fos activity is used as a neuronal marker in terms of showing neuronal activation [8]. It has been emphasized that MMP-9 is very important in the physiology and pathology of the brain. In fact, its amount is quite low in healthy brain tissue. In physiological stimulation and pathological conditions, protein synthesis, gene expression and enzymatic there is an increase in the level of activity [9].

In the study conducted by Feng *et al*. [10], have demonstrated that transient receptor potential vanilloid 1 (TRPV1) stimulates sensory neurons involved in the transport and determination of pain sensation and these channels have been shown to be highly important regulators of nociceptive and inflammatory pain.

Zona pellucida glycoproteins have important roles in fertilization. Therefore, any deficiency or defect in zona pellucida glycoproteins may adversely affect fertilization [11].

It was aimed to determine the expressions of c-Fos, Matrix metalloproteinase-9 (MMP-9), TRPV1 and Zona Pellucida 3 (ZP3) in the brainstem, brain frontal cortex and ovarian tissues where migraine and FA were studied together and to show the interactions between ovarian and brain tissue as a result of oogenesis stimulation in rats with a migraine model. The objectives set forth set this study apart from similar studies.

MATERIAL AND METHODS

In this study, 32 8-week-old adult female Wistar albino rats (*Rattus norvegicus*) with a body weight of 200-250 grams were used in the Experimental Animal Laboratory. The animals were fed with standard pellet feed and tap water during the experiment and then the animals were divided into a total of four groups: a control group and three experimental groups.

The first experimental group animals were injected with NG, the second experimental group animals were injected with FA, and the third experimental group animals were injected with both NG and FA (Gonal-f 900 IU/1.5 mL).

For immunohistochemical staining, c-Fos, ZP3, MMP-9 and TRPV1 were used, the tissues obtained were compared and evaluated and photographs were taken from the appropriate areas. For semiquantitative evaluation of tissue samples from the brain, brainstem and ovary, 5 preparations per group were scoring was done by selecting. A semi-quantitative scoring method was applied to evaluate staining intensity and distribution. Specifically, three independent observers visually assessed the histochemical intensity in each structure, and the average of their scores was used to describe the final expression levels.

Immunohistochemical staining

Serial 3 µm sections from tissue samples taken from paraffin blocks were incubated in an oven overnight, and the following steps were applied. Deparaffinization was performed in xylol. Subsequently, the samples were incubated in 100 %, 95 %, 80 %, and 70 % alcohol series for 2 minutes (min) each, followed by a 5-min rinse in distilled water. Hydrogen peroxide was added and allowed to stand at room temperature for 10 min. Sodium citrate buffer (pH = 6) was used for the boiling solution, and the samples were boiled in the microwave for a total of 10 min.

The edges of the tissue samples were outlined with a Pap pen (Daido Sangyo Co., Ltd. Tokyo, Japan). They were then washed in distilled water. To prevent nonspecific transfer of immunoglobulin, the sections were incubated in SuperBlock (Sky Tech Lab, USA) solution at room temperature for approximately 30 min. Tissue samples were incubated with primary antibodies at 4 °C in a humid, dark environment overnight.

The primary antibody used in this study was MMP-7, and the antibody dilution ratio was 1:100. The primary antibody (ab5706, Abcam) was diluted with antibody diluent reagent (Invitrogen, USA) at pH 7.4. They were washed with distilled water. After 20 min of Streptavidin/Biotin protein binding, the samples were finally washed with distilled water. Counterstaining was performed with hematoxylin for 1 min and then washed. Coverslip medium was used to cover the tissues, and the tissues were then covered.

Semiquantitative Assessment

All sections were examined by two independent observers, and the ovary tissues were evaluated according to the degree of antibody staining: no staining was considered negative (-), mild staining was considered (+), moderate staining was considered (++), strong staining was considered (+++), and very strong staining was considered (++++).

RESULTS AND DISCUSSION

In nitroglycerin group animals, very intense c-Fos expression was observed in granular neurons in the Trigeminal Nucleus Caudalis (TNC) region of the brain stem, while localization was not observed in control group animals (FIG. 1).

