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RESEARCH

Effects of Sumatriptan on PACAP, PAC-1, CGRP, VIP, and TRPV-1 Molecules in an Experimental Migraine Model

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Abstract
Migraine is a chronic neurological disorder characterized by severe headache. In the pathology of migraine, it is suggested that increased parasympathetic output to intracranial arteries due to excessive stimulation of the trigeminovascular system is responsible. The release of PACAP, VIP, and CGRP signaling molecules due to excessive stimulation leads to the dilation of intracranial arteries, causing headaches. Sumatriptan exerts its therapeutic effect by causing vasoconstriction of dilated meningeal blood vessels in migraine and suppressing the release of vasoactive neuropeptides from trigeminal sensory neurons. However, the relationship between the Sumatriptan and the signaling pathways in migraine pathology has not been sufficiently studied. In this study, we aimed to determine the potential therapeutic efficacy of the Sumatriptan on PACAP, PAC-1, CGRP, VIP, and TRPV1 molecules in an experimental migraine model. In the study, 62 Sprague Dawley rats (31 males and 31 females) weighing 250–300 g were randomly divided into five experimental groups: Control, Sham, Migraine, Sumatriptan, and Migraine-Sumatriptan, with procedures followed accordingly, with Sumatriptan administered as a 14-day chronic treatment regimen. At the end of the study, histopathological evaluation of brain tissues taken from the animals was performed, and the immunoreactivity of PACAP, PAC-1, CGRP, VIP, and TRPV1 proteins was assessed immunohistochemically. As a result, it was observed that the histopathological findings were observed to decrease in the migraine group treated with sumatriptan compared to the migraine group ($p<0.0001$). Based on the obtained data, it was determined that sumatriptan significantly affected the neuropeptides associated with pain in migraine. However, more detailed analyses are still needed in this regard.

Keywords PACAP pathway · Neurovascular Theory · Neuropeptide signaling · CGRP modulation · Trigeminal neurons

1 Introduction

Migraine, the second leading cause of disability worldwide, is a common neurological disorder affecting approximately 12% of the population [8, 43]. Migraine is defined as a recurrent primary headache disorder resulting in attacks lasting 4–72 h. The headache seen in migraine is typically unilateral, episodic, of moderate or severe intensity, and can be aggravated by routine physical activity. Migraine attacks can be associated with nausea, photophobia, and phonophobia ([28]). Despite these data, while the role of the trigeminovascular system in the pathology of migraine is suggested, this condition is not yet fully understood [4, 9]. In this context, it has been stated that the neurovascular theory induces functional and anatomical changes in the pathophysiology of migraine [36]. In the development of migraine, excessive stimulation of the trigeminovascular

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system [29] results in parasympathetic output to the intracranial arteries. This output, in turn, leads to the dilation of intracranial arteries due to the release of signaling molecules such as pituitary adenylate cyclase-activating peptide (PACAP) and calcitonin gene-related peptide (CGRP) [3]. In this context, targeting signaling molecules like PACAP and CGRP in new approaches could have therapeutic importance. PACAP and its binding receptor, pituitary adenylate cyclase-activating polypeptide type 1 (PAC-1), belong to the vasoactive intestinal peptide (VIP)/secretin/growth hormone-releasing neuropeptide superfamily, which is widely expressed in tissues [2, 49]. The existing literature suggests that PACAP and its receptors may play a role in the pathogenesis of migraine-associated headaches [14]. CGRP, a neuropeptide related to signaling pathways in migraine, is produced in peripheral and central neurons and exhibits a potent vasodilatory effect [7, 42]. In this context, drug trials targeting CGRP in migraine-related attacks have come to the forefront [47]. According to the presented data, signaling molecules such as PACAP, PAC-1, VIP, and CGRP stand out as critical molecules in the occurrence of episodic attacks in migraine disease [15, 22]. Additionally, molecules that increase pain sensitivity in migraine-related attacks may also contribute to the enhancement of symptoms. Transient receptor potential (TRP) channels, which are among the molecules that increase pain sensitivity in the perception of migraine-related attacks, consist of six subfamilies: TRPA, TRPC, TRPM, TRPP, TRPL, and TRPV. These channels are primarily expressed on the cell membrane and are part of the cation channel family [30]. TRP channels contribute to various physiological processes, including temperature regulation and the perception of pain. In this context, transient receptor potential vanilloid 1 (TRPV1) channels, particularly among the TRP channels, are associated with migraine pathogenesis due to the stimulation of meningeal nociceptors related to pain [5]. Furthermore, the activation of TRP channels can positively affect the release of CGRP from sensory nerve endings [13]. Therefore, it can be considered that TRPV1, from the TRP channel family, may play a role in the pathophysiological mechanisms related to migraine. In light of all the presented data, targeting PACAP, CGRP, VIP, which cause vasodilation, and TRPV1 channels, which increase pain sensitivity, with potential pharmacological trials in migraine-related attacks may have a positive effect on the acute treatment of migraine. Sumatriptan, one of the drugs used in the acute treatment of migraine, is a 5-hydroxytryptamine 1 (5-HT1) agonist that selectively targets the 5-HT1B and 5-HT1D receptors. Sumatriptan exerts its therapeutic effect in migraine by causing vasoconstriction of dilated meningeal blood vessels, suppressing the release of vasoactive neuropeptides from perivascular trigeminal sensory neurons, and reducing the transmission of pain signals in the trigeminal dorsal horn [17, 19]. However, although these mechanisms explain the acute action of triptans, the present study employed a 14-day daily dosing regimen. Therefore, this chronic administration paradigm was intentionally selected to investigate how prolonged triptan exposure influences migraine-related neurobiological pathways, allowing us to examine both therapeutic and potential maladaptive mechanisms. In another study, the relationship of Sumatriptan with TRPV channels in trigeminal neurons was examined. It was shown that TRPV1 channels are functional in neurons projecting to the cerebral dura and that Sumatriptan suppresses TRPV1 in trigeminal neurons [16]. TRPV1 channels may have a specific role in the pathology of migraine. Additionally, a study conducted in 2021 on 26 migraine patients indicated that early treatment with intravenously administered Sumatriptan prevented PACAP-38-induced migraine [48]. In light of these data, it can be stated that Sumatriptan may affect PACAP-38 in relation to the trigeminovascular system. In the study, the potential effects of Sumatriptan, an used in the acute treatment of migraine, on PACAP and its receptor PAC-1, CGRP and VIP, which modulates CGRP, and the TRPV1 channel, which increases pain sensitivity, were investigated, as these have been shown to be related to migraine pathogenesis in the literature. Following this, a treatment group given the Sumatriptan was formed, and the animals were dissected. In this context, the immunoreactivity of PACAP, PAC-1, CGRP, VIP, and TRPV1 was evaluated using the immunohistochemistry method.

2 Materials and methods

An experimental migraine animal model was created in the project and approval was received for the study from the Erciyes University Animal Experiments Local Ethics Committee (Ethics committee protocol number: 22/076). In the study, 62 Sprague Dawley animals, 31 females and 31 males, weighing 250–300 g, were randomly divided into 5 experimental groups, and the procedures were observed. During the experiment, rats were housed in cages with a constant ambient temperature (24 ± 2 °C), humidity (60 ± 5%), and alternating 12-h light and 12-h dark cycles. Experimental groups were designed as follows.

2.1 Control group (*n* = 10, 5 females, 5 males)

Rats in this group were fed rodent rearing pellet with free access to food and water for 14 days.

2.2 Sham group (*n* = 10, 5 females, 5 males)

Rats were given 1 ml of saline solution (0.9%) intraperitoneally (i.p.) for 14 days.

2.3 Nitroglycerin Migraine group (*n* = 14, 7 females, 7 males)

Nitroglycerin was administered at 10 mg/kg i.p. to 14 Spraque dawley rats, 7 females and 7 males, in the nitroglycerin-induced migraine group for 14 days. Nitroglycerin was dissolved in 30% alcohol, 30% propylene glycol and water, and this solution was diluted in 0.9% saline in a polypropylene tube and injected i.p. into rats [34].

2.4 Sumatriptan group (*n* = 14, 7 females, 7 males)

Rats in this group were given sumatriptan i.p. at a dose of 600 µg/kg for 14 days [9, 32]. This dose and duration were selected based on previous studies demonstrating that repeated daily administration of sumatriptan at 0.6 mg/kg for more than seven days induces latent sensitization, a well-established preclinical model of medication-overuse headache (MOH). Thus, rather than modeling an acute therapeutic response, the study intentionally employed a chronic 'overuse-like' regimen to investigate how prolonged triptan exposure modulates migraine-related neurobiological pathways and to capture both therapeutic and sensitization-related adaptations.