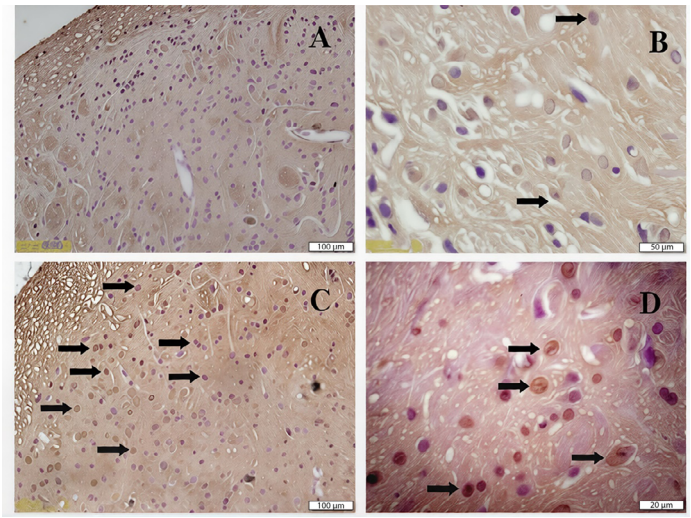

FIGURE 1. C-Fos immunolocalization of the (A, B) control group and (C, D) nitroglycerin group in the brain stem. A) x40 scale bar: 20 µm, B) x100 scale bar: 10 µm, C) x40 scale bar: 20 µm D) x100 scale bar: 10 µm. Granular neurons (black arrow).

In the control group, non-localized MMP-9 was expressed in the cerebral cortex region, but mostly in granular and occasionally motor neurons in the lamina granularis interna and externa regions in the experimental groups. MMP-9 was localized especially in the peripheral parts of granular neurons (FIG. 2).

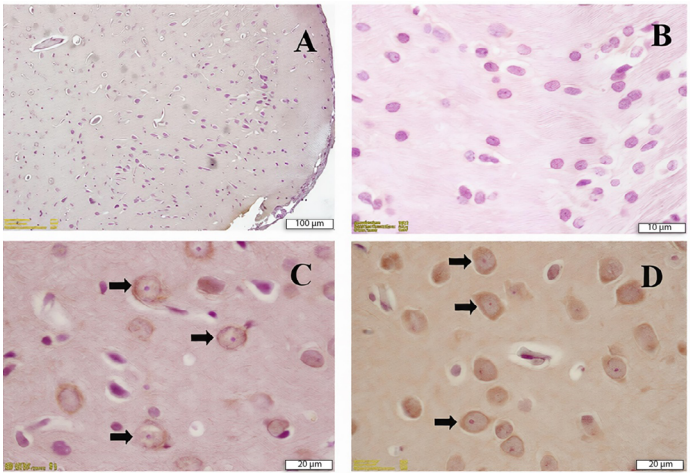

FIGURE 2. MMP-9 immunolocalization of (A, B) control group and (C) nitroglycerin group and (D) nitroglycerin + follitropin alpha group in the brain. A) x40 scale bar: 20 µm, B) x100 scale bar: 10 µm, C) x100 scale bar: 10 µm D) x100 scale bar: 10 µm. Granular neurons (black arrow).

Transient receptor potential vanilloid 1, showed intense expression, especially in the NG group. Although TRPV1 was strongly observed in the cytoplasm of pyramidal neurons, its density was quite evident in both large and small motor neurons, but TRPV1 localization was not observed in granular neurons. TRPV1 localization was moderate in granular neurons in the FA + NG group. TRPV1 expression was not observed in the FA group (FIG. 3).