2.5 Nitroglycerin Migraine + Sumatriptan group (*n* = 14, 7 females, 7 males)

Rats in this group were given 10 mg/kg i.p. nitroglycerin for 14 days. Nitroglycerin was dissolved in 30% alcohol, 30% propylene glycol and water. This solution was diluted in 0.9% saline in a polypropylene tube and injected i.p. into rats, and 600 µg/kg sumatriptan was given i.p. 5 min after nitroglycerin induction ([34]; Kural et al., 2008; [9]).

While the rats were under anesthesia, they were sacrificed with the exsanguination method, and the hippocampus and cortex tissues were dissected [1, 10, 24, 39]. Neuronal changes were considered degenerative when they exhibited a region-specific and biologically plausible pattern consistent with the migraine model, rather than the random, surface-associated distribution typical of mechanically induced artifacts. Degenerating neurons were identified based on a coherent set of morphological features, including eosinophilic cytoplasm, nuclear pyknosis, and disruption of somatodendritic integrity.

2.6 Histological Analysis

The tissues were fixed with 10% formaldehyde solution and then washed in tap water (1 night), then dehydration was performed by passing the tissues through increasing alcohol series (70%, 80%, 90% and 100%). Then, the tissue samples were left in xylene (1082984000, Merck) to make them transparent, and after passing through paraffin series, they were embedded in clean paraffin. 5 µm thick sections taken from paraffin-embedded brain tissue samples using a microtome were stained with Harris hematoxylin and eosin. Histomorphological examination was performed under a light microscope (Olympus BX53) and imaged with a ZEISS Axiscope Colibri 3 digital camera. Neuronal degeneration and vascular diameter in the hippocampus and cortex were evaluated in the brain tissues of the experimental groups, with the understanding that measurements obtained from immersion-fixed sections reflect structural vessel dimensions rather than functional vasodilation. Eosinophilic neuron with pyknotic nuclei, cell swelling or shrinkage was considered as neuron degeneration [10, 46]. Histopathological results in each category were scored as 0 = none, 1 = mild, 2 = moderate and 3 = severe. Quantification was performed randomly and blindly by two researchers. A minimum of 10 images were used for histomorphological scoring for at least 10 rats (5 females + 5 males) per experimental group.

2.7 Immunohistochemistry Analysis

Anti-PACAP (Affinity, DF7197), Anti-PAC-1 (Bioss, bs-0198R), Anti-CGRP (Affinity, DF7277), Anti-VIP (Affinity, DF6627) and Anti-TRPV-1 (Cloud-Clone Corp., PAF839Hu01) immunoreactivities were detected by immunohistochemical analysis [10, 11, 38]. In summary, after deparaffinization of 5 µm thick sections, citrate buffer was used to open the epitopes (pH: 6.0; Thermo Fischer Scientific, UK, AP-9003-500). The slides were then placed in a 3% hydrogen peroxide solution in methanol to prevent endogenous peroxidase activity. Ultra V block solution (Thermo Fischer Scientific, UK, TA-125-UB) was applied to prevent nonspecific staining. Then, it was incubated with the primary antibody at 4 °C overnight. Then, it was incubated with biotinylated goat anti polyvalent secondary antibody (Thermo Fischer Scientific, UK, TP-125-BN) in an oven at 37 °C for 40 min. After washing several times with PBS, it was incubated with streptavidin peroxidase (Thermo Fischer Scientific, UK, TS-125-HR) in an oven at 37 °C for 30 min. The antibody complex was visualized by incubation with diaminobenzidine (DAB) chromogen (Thermo Fischer Scientific, UK, TA-125-HD). Then, the sections were counterstained with Gill III Hematoxylin (Merck, Germany,

1.05174.1000). It was dehydrated by passing through an increasing series of alcohol and sealed with a sealer called Entellan. Sections were examined with a ZEISS Axiscope Colibri 3 light microscope. Assessment of immunoreactivity levels was performed with Image J Version 1.46 (National Institutes of Health, Bethesda, Maryland).

2.8 Statistical Analysis

Data were expressed as mean ± SD and analyzed by One-way ANOVA test and Tukey's post-hoc test for parametric tests. In non-parametric tests, independent sample-t test with median minimum-maximum values will be applied. $p < 0.05$ was considered significant in the analyses.

3 Results

3.1 Histological Findings

Histopathological changes in the brain hippocampus and prefrontal cortex areas of the rats in the experimental groups were evaluated under a light microscope after hematoxylin and eosin staining (Fig. 1). When neuron degeneration in the hippocampus in the nitroglycerin groups was compared, it was observed that it was more severe in males than in females (***$p = 0.0004$). While vascular dilation increased in males compared to females in the sumatriptan groups ($p = 0.0045$), more dilated blood vessels were observed in females than males in the nitroglycerin groups ($p < 0.0001$).

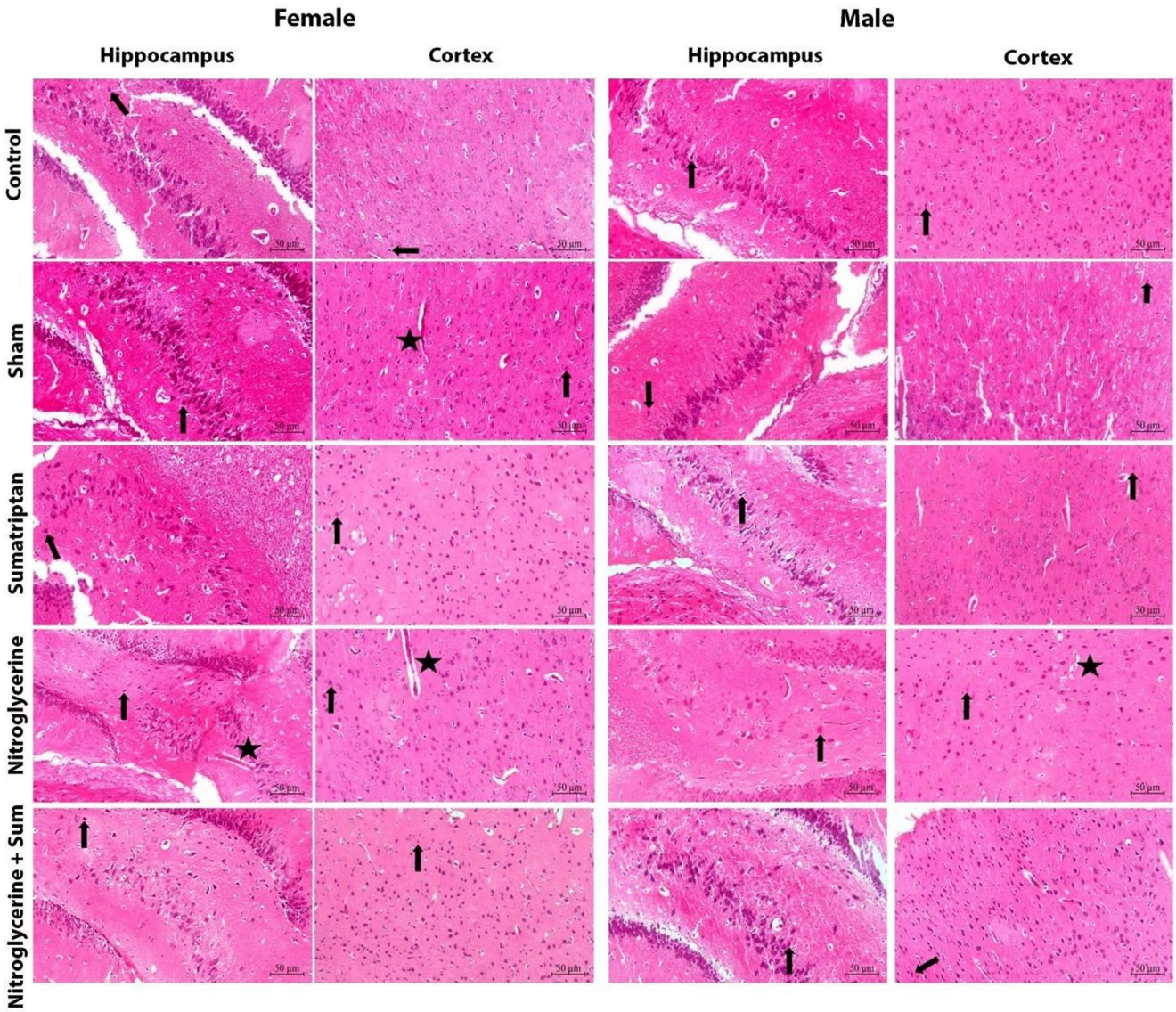


Fig. 1 Hematoxylin and eosin staining images of the brain tissues of the experimental groups. The black arrow indicates neuron degeneration. Asterisk indicates vessel diameter. Magnification is 20X and scale bar is 50 µm (Histomorphological scoring for at least 10 rats (5 females + 5 males) per experimental group, at least 10 images per animal were recorded)

When the cortex was examined, it was found that there was no difference between the experimental groups in terms of neuron degeneration. Vascular diameter in the cortex was observed to be greater in males than in females in the sumatriptan groups ($p=0.0223$). Neuron degeneration and vascular diameter in the brain were observed to be significantly increased in the nitroglycerin groups in both male and female groups (Table 1). It was determined that neuron degeneration in the female and male cortices was significantly reduced in the nitroglycerin +sumatriptan groups compared to the nitroglycerin groups (Table 1). It was observed that the vascular dilation observed in the hippocampus of nitroglycerin +sumatriptan females was less severe than that of nitroglycerin females (Table 1).

Table 1 Histopathological damage rates in brain tissues of experimental groups

Groups	Hippocampus		Cortex	
	Neuron Degeneration	Vascular diameter	Neuron Degeneration	Vascular diameter
Control female	0.3±0.07	0.32±0.13	0.34±0.08	0.52±0.19
Sham female	0.22±0.08	0.28±0.04	0.34±0.15	0.5±0.36
Sumatriptan female	0.41±0.13	0.3±0.14	0.45±0.19	0.34±0.16
Nitroglycerin female	1.37 ± 0.61^{abc}	1.77 ± 0.17^{abc}	2.12 ± 0.14^{abc}	1.55 ± 0.32^{abc}
Nitroglycerin +Sumatriptan female	1.55 ± 0.19^{abc}	0.78 ± 0.19^{abcd}	1.52 ± 0.17^{abcd}	0.91 ± 0.31^{cd}
Control male	0.18±0.08	0.38±0.30	0.4±0.14	0.48±0.29
Sham male	0.26±0.05	0.28±0.08	0.38±0.13	0.42±0.13
Sumatriptan male	0.54±0.27	0.67 ± 0.38^{f}	0.3±0.14	0.75±0.22
Nitroglycerin male	1.98 ± 0.19^{efg}	0.88 ± 0.10^{ef}	2.18 ± 0.19^{efg}	1.39 ± 0.21^{efg}
Nitroglycerin +Sumatriptan male	1.77 ± 0.24^{efg}	0.61 ± 0.08^{f}	1.7 ± 0.23^{efgh}	1.13 ± 0.30^{ef}

Statistically, the data obtained from the groups were compared using two-way analysis of variance and Sidak's multiple comparison tests. Data shown in the table are expressed as mean±SD. In female experimental groups, a $p<0.05$ vs. control group; b $p<0.05$ vs. sham group; c $p<0.05$ vs. sumatriptan group and d $p<0.05$ vs. nitroglycerin group. In male experimental groups, e $p<0.05$ vs. control group; f $p<0.05$ vs. sham group; g $p<0.05$ vs. sumatriptan group and h $p<0.05$ vs. nitroglycerin group (Histomorphological scoring for at least 10 rats (5 females+5 males) per experimental group, at least 10 images per animal were recorded)

3.2 Immunohistochemistry Findings

TRPV1 expression in the hippocampus was lower in nitroglycerin and nitroglycerin+sumatriptan groups compared to control, sham and sumatriptan groups. In both nitroglycerin and nitroglycerin +sumatriptan groups, TRPV1 expression in the hippocampus was higher in females than in males (Fig. 2, $p<0.0001$). TRPV1 expression in the cortex was significantly different between males and females in sham ($p<0.0001$), sumatriptan ($p=0.0403$) and nitroglycerin+sumatriptan ($p=0.0459$) groups (Fig. 2). In the cortex, TRPV1 expression was decreased in sumatriptan, nitroglycerin and nitroglycerin+sumatriptan groups in females and males compared to the control group (Fig. 2, $p<0.05$).

When the control and sham groups were compared within themselves in terms of VIP expression, a difference was observed between female and male hippocampi. In female hippocampuses, VIP expression decreased in all groups except the control group, whereas in male hippocampuses, VIP expression increased in the nitroglycerin +sumatriptan group compared to the other groups (Fig. 3). When VIP expression in the cortex was compared between the control, sham and nitroglycerin groups, a difference was observed between male and female rats (Fig. 3). In the cortex, VIP expression was decreased in the other experimental groups compared to the control group in both females and males (Fig. 3).

When the control groups were compared within themselves in the hippocampus, a difference was observed between male and female rats in terms of PACAP expression. In Nitroglycerin+Sumatriptan groups, increased PACAP expression was observed in both male and female rats in the hippocampus (Fig. 4). When the control, sham and sumatriptan groups were compared within themselves in the cortex, it was found that there was a difference between male and female rats in terms of PACAP expression. In both male and female rats, PACAP expression in the hippocampus increased in nitroglycerin+sumatriptan groups, whereas in the cortex it increased in nitroglycerin groups (Fig. 4).

In the hippocampus, when the nitroglycerin group was compared for PAC1 expression in females and males, it was observed that PAC1 expression was higher in females than in males. Increased PAC1 expression was observed in the hippocampus and cortex of female nitroglycerin and nitroglycerin+sumatriptan groups. In addition, PAC1 expression was increased in male hippocampus in nitroglycerin+sumatriptan group and decreased in nitroglycerin only groups. In the cortex, PAC1 expression was observed to be different in control, sham, nitroglycerin and nitroglycerin +sumatriptan groups in males and females. In male