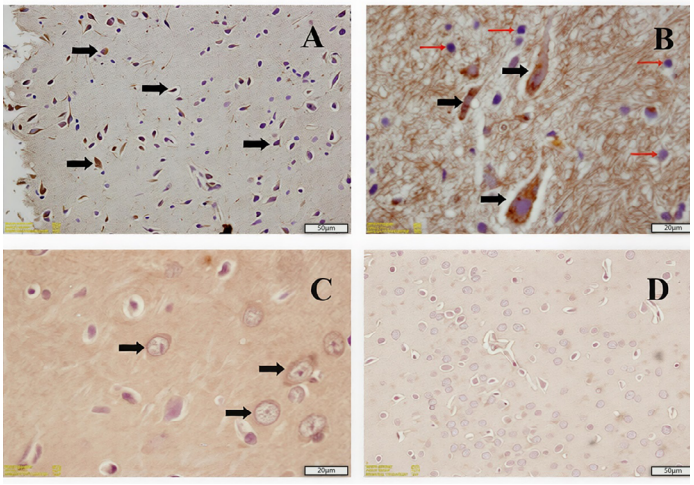

FIGURE 3. MMP-9 immunolocalization of (A, B) nitroglycerin group, (C) nitroglycerin + follitropin alpha group, and (D) follitropin alpha group in the brain. A) x40 scale bar: 20 µm, B) x100 scale bar: 10 µm, C) x100 scale bar: 10 µm D) x40 scale bar: 20 µm. Motor and granular neurons (black arrow).

In the ovary, MMP-9 was observed in the most prominent corpus luteum, cytoplasmic areas of granulosa lutein and theca lutein cells in the control group, while MMP-9 localization was observed in the ovarian epithelium and primordial follicle epithelium. When the ovarian samples taken from the NG

injection group were examined, MMP-9 staining was generally weak. MMP-9 expression was significantly higher in the FA group compared to the control and NG groups. In the FA + NG group, a very severe MMP-9 staining was observed in the medulla region and granulosa lutein and theca lutein cells (FIG. 4).

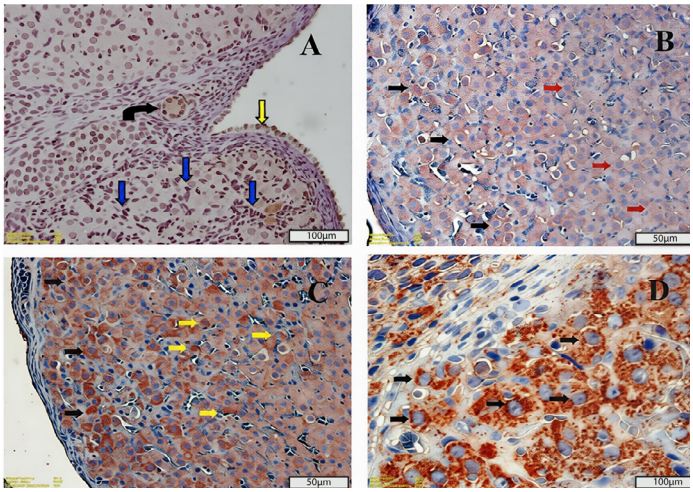

FIGURE 4. MMP-9 immunolocalization of (A) control group, (B) nitroglycerin group, (C) follitropin alpha group and (D) nitroglycerin + follitropin alpha group in the ovary. A) x40 scale bar: 20 µm, B) x40 scale bar: 20 µm, C) x40 scale bar: 20 µm D) x100 scale bar: 10 µm. Theca lutein cells (black arrow), primordial follicle (curved black arrow), germinal epithelium (yellow arrow with black stripes), granulosa lutein cells (yellow, blue arrow).

Transient receptor potential vanilloid 1 immunolocalization appeared more prominent in the ovaries of the FA and FA + NG groups compared to the control and NG groups. High level of TRPV1 expression was observed in the medulla layer, corpus luteum cells, granulosa lutein and theca lutein cells, and intermediate connective tissue cells (FIG. 5).