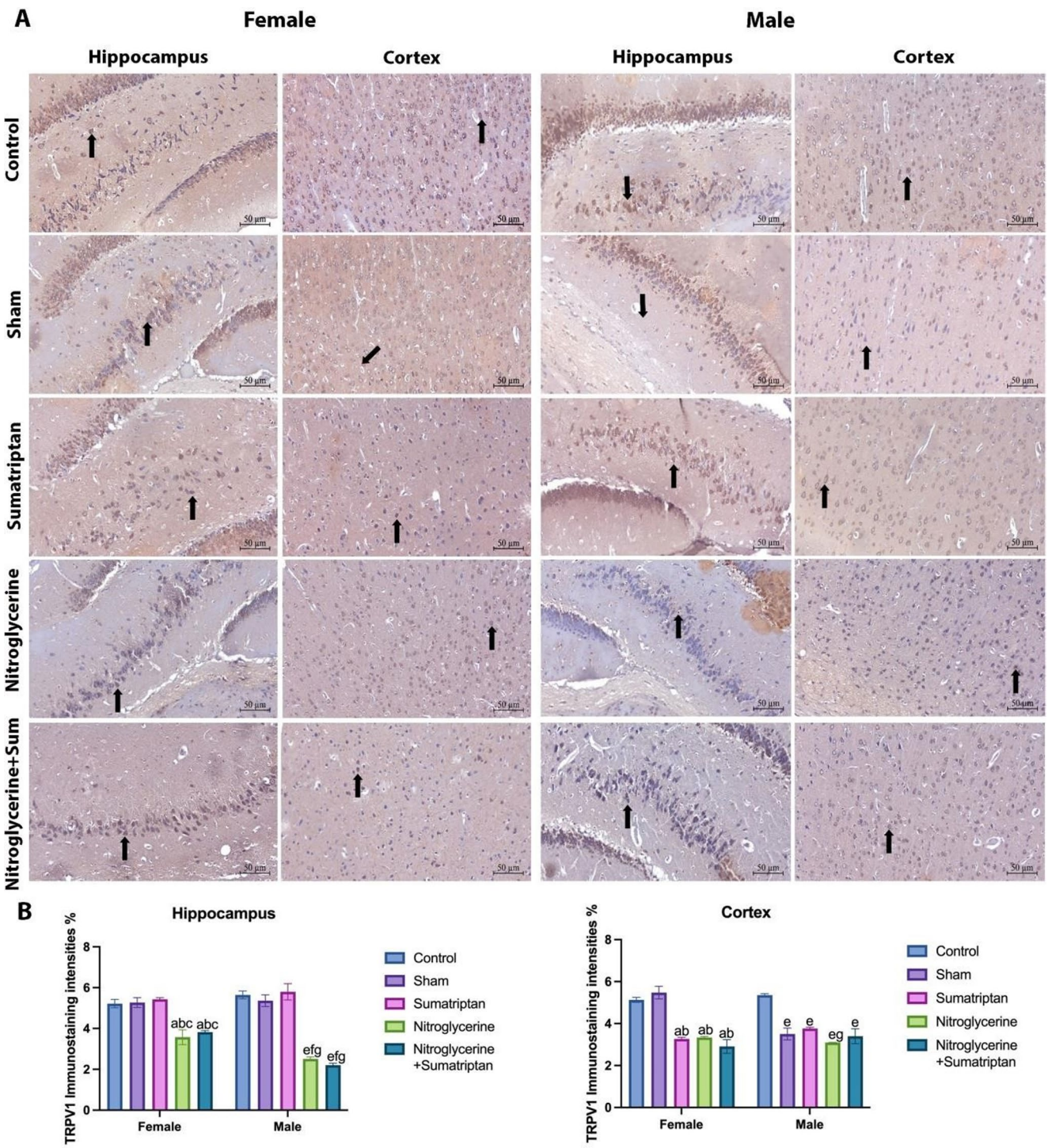


Fig. 2 **A**) Immunohistochemistry staining images of TRPV1 in the hippocampus and cortex areas of the experimental groups. Black arrow indicates immunoreactive cells. Magnification 20X and scale bar 50 µm. **B**) Bar graphs showing the intensity of immunostaining. Two-way analysis of variance, Tukey's and Sidak's multiple comparison tests were applied. Data shown in the bar graph are expressed as mean ± SD. In female experimental groups, a $p < 0.05$ vs. control group; b $p < 0.05$ vs. sham group; c $p < 0.05$ vs. sumatriptan group and d $p < 0.05$ vs. nitroglycerin group. In male experimental groups, e $p < 0.05$ vs. control group; f $p < 0.05$ vs. sham group; g $p < 0.05$ vs. sumatriptan group and h $p < 0.05$ vs. nitroglycerin group. Immunoreactivity intensity was measured separately for hippocampus and cortex areas for at least 6 rats (3 females and 3 males) per experimental group

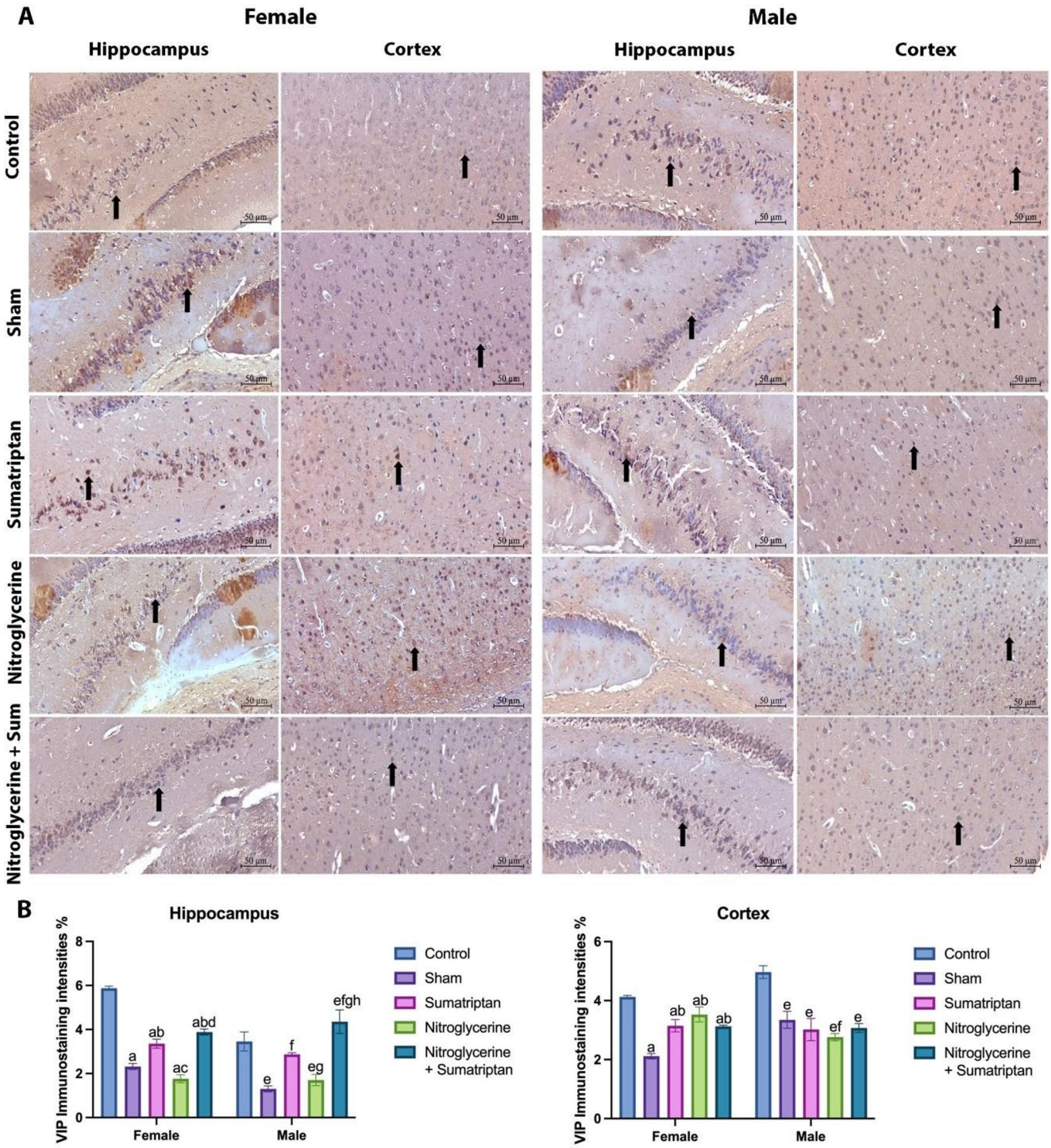


Fig. 3 **A**) Immunohistochemistry staining images of VIP in the hippocampus and cortex areas of the experimental groups. Black arrow indicates immunoreactive cells. Magnification 20X and scale bar 50 µm. **B**) Bar graphs showing the intensity of immunostaining. Two-way analysis of variance, Tukey's and Sidak's multiple comparison tests were applied. Data shown in the bar graph are expressed as mean ± SD. In female experimental groups, a $p < 0.05$ vs. control group; b $p < 0.05$ vs. sham group; c $p < 0.05$ vs. sumatriptan group and d $p < 0.05$ vs. nitroglycerin group. In male experimental groups, e $p < 0.05$ vs. control group; f $p < 0.05$ vs. sham group; g $p < 0.05$ vs. sumatriptan group and h $p < 0.05$ vs. nitroglycerin group. Immunoreactivity intensity was measured separately for hippocampus and cortex areas for at least 6 rats (3 females and 3 males) per experimental group

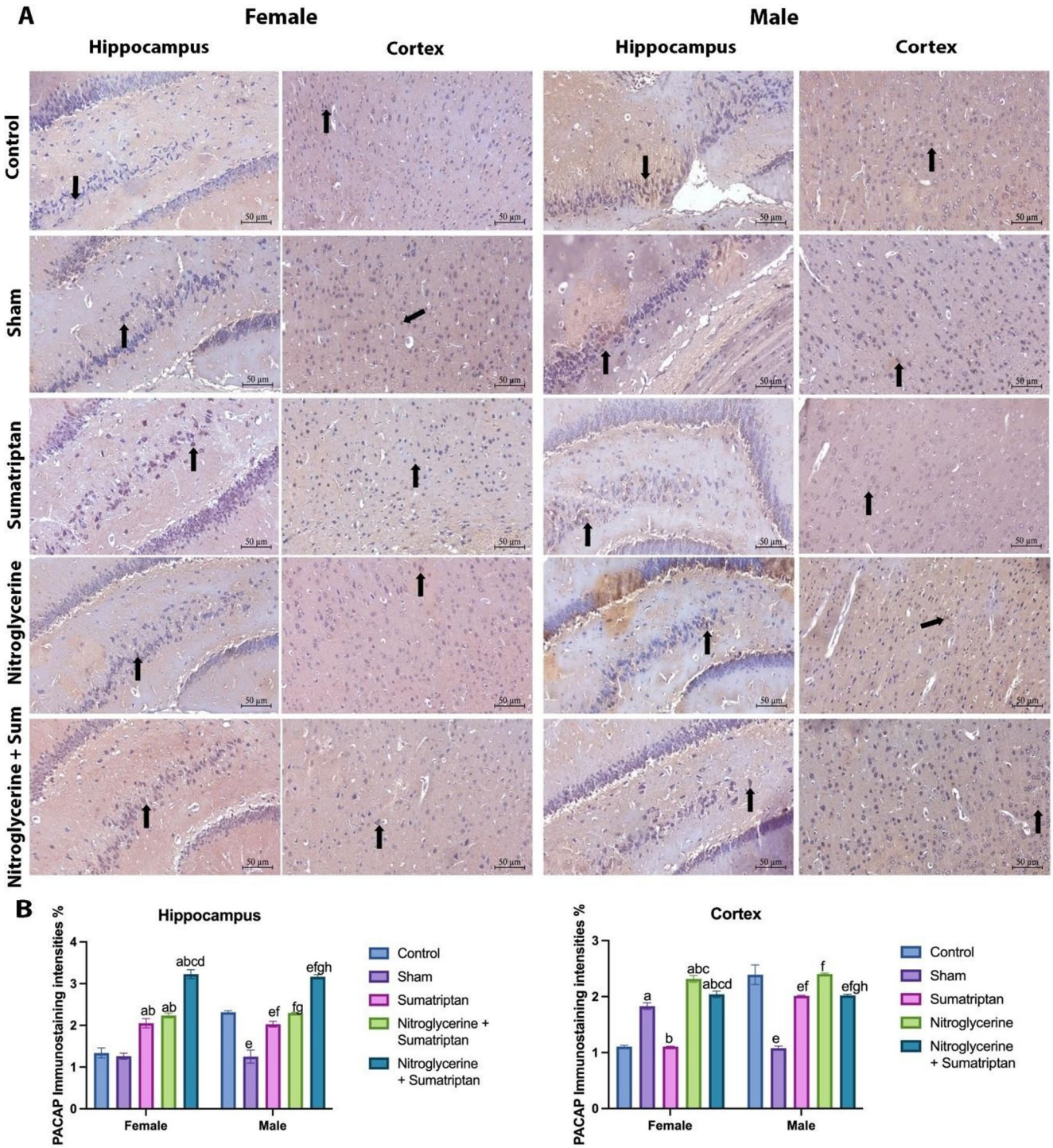


Fig. 4 **A**) Immunohistochemistry staining images of PACAP in the hippocampus and cortex areas of the experimental groups. Black arrow indicates immunoreactive cells. Magnification 20X and scale bar 50 µm. **B**) Bar graphs showing the intensity of immunostaining. Two-way analysis of variance, Tukey's and Sidak's multiple comparison tests were applied. Data shown in the bar graph are expressed as mean ± SD. In female experimental groups, a $p < 0.05$ vs. control group; b $p < 0.05$ vs. sham group; c $p < 0.05$ vs. sumatriptan group and d $p < 0.05$ vs. nitroglycerin group. In male experimental groups, e $p < 0.05$ vs. control group; f $p < 0.05$ vs. sham group; g $p < 0.05$ vs. sumatriptan group and h $p < 0.05$ vs. nitroglycerin group. Immunoreactivity intensity was measured separately for hippocampus and cortex areas for at least 6 rats (3 females and 3 males) per experimental group

nitroglycerin +sumatriptan groups, PAC1 expression in the cortex was significantly increased (Fig. 5).

In the hippocampus, when sumatriptan and nitroglycerin groups were compared within themselves, a difference was observed in terms of CGRP expression in males and females. CGRP expression was significantly increased in the sumatriptan and nitroglycerin sumatriptan groups compared to the control group in males and females (Fig. 6). When the control, sumatriptan and nitroglycerin + sumatriptan groups were compared within themselves in the cortex, a difference was observed in terms of CGRP expression in males and females. Compared to the control group, CGRP expression increased in female sumatriptan groups, while it decreased in males. CGRP expression was similar in female and male nitroglycerin groups. While CGRP expression decreased in female nitroglycerin + sumatriptan group compared to nitroglycerin group, CGRP expression increased in male nitroglycerin + sumatriptan groups compared to nitroglycerin group (Fig. 6).

4 Discussion

In this study, the effect of sumatriptan on neuropeptides in migraine disease was examined immunoexpressively. Sumatriptan-induced changes in PACAP, PAC-1, CGRP, VIP and TRPV-1 molecules were evaluated immunohistochemically. In both male and female groups, neuronal degeneration and vascular diameter in the brain were significantly increased in nitroglycerin groups. In the nitroglycerin-treated groups, sumatriptan was associated with a reduced number of neurons displaying degenerative morphology. While this finding may reflect a mitigation of nitroglycerin (NTG)-induced stress responses, its interpretation must be made with caution, as immersion fixation carries a risk of dark-neuron artifacts that can mimic true degeneration. In line with the data obtained, it was determined that sumatriptan significantly affected the neuropeptides associated with pain in migraine. Although triptan effective in the treatment of acute migraine are well tolerated, the mechanism of action is still debated. Our findings indicate that chronic Sumatriptan administration modulates the expression of CGRP and PACAP in the hippocampus and cortex, potentially leading to intracellular accumulation of these nociceptive peptides.

In the literature, the relationship between sumatriptan and TRPV1 channels in trigeminal neurons has been examined. It has been shown that TRPV1 channels are functional in neurons projecting to the cerebral dura and sumatriptan suppresses TRPV1 in trigeminal neurons [16]. Our results revealed that TRPV1 immunoactivity in the hippocampus was lower in both the experimental migraine group and the sumatriptan-treated migraine group compared to the control group. These data were significantly higher in the female group compared to males. In the cortex, TRPV1 was significantly decreased in both sexes in the migraine group receiving sumatriptan, the migraine group and the migraine group receiving the sumatriptan compared to the control. In line with these data, TRPV1 molecule may play a role in the brain in migraine-induced craniofacial pain. Sumatriptan may be involved in the reduction of migraine-related pain by affecting TRPV channels at the molecular level. Progestogens, estrogens and androgens may play a role in affecting migraine-induced TRPV1 in sumatriptan treatment. TRPV expression may be transcriptionally regulated by sex hormones [31]. Based on these data, the role of sex hormones in the activation, modulation and regulation of the main TRPV channels involved in migraine pathophysiology should be considered.

Parasympathetic neurotransmitters, PACAP38 and VIP, can be released from parasympathetic fibers and activate sensory nerve fibers during migraine attacks [35]. It is known that 5-HT1B/D receptor activation has no effect on circulating VIP and PACAP levels in humans without trigeminovascular activation [26]. In our study, VIP expression decreased in female hippocampi except for the control group, whereas VIP expression increased in male hippocampi in the nitroglycerin+sumatriptan group compared to the other groups. In the cortex, VIP expression decreased in the other experimental groups compared to the control group in both females and males. In the literature, it has been shown that 2-hour VIP infusion triggers migraine attacks in migraine patients without aura. VIP was found to have a migraine triggering rate of 71% [40]. In this context, VIP values that differ from the literature should be re-evaluated in the attack pathology of migraine disease in the brain [26]. Given that neuropeptides play an important role in migraine, it may be important to conduct a similar study in the migraine population [20].

Experimental studies have shown that PACAP isoforms (PACAP27 and PACAP38) cause migraine attacks after 20 min of infusion in migraine patients without aura [18]. Our results revealed that PACAP immune reactivity was increased in the hippocampus in the sumatriptan-treated migraine group in both females and males. In the cortex, increased PACAP expression was observed in migraine groups of both sexes. Similar to the literature, the relationship between PACAP increase and migraine disease has been shown histopathologically [6]. A study reported that early treatment with intravenously administered sumatriptan prevented migraine caused by PACAP-38 [48]. In 2022, it was reported that sumatriptan had no effect on PACAP38-induced hypersensitivity associated with migraine [21]. In our results, increased PACAP expression was observed in