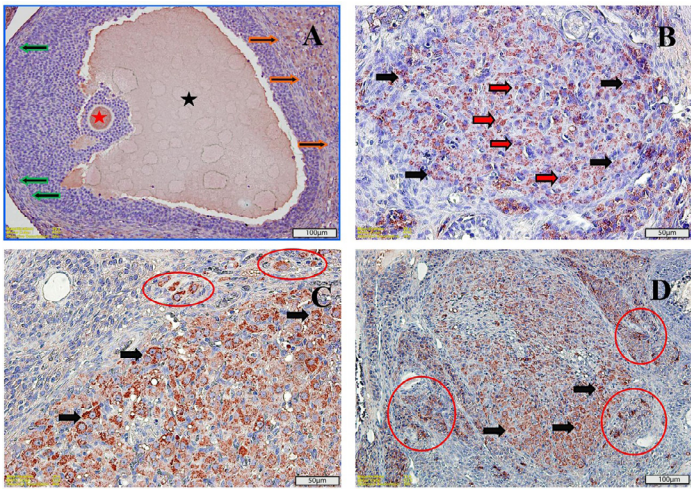

FIGURE 5. TRPV1 immunolocalization in the ovary (A) control group, (B) nitroglycerin group, (C) follitropin alpha group and (D) nitroglycerin + follitropin alpha group. A) x40 scale bar: 20 µm, B) x40 scale bar: 20 µm, C) x40 scale bar: 20 µm D) x40 scale bar: 20 µm. Theca lutein cells (black arrow), granulosa lutein cells (red arrow with black stripes), antrum (black star), secondary follicle (red star), theca interna (black arrow with green stripe), theca externa (black arrow with orange stripe), connective tissue areas (ring).

The zona pellucida 3 was intensely expressed in the zona pellucida regions of the ovarian follicles, especially in the secondary follicles (FIG. 6).

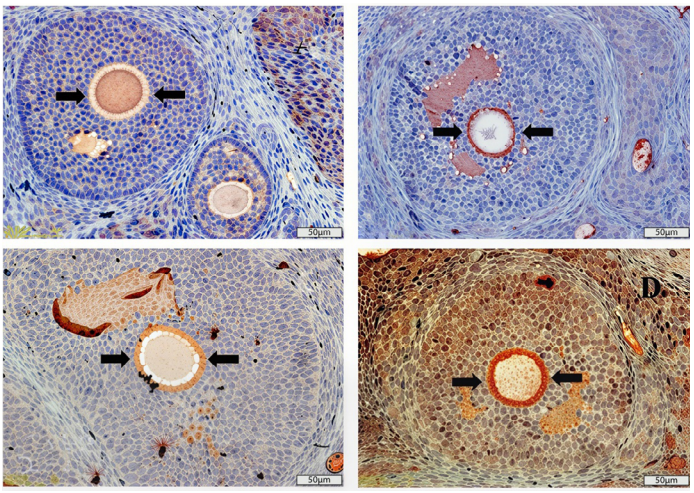

FIGURE 6. ZP3 immunolocalization in the ovary, (A) control group, (B) nitroglycerin group, (C) follitropin alpha group and (D) nitroglycerin + follitropin alpha group. A) x40 scale bar: 20 µm, B) x40 scale bar: 20 µm, C) x40 scale bar: 20 µm D) x40 scale bar: 20 µm. Zona pellucida (black arrow).

Many studies have been conducted to elucidate the pathophysiology of migraine, however the exact mechanism remains unclear due to the complex nature of migraine pain. Systemic administration of NG, a nitric oxide donor, brain nuclei are activated involved in nociceptive transmission [12 , 13 , 14]. In this study, we think that the high localization of c-Fos in the TNC region in animals in which we created an experimental migraine model compared to normal tissues may be due to the increased expression of Fos proteins by growth factors and c-Fos gene, mitogens and many extracellular factors and the relationship between this increase and very important biological processes such as proliferation, differentiation, growth and apoptosis occurring in the cell.

In addition, since c-Fos activity is used as a neuronal marker in terms of showing neuronal activation [13], it is possible to say that the significant difference in expression and its higher intensity in the experimental group may be due to NG. FAis a follicle-stimulating hormone belonging to the gonadotropins hormone family. In adult women who are unable to ovulate and have not responded to treatment with clomiphene citrate, Gonal-f is used to promote the development of multiple follicles in IVF such as in vitro fertilization, which is used to induce ovulation [14].

In this study, c-Fos localization decreased in the brainstem samples of the FA group, especially in the NG group, suggesting that c-Fos gene is one of the first genes may be due to the fact that the expression and accumulation of this core protein in the cell can be seen without any effect [15], and it can also accept it as an indication that FA that it does not exert an effect externally on pain. It has been emphasized that MMP-9 is very important in the physiology and pathology of the brain. In fact, its amount is quite low in healthy brain tissue.