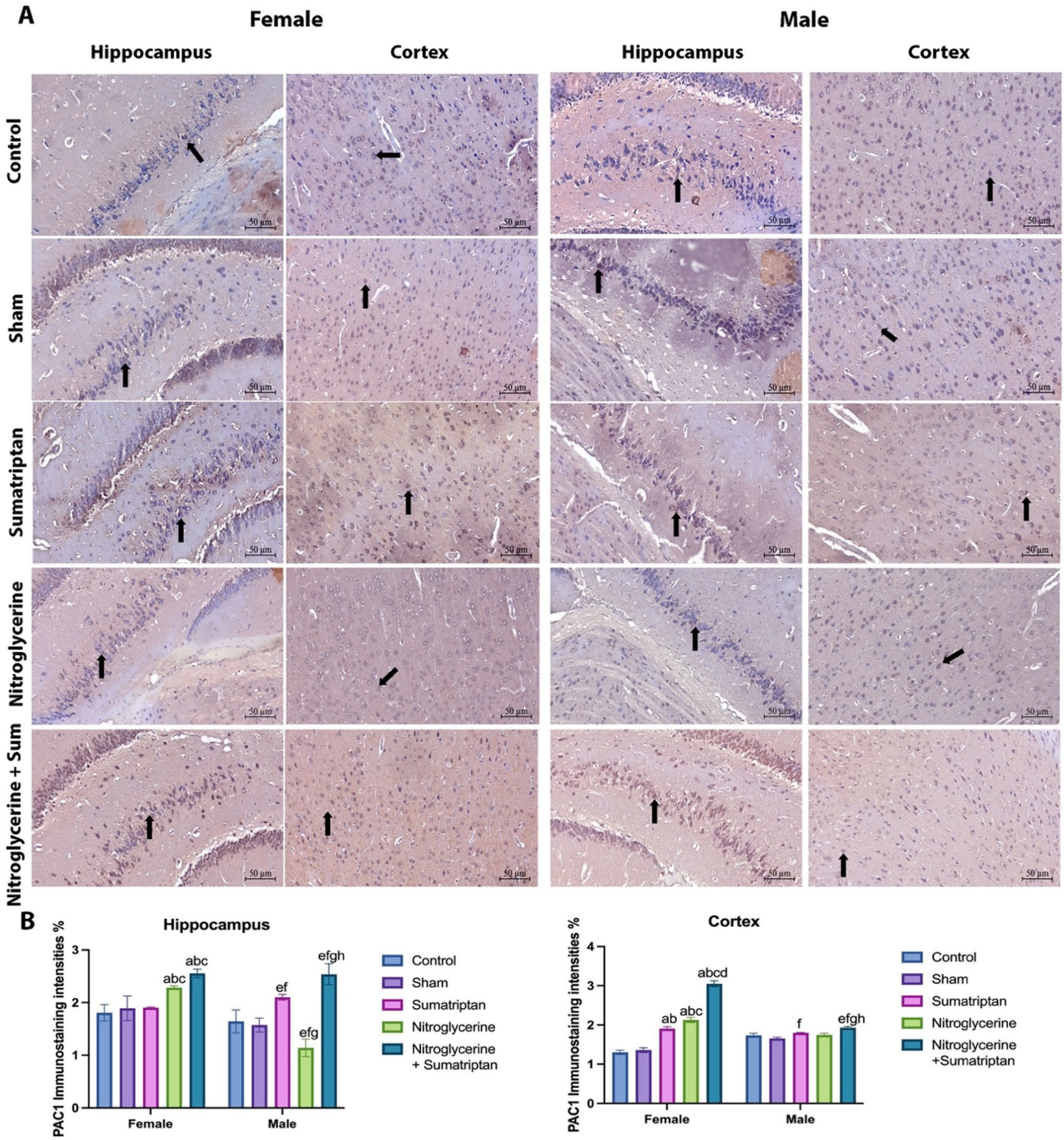


Fig. 5 **A**) Immunohistochemistry staining images of PAC1 in the hippocampus and cortex areas of the experimental groups. Black arrow indicates immunoreactive cells. Magnification 20X and scale bar 50 µm. **B**) Bar graphs showing the intensity of immunostaining. Two-way analysis of variance, Tukey's and Sidak's multiple comparison tests were applied. Data shown in the bar graph are expressed as mean ± SD. In female experimental groups, a $p<0.05$ vs. control group; b $p<0.05$ vs. sham group; c $p<0.05$ vs. sumatriptan group and d $p<0.05$ vs. nitroglycerin group. In male experimental groups, e $p<0.05$ vs. control group; f $p<0.05$ vs. sham group; g $p<0.05$ vs. sumatriptan group and h $p<0.05$ vs. nitroglycerin group. Immunoreactivity intensity was measured separately for hippocampus and cortex areas for at least 6 rats (3 females and 3 males) per experimental group

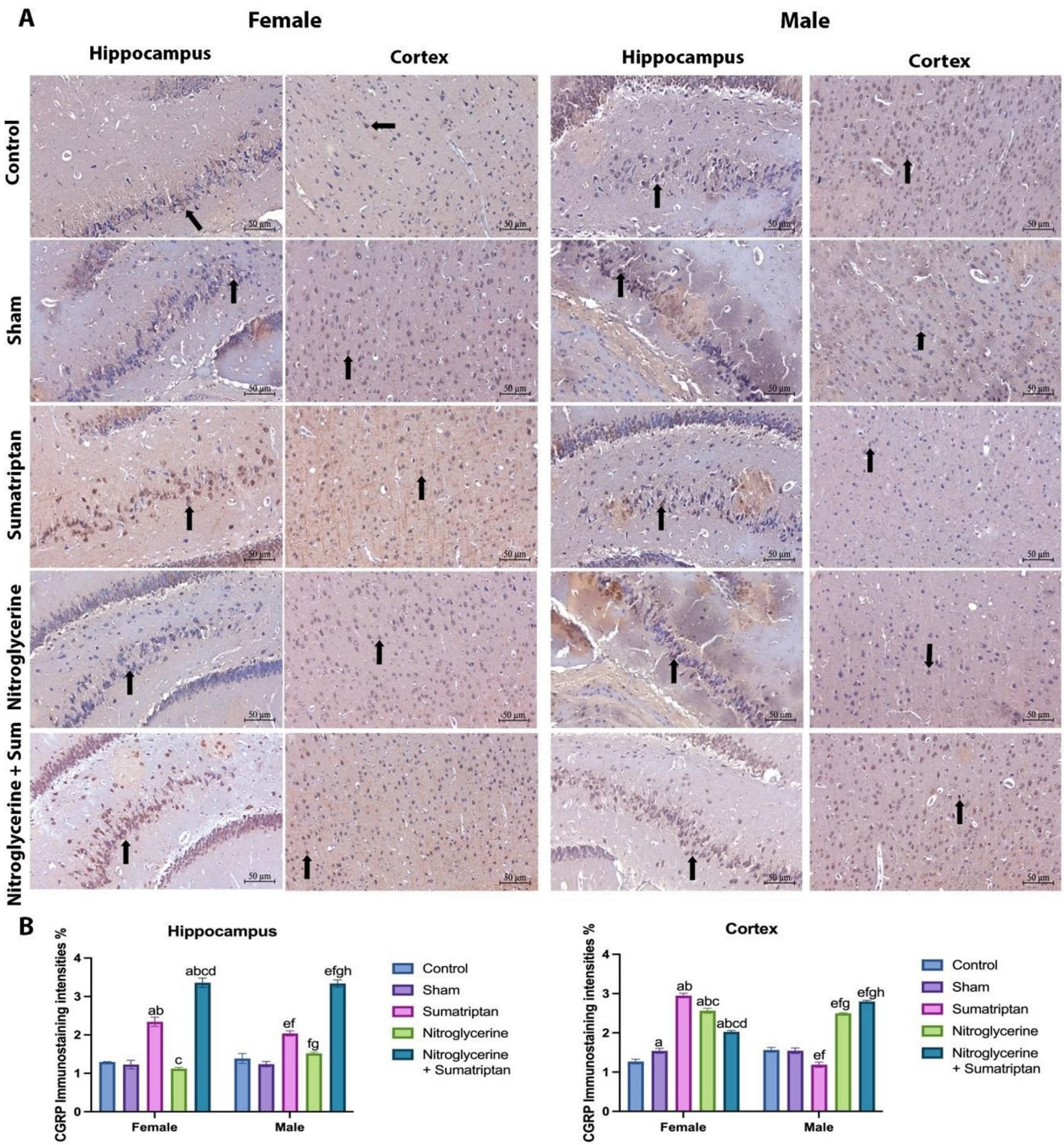


Fig. 6 **A**) Immunohistochemistry staining images of CGRP in hippocampus and cortex areas of experimental groups. Black arrow indicates immunoreactive cells. Magnification 20X and scale bar 50 µm. **B**) Bar graphs showing the intensity of immunostaining. Two-way analysis of variance, Tukey's and Sidak's multiple comparison tests were applied. Data shown in the bar graph are expressed as mean ± SD. In female experimental groups, a $p < 0.05$ vs. control group; b $p < 0.05$ vs. sham group; c $p < 0.05$ vs. sumatriptan group and d $p < 0.05$ vs. nitroglycerin group. In male experimental groups, e $p < 0.05$ vs. control group; f $p < 0.05$ vs. sham group; g $p < 0.05$ vs. sumatriptan group and h $p < 0.05$ vs. nitroglycerin group. Immunoreactivity intensity was measured separately for hippocampus and cortex areas for at least 6 rats (3 females and 3 males) per experimental group

the hippocampus of the migraine experimental group treated with sumatriptan. Therefore, the activity of sumatriptan on PACAP in the brain should be repeated in different groups. Our finding may be interpreted as additional evidence that PACAP-mediated hypersensitivity is different from the known pathways in migraine [41].