In physiological stimulation and pathological conditions, protein synthesis, gene expression and enzymatic activity there is an increase in the level [9]. The absence of MMP-9 localization in the brain tissue of the control group in this study may be due to the very low amount of MMP-9 in the healthy brain tissue. Studies have shown that cell death occurs in the extracellular matrix area of neurons as a result of MMP-9 disruption [16], and that MMP-9 expression may occur in ischemic neurons due to apoptosis, one of the important mechanisms of programmed cell death [17].

In this study, MMP-9 expression occurred in the periphery of the cytoplasmic areas of granular neurons and in certain accompanying extracellular matrix areas in the experimental group in the NG-induced migraine model. It is conceivable that the intense MMP-9 expression in granular neurons may be triggered by migraine, in line with the possibility that apoptosis may have occurred in granular neurons, especially in cells close to their peripheral regions, since MMP-9 can be expressed in cells undergoing apoptosis.

In the ovary, MMP-9 was observed intensely among the staining patterns, especially in the granulosa lutein and teka lutein cells of the control group corpus luetum. We think that NG may have affected Follicle-stimulating hormone Nitric oxide synthase levels and suppressed folliculogenesis. Therefore, MMP-9, whose expression increases with the progression of follicular development, may be one of the proteins that can be used to determine follicular development in migraine tissues.

In addition, the fact that MMP-9 activity was predominantly observed in interstitial glandular cells and luteal tissue due to the known reproductive effect of FA, which we used in this study, and the fact that hormonal differences may cause changes in localization levels, in light of the studies mentioned above, clarifies that the difference in MMP-9 staining is seen in the interstitial cells of the follicle, which are open to hormonal interactions.

However, the fact that localization was predominantly observed in the corpus luteum in the FA + NG group may be due to the fact that female sex hormones are effective in migraine pathophysiology [17] and FA had an increasing effect on MMP-9 expression in the NG-induced migraine model.

It is also known that TRPV1 is an effective control mechanism in the initiation of various pathological phenomena such as pain sensation [18 , 19] and is effective in the development of pathological conditions such as inflammation, excessive pain sensitivity and visceral hyperreflexia [20 , 21].

In the experimental group with migraine, TRPV1 localization in pyramidal neurons was observed intensely. This is similar to the information that TRPV1 increases in migraine disease states and is localized in neurons. Since it is also known that TRPV1 triggers pain and inflammation in the central nervous system, it is an expected result that V1 shows a different course from the control group with the effect of NG.

The localization of TRPV1 in pyramidal neurons in the brain tissue of migraine group animals and the fact that the study showed a difference compared to the control group supports that the study is in the right direction. Therefore, in line with the findings obtained, the idea that TRPV1 can be used in studies to determine pain is supported and brings to mind the idea that it may be one of the determinant proteins of migraine disease. FA group samples did not show TRPV1 expression in brain tissue. When animals given FA together with NG were compared with animals that received only FA injection, a significant difference was found in terms of c-Fos expression in granular neurons

Although Transient Receptor Potential channels are not selective against cations, they are known to be highly sensitive to calcium (Ca+2). In this study, there is no localization due to the fact that FA does not have a NG-like pain-inducing effect and possibly interferes with Ca+2 influx or FA suppresses the excitatory pathway of TRPV1.

It is noteworthy that the expression of TRPV1 is more intense in the teca interna and teca externa layer of tertiary follicles, intermediate connective tissue, granulosa cells and cytoplasmic areas of cells. In the realization of TRPV1 expression, it can be said that functional information may have been received through neurons and sent to hypothalamic centers via receptors located around the follicle, or TRPV1 activity may have occurred through sensory nerves [22].

In the absence of ZP3, one of the major components of the zona pellucida, folliculogenesis continues in mice, but these mice are infertile because oocytes adhere to the oviducts after ovulation. Zona pellucida glycoproteins have important roles in fertilization. ZP3 glycoprotein acts as the primary receptor in the acrosome reaction. Therefore, any deficiency or defect in zona pellucida glycoproteins negatively affects fertilization [23].

The observation of ZP3 expression in multilayered primary, secondary and tertiary follicles in ovarian control group samples indicates the presence of ZP3 protein in the zona pellucida areas of follicles. ZP3 protein is a protein capable of recognizing carbohydrate domains [24].

The reason for the intense localization of ZP3 in the NG group in this study is that NG also recognizes carbohydrate binding sites like ZP3, and the fact that no localization was observed in the tertiary follicle in the FA + NG group suggests that NG may have disrupted the functional aspect of ZP3 together with FA.

Several differences were found between the expressions. The results of this study support the differential immunolocalization of c-Fos, MMP-9, TRPV1 and ZP3 proteins in rat ovarian and brain tissues, their widespread presence in these tissues and their important roles in migraine and FA. c-Fos, MMP-9 and TRPV1 may have important roles in the pathogenesis of migraine, since the physiopathology of pain is quite complex, determining the cause of pain, It is thought that TRPV1 and MMP-9 may have roles in finding the right treatment approaches and maintaining the treatments effectively, MMP-9 and ZP3 may have roles in the results that can be obtained by comparing migraine with hormonal level differences.

CONCLUSION

This study, demonstrated for the first time the relationship between c-Fos, MMP-9, TRPV1 and ZP3 expression in brainstem, brain frontal cortex and ovarian tissues in which migraine and FA were studied together. MMP-9, TRPV1 and ZP3 proteins can be used to show the relationship between female sex hormones and migraine.

In addition, it has been shown that experimental migraine can be induced by showing c-Fos expression in the brain stem as a result of administration of NG (10 mg/kg) to rats, and in brain and ovarian tissue samples taken from animals as a result of administration of Gonal-F to rats with a total of 4 IU.

ACKNOWLEDGEMENT

This work was supported by Sivas-Cumhuriyet University Scientific Research Project Unit (No: T-2021-799)

Conflict of Interest

There is no conflict of interest between the authors

BIBLIOGRAPHIC REFERENCES

[1] Lisicki M, Schoenen J. Metabolic treatments of migraine. Expert. Rev. Neurother. [Internet]. 2020; 20(3):295-302 doi: https://doi.org/gk7wrw

[2] Goadsby P, Holland P, Martins-Oliveira M, Hoffmann J, Schankin C, Akerman S. Pathophysiology of Migraine: A Disorder of Sensory Processing. Physiol. Rev. [Internet]. 2017; 97(2):553-622. doi: https://doi.org/gh5s8v

[3] Costa D, Benincasa G, Lucchese R, Infante T, Nicoletti GF, Napoli C. Effect of nitric oxide reduction on arterial thrombosis. Scand. Cardiovasc. J. [Internet]. 2019; 53(1):1–8. doi: https://doi.org/ghkck6

[4] Pradhan AA, Smith ML, McGuire B, Tarash I, Evans CJ, Charles A. Characterization of a novel model of chronic migraine. Pain. [Internet]. 2014; 155(2):269–274. doi: https://doi.org/f5pb7p

[5] Stratford JM, Thompson JA. MSG-Evoked c-Fos activity in the nucleus of the solitary tract is dependent upon fluid delivery and stimulation parameters. Chem. Senses. [Internet]. 2016; 41(3):211–220. doi: https://doi.org/f8dnwd

[6] Maleki N, Androulakis XM. Is there any MRI pattern that discriminates female from male migraine patients? Fro. nt Neurol. [Internet]. 2019; 10:961. doi: https://doi.org/g9hc9j

[7] Yan Z, Dai Y, Fu H, Zheng Y, Bao D, Yin Y, Chen Q, Nie X, Hao Q, Hou D, Cui Y. Curcumin exerts a protective effect against premature ovarian failure in mice.J. Mol. Endocrinol. [Internet]. 2018;60(3):261-271. doi: https://doi.org/gc7dmj

[8] Dong C, Ye DX, Zhang WB, Pan HY, Zhang ZY, Zhang L. Overexpression of c-fos promotes cell invasion and migration via CD44 pathway in oralsquamous cell carcinoma. J. Oral Pathol. Med. [Internet]. 2015; 44(5):353-360. doi: https://doi.org/qpv9

[9] Vafadari B, Salamian A, Kaczmarek L. MMP-9 in translation: from molecule to brain physiology, pathology, and therapy. J. Neurochem. [Internet]. 2016; 139(S2):91-114. doi: https://doi.org/f89x5s