Preclinical evidence suggests that PACAP38 modulates trigeminal nociceptive activity mainly through PAC1 receptors, while clinical studies report that plasma concentrations of PACAP38 are elevated in cluster headache and migraine spontaneous attacks and return to normal after sumatriptan treatment [45, 46, 50]. In our study, more PAC1 expression was observed in the hippocampus in females. There is insufficient information on gender-related PAC1 expression in the literature. In this context, PAC1 expression may be modulated in migraine due to gender differences and estrogen dominance. In addition, PAC1 expression increased in the hippocampus and cortex in female nitroglycerin and nitroglycerin + sumatriptan groups. PAC1 expression was found to be significantly increased in the cortex in male nitroglycerin +sumatriptan groups. In addition, PAC1 expression was increased in the male hippocampus in the nitroglycerin +sumatriptan group and decreased in the nitroglycerin only group. These data suggest that the efficacy of sumatriptan may vary in males and females. Our finding draws attention to gender differences in the mechanism of PAC1-mediated PACAP in the known pathways of sumatriptan activity in migraine.

Sumatriptan does not alter cephalic and extrencephalic circulating CGRP in healthy volunteers [27]. The observation that CGRP immunoreactivity increased in the sumatriptan-treated groups may appear paradoxical, as triptans are known to inhibit CGRP release from trigeminal neurons. However, chronic daily administration of sumatriptan can alter peptide dynamics differently from acute dosing. Increased CGRP staining in fixed tissue likely reflects intracellular accumulation of the peptide, as synthesis may continue while release is pharmacologically suppressed. This phenomenon aligns with mechanistic studies demonstrating that sumatriptan inhibits CGRP secretion through a slow, sustained rise in intracellular calcium and the activation of an okadaic acid–sensitive phosphatase, which together suppress vesicular release without necessarily reducing peptide production [12]. Such chronic suppression may also trigger compensatory upregulation of CGRP synthesis. Therefore, the heightened immunoreactivity observed in our study should not be interpreted as increased functional CGRP release but rather as an adaptive intracellular response to prolonged triptan-mediated blockade. In our study, in the experimental migraine model, CGRP expression was found to be significantly increased in the hippocampus in male and female sumatriptan and nitroglycerin sumatriptan groups compared to the control group. In the cortex, CGRP expression was increased in female sumatriptan groups and decreased in males compared to the control group. In the female nitroglycerin + sumatriptan group, CGRP expression decreased compared to the nitroglycerin group, while in the male nitroglycerin + sumatriptan groups, CGRP expression increased compared to the nitroglycerin group. In this context, the efficacy of sumatriptan on CGRP in migraine may vary depending on the gender parameter. Fluctuations in estrogen levels modulate CGRP receptor signaling in the trigeminovascular system [33]. The presence of sex hormone receptors in the trigeminovascular system may indicate that trigeminal neurons are sensitive to changes in the levels of these hormones [23].

Our study revealed that sumatriptan, which is used in migraine attacks and belongs to the triptan family, may modulate neuropeptides in the hippocampus and cortex that have been implicated in migraine pathology. Significant sex-related changes in CGRP and TRPV1 immuno expression may be due to endocrine factors such as estrogen hormone. Considering that estrogens modulate the action of nociceptive receptors, female mice may have a lower pain threshold than male mice [37]. However, it may also be important to evaluate the condition during menstruation in females in migraine pathology. The effect of sumatriptan on the menstrual period and migraine attack in females can be discussed in more detail.

In pathologies with trigeminal activation such as migraine, 5-HT1B/D receptor activation such as sumatriptan may regulate neuronal VIP and PACAP. The effect of sumatriptan on PACAP- related sensitization in migraine should be evaluated. In our study, increased PACAP expression was observed in the hippocampus of the migraine experimental group treated with sumatriptan. In this context, the significant increase in neuronal PACAP expression may be considered as an effective factor on hypersensitivity. In addition, the mechanism of the PAC1- mediated PACAP-mediated PACAP activity in sumatriptan may also draw attention to gender differences in known pathways in migraine.

These data suggest that 5HT1 agonists such as sumatriptan may mediate neuronal neuropeptide changes by regulating the activation of the trigeminal system. This potential therapeutic mechanism of sumatriptan can be evaluated in systems such as migraine or cluster headache to establish its relevance. On the other hand, more detailed analyses are needed to analyze all proteins associated with the trigeminal system, to show which molecules are involved in regulatory mechanisms and to suggest therapeutic approaches for these molecules. Similarly, it is important to reveal the data in this study with different detection methods such as western blot.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

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References

1. Akyuz E, Doganyigit Z, Paudel YN, Koklu B, Kaymak E, Villa C, et al. Immunoreactivity of muscarinic acetylcholine M2 and serotonin 5-HT2B receptors, norepinephrine transporter and Kir channels in a model of epilepsy. Life (Basel, Switzerland). 2021;11(4):276.
2. Arimura A. PACAP: the road to discovery. Peptides. 2007;28(9):1617–9.
3. Ashina M, Hansen JM, Dunga Á, Olesen BO, J. Human models of migraine - short-term pain for long-term gain. Nat reviews Neurol. 2017;13(12):713–24.
4. Ashina M, Hansen JM, Do TP, Melo-Carrillo A, Burstein R, Moskowitz MA. Migraine and the trigeminovascular system-40 years and counting. Lancet Neurol. 2019;18(8):795–804.
5. Benemei S, Dussor G. TRP channels and migraine: recent developments and new therapeutic opportunities. Pharmaceuticals. 2019;12(2):54.
6. Boni LJ, Ploug KB, Olesen J, Jansen-Olesen I, Gupta S. The in vivo effect of VIP, PACAP-38 and PACAP-27 and mRNA expression of their receptors in rat middle meningeal artery. Cephalalgia. 2009;29(8):837–47.
7. Brain SD, Williams TJ, Tippins JR, Morris HR, MacIntyre I. Calcitonin gene-related peptide is a potent vasodilator. Nature. 1985;313(5997):54–6.
8. Burch RC, Buse DC, Lipton RB. Migraine: Epidemiology, Burden, and Comorbidity. Neurol Clin. 2019;37(4):631–49.
9. Casili G, Lanza M, Filippone A, Campolo M, Paterniti I, Cuzzocrea S, Esposito E. (2020). Dimethyl fumarate alleviates the nitroglycerin (NTG)-induced migraine in mice. Journal of neuroinflammation, 17(1), 1-Charles A. (2018). The pathophysiology of migraine: implications for clinical management. The Lancet. Neurology, 17(2), 174–182.
10. Doğanyiğit Z, Okan A, Akyüz E, Yılmaz S, Ateş Ş, Taheri S, et al. Can endoplasmic reticulum stress observed in the PTZ-kindling model seizures be prevented with TUDCA and 4-PBA? Eur J Pharmacol. 2023;960:176072. https://doi.org/10.1016/j.ejphar.2023.176072.
11. Doğanyiğit Z, Okan A, Kaymak E, Pandır D, Silici S. Investigation of protective effects of apilarnil against lipopolysaccharide induced liver injury in rats via TLR 4/HMGB- 1/NF-κB pathway. Biomed Pharmacother. 2020;125:109967.
12. Durham PL, Russo AF. Regulation of calcitonin gene-related peptide secretion by a serotonergic antimigraine drug. J Neurosci. 1999;19(9):3423–9.
13. Dussor G, Yan J, Xie JY, Ossipov MH, Dodick DW, Porreca F. Targeting TRP channels for novel migraine therapeutics. ACS Chem Neurosci. 2014;5(11):1085–96.
14. Edvinsson L, Tajti J, Szalárdy L, Vécsei L. PACAP and its role in primary headaches. J Headache Pain. 2018;19(1):1–7.
15. Erdling A, Sheykhzade M, Maddahi A, Bari F, Edvinsson L. VIP/PACAP receptors in cerebral arteries of rat: characterization, localization and relation to intracellular calcium. Neuropeptides. 2013;47(2):85–92.
16. Evans MS, Cheng X, Jeffry JA, Disney KE, Premkumar LS. Sumatriptan inhibits TRPV1 channels in trigeminal neurons. Headache. 2012;52(5):773–84.
17. Ferrari MD, Goadsby PJ, Roon KI, Lipton RB. Triptans (serotonin, 5- HT1B/1D agonists) in migraine: detailed results and methods of a meta-analysis of 53 trials. Cephalalgia. 2002;22(8):633–58.
18. Ghanizada H, Al-Karagholi MA, Arngrim N, Olesen J, Ashina M. PACAP27 induces migraine-like attacks in migraine patients. Cephalalgia: Int J headache. 2020;40(1):57–67.
19. Goadsby PJ. Recent advances in understanding migraine mechanisms, molecules and therapeutics. Trends Mol Med. 2007;13(1):39–44.
20. Goadsby PJ, Edvinsson L, Ekman R. Release of vasoactive peptides in the extracerebral circulation of humans and the cat during activation of the trigeminovascular system. Ann Neurol. 1988;23(2):193–6.
21. Guo S, Ernstsen C, Hay-Schmidt A, Kristensen DM, Ashina M, Olesen J, et al. PACAP signaling is not involved in GTN- and levcromakalim-induced hypersensitivity in mouse models of migraine. J Headache Pain. 2022;23(1):155.
22. Guo Z, Czerpaniak K, Zhang J, Cao YQ. Increase in trigeminal ganglion neurons that respond to both calcitonin gene-related peptide and pituitary adenylate cyclase-activating polypeptide in mouse models of chronic migraine and posttraumatic headache. Pain. 2021;162(5):1483–99.
23. Gupta S, McCarson KE, Welch KMA, Berman NE. Mechanisms of pain modulation by sex hormones in migraine. Headache: The Journal of Head and Face Pain. 2011;51(6):905–22.
24. Hamamci M, Doganyigit Z, Silici S, Okan A, Kaymak E, Yilmaz S, Tokpinar A, Inan LE. Apilarnil: A Novel Neuroprotective Candidate. Acta Neurol Taiwanica. 2020;29(2):33–45.
25. Hanci F, Kilinc YB, Kilinc E, Turay S, Dilek M, Kabakus N. Plasma levels of vasoactive neuropeptides in pediatric patients with migraine during attack and attack-free periods. Cephalalgia. 2021;41(2):166–75.
26. Hansen JM, Fahrenkrug J, Petersen J, Wienecke T, Olsen KS, Ashina M. Vasoactive intestinal peptide (VIP) and pituitary