[10] Feng J, Yang P, Mack MR, Dryn D, Luo J, Gong X, Liu S, Oetjen LK, Zholos AV, Mei Z, Yin S, Kim BS, Hu H. Sensory TRP channels contribute differentially to skin inflammation and persistent itch. Nat. Commun. [Internet]. 2017; 8:980. doi: https://doi.org/gchz82

[11] Litscher ES, Wassarman PM. Zona Pellucida Proteins, Fibrils and Matrix. Annu. Rev. Biochem. [Internet]. 2020; 89:695-715. doi: https://doi.org/gn8ktc

[12] Pietrobon D, Moskowitz MA. Pathophysiology of migraine. Annu. Rev. Physiol. [Internet]. 2013; 75:365–391. doi: https://doi.org/gm4h2n

[13] Yang DG, Gao YY, Yin ZQ, Wang XR, Meng XS, Zou TF, Duan YJ, Chen YL, Liao CZ, Xie ZL, Fan XD, Sun L, Han JH, Yang XX. Roxadustat alleviates nitroglycerin-induced migraine in mice by regulating HIF-1α/NF-κB/inflammation pathway. Acta Pharmacol. Sin. [Internet]. 2023; 44(2):308-320. doi: https://doi.org/qpwc

[14] Pacchiarotti A, Selman H, Valeri C, Napoletano S, Sbracia M, Antonini G, Biagiotti G, Pacchiarotti A. Ovarian Stimulation Protocol in IVF: An Up-to-Date Review of the Literature. Curr. Pharm. Biotechnol. [Internet]. 2016; 17(4):303–315. doi: https://doi.org/qpwd

[15] Velazquez FN, Caputto BL, Boussin FD. c-Fos importance for brain development. Aging (Albany NY). [Internet]. 2015; 7(12):1028-1029. doi: https://doi.org/qpwf

[16] Rodriguez C, Agulla J, Delgado-Esteban M. Refocusing the Brain: New Approaches in Neuroprotection Against Ischemic Injury. Neurochem. Res. [Internet]. 2021; 46(1):51-63. doi: https://doi.org/qpwg

[17] Kaler S, Dhar P, Bhattacharya A, Mehra RD. Preliminary morphological and immunohistochemical changes in rat hippocampus following postnatal exposure to sodium arsenite. Toxicol. Int. [Internet]. 2013; 20(2):160-169. doi: https://doi.org/qpwh

[18] Aghazadeh-Tabrizi M, Baraldi PG, Baraldi S, Gessi S, Merighi S, Borea PA. Medicinal Chemistry, Pharmacology, and Clinical Implications of TRPV1 Receptor Antagonists. Med. Res. Rev. [Internet]. 2017; 37(4):936-983. doi: https://doi.org/f9g7t3

[19] Li F, Wang F. TRPV1 in Pain and Itch. In: Zhou, L. (eds). Ion Channels in Biophysics and Physiology. Advances in Experimental Medicine and Biology. Singapore: Springer. [Internet]. 2021; 1349:249-273. doi: https://doi.org/gtn83j

[20] Kolesár D, Kolesárová M, Kyselovič J. Distribution pattern of dorsal root ganglion neurons synthesizing nitric oxide synthase in different animal species. Can. J. Physiol. Pharmacol. [Internet]. 2017; 95(4):328-332. doi: https://doi.org/qpwj

[21] Iglesias LP, Aguiar DC, Moreira FA. TRPV1 blockers as potential new treatments for psychiatric disorders. Behav. Pharmacol. [Internet]. 2022; 33(1):2-14. doi: https://doi.org/qpwk

[22] Ren M, Wang T, Huang L, Ye X, Xv Z, Ouyang C, Han Z. Role of VR1 in the differentiation of bone marrow-derived mesenchymal stem cells into cardiomyocytes associated with Wnt/β-catenin signaling. Cardiovasc. Ther. [Internet]. 2016; 34(6):482-488. doi: https://doi.org/f9chpp

[23] Wassarman PM, Litscher ES. The Mouse Egg's Zona Pellucida. Curr. Top. Dev. Biol. [Internet]. 2018; 130:331-356. doi: https://doi.org/qpwm

[24] Litscher ES, Wassarman PM. Zona Pellucida Proteins, Fibrils, and Matrix. Annu. Rev. Biochem. [Internet]. 2020; 89:695-715. doi: https://doi.org/gn8ktc