adenylate cyclase-activating polypeptide (PACAP) in the circulation after Sumatriptan. Scand J Pain. 2013;4(4):211–6.
27. Hansen JM, Petersen J, Wienecke T, Olsen KS, Jensen LT, Ashina M. Sumatriptan does not change calcitonin gene-related peptide in the cephalic and extracephalic circulation in healthy volunteers. J Headache Pain. 2009;10:85–91.
28. Headache Classification Committee of the International Headache Society (IHS). The International Classification of Headache Disorders, 3rd edition (beta version). Cephalalgia. 2013;33(9):629–808.
29. Hoffmann J, Baca SM, Akerman S. Neurovascular mechanisms of migraine and cluster headache. J Cereb Blood Flow Metab. 2019;39(4):573–94.
30. Jardín I, López JJ, Diez R, Sánchez-Collado J, Cantonero C, Albarrán L, Woodard GE, Redondo PC, Salido GM, Smani T, Rosado JA. TRPs in Pain Sensation. Front Physiol. 2017;8:392.
31. Jung C, Fandos C, Lorenzo IM, Plata C, Fernandes J, Gené GG, et al. The progesterone receptor regulates the expression of TRPV4 channel. Pflugers Arch. 2009;459:105–13.
32. Kurul SH, Demirpence S, Kiray M, Tugyan K, Yilmaz O, Kose G. Investigation of the immunoreactivities of NOS enzymes and the effect of Sumatriptan in adolescent rats using an experimental model of migraine. J Headache Pain. 2008;9(5):317–23.
33. Labastida-Ramírez A, Rubio-Beltrán E, Villalón CM, Maassen-VanDenBrink A. Gender aspects of CGRP in migraine. Cephalalgia. 2019;39(3):435–44.
34. Lai T, Chen L, Chen X, He J, Lv P, Ge H. Rhynchophylline attenuates migraine in trigeminal nucleus caudalis in nitroglycerin-induced rat model by inhibiting MAPK/NF-κB signaling. Mol Cell Biochem. 2019;461(1–2):205–12.
35. Liu J, Wang G, Dan Y, Liu X. CGRP and PACAP-38 play an important role in diagnosing pediatric migraine. J Headache Pain. 2022;23(1):68.
36. Marichal-Cancino BA, González-Hernández A, Guerrero-Alba R, Medina-Santillán R, Villalón CM. A critical review of the neurovascular nature of migraine and the main mechanisms of action of prophylactic antimigraine medications. Expert Rev Neurother. 2021;21(9):1035–50.
37. Mitrovic I, Margeta-Mitrovic M, Bader S, Stoffel M, Jan LY, Basbaum AI. Contribution of GIRK2-mediated postsynaptic signaling to opiate and alpha 2-adrenergic analgesia and analgesic sex differences. Proc Natl Acad Sci USA. 2003;100(1):271–6.
38. Okan A, Demir N, Doğanyiğit Z. Linagliptin in combination with insülin suppresses apoptotic unfolded protein response in ovaries exposed to type 1 diabetes. Cell Biochem Funct. 2024;42(2):e3898.
39. Ozkul Y, Taheri S, Bayram KK, Sener EF, Mehmetbeyoglu E, Öztop DB, Aybuga F, Tufan E, Bayram A, Dolu N, Zararsiz G, Kianmehr L, Beyaz F, Doganyigit Z, Cuzin F, Rassoulzadegan M. A heritable profile of six miRNAs in autistic patients and mouse models. Sci Rep. 2020;10(1):9011.
40. Pellesi L, Al-Karagholi MA, Chaudhry BA, Lopez CL, Snellman J, Hannibal J, Amin FM, Ashina M. Two-hour infusion of vasoactive intestinal polypeptide induces delayed headache and extracranial vasodilation in healthy volunteers. Cephalalgia: Int J headache. 2020;40(11):1212–23.
41. Pérez-Pereda S, Toriello-Suárez M, Ocejo-Vinyals G, Guiral-Foz S, Castillo-Obeso J, Montes-Gómez S, Martínez-Nieto RM, Iglesias F, González-Quintanilla V, Oterino A. Serum CGRP, VIP, and PACAP usefulness in migraine: a case-control study in chronic migraine patients in real clinical practice. Mol Biol Rep. 2020;47(9):7125–38.
42. Rosenfeld MG, Mermod JJ, Amara SG, Swanson LW, Sawchenko PE, Rivier J, Vale WW, Evans RM. Production of a novel neuropeptide encoded by the calcitonin gene via tissue-specific RNA processing. Nature. 1983;304(5922):129–35.
43. Stovner Lj, Hagen K, Jensen R, Katsarava Z, Lipton R, Scher A, Steiner T, Zwart JA. The global burden of headache: a documentation of headache prevalence and disability worldwide. Cephalalgia: Int J headache. 2007;27(3):193–210.
44. Syed AU, Koide M, Braas KM, May V, Wellman GC. Pituitary adenylate cyclase-activating polypeptide (PACAP) potently dilates middle meningeal arteries: implications for migraine. J Mol Neurosci. 2012;48(3):574–83.
45. Takács-Lovász K, Kun J, Aczél T, Urbán P, Gyenesei A, Bölcskei K, et al. PACAP-38 induces transcriptomic changes in rat trigeminal ganglion cells related to neuroinflammation and altered mitochondrial function presumably via PAC1/VPAC2 receptor-independent mechanism. Int J Mol Sci. 2022;23(4):2120.
46. Tambe R, Jain P, Patil S, Ghumatkar P, Sathaye S. Antiepileptogenic effects of borneol in pentylenetetrazole-induced kindling in mice. Naunyn Schmiedebergs Arch Pharmacol. 2016;389(5):467–75.
47. Tepper SJ. CGRP and headache: a brief review. Neurol sciences: official J Italian Neurol Soc Italian Soc Clin Neurophysiol. 2019;40(Suppl 1):99–105.
48. Wienholtz N, Christensen CE, Zhang DG, Coskun H, Ghanizada H, Al-Karagholi MA, Hannibal J, Egeberg A, Thyssen JP, Ashina M. Early Treat Int J headache. 2021;41(6):731–48.
49. Wong AO, Leung MY, Shea WL, Tse LY, Chang JP, Chow BK. Hypophysiotropic action of pituitary adenylate cyclase-activating polypeptide (PACAP) in the goldfish: Immunohistochemical demonstration of PACAP in the pituitary, PACAP stimulation of growth hormone release from pituitary cells, and molecular cloning of pituitary type I PACAP receptor. Endocrinology. 1998;139(8):3465–79.
50. Zagami AS, Edvinsson L, Goadsby PJ. Pituitary adenylate cyclase activating polypeptide and migraine. Ann Clin Transl Neurol. 2014;1(12):1036–40.

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