SEROTONIN-INDUCED PAIN SIGNALING IN TRIGEMINAL NEURONS AND THE SENSITIZATION ACTION OF THE MIGRAINE MEDIATOR CGRP

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Abstract

Migraine is the most common neurological disorder originating from the activation of trigeminal in meninges. Migraine has been estimated to be the most expensive neurological disorder for society that occurs among 12–20% of adult population. Serotonin or 5-hydroxytryptamine is a monoamine neurotransmitter that is likely involved in migraine pathophysiology. The pain caused by serotonin and mediated via nociceptive fibers to activate trigeminal nociceptors. Even it was proposed that serotonin receptors participates in migraine pain, the pro-nociceptive mechanisms of this neurotransmitter remain largely unclear. The aim in this project was to analyse the serotonin-induced currents in rat trigeminal neurons and to test the sensitizing action of the migraine mediator neuropeptide calcitonin gene-related peptide on serotonin receptors. Experiments were performed by using patch-clamp recording of membrane currents induced by serotonin in rat trigeminal neurons in control conditions and after modelling migraine attack by exposure to calcitonin gene-related peptide. We found that serotonin induced fast desensitising membrane currents suggesting the functional expression of 5-HT3 receptors in trigeminal neurons. These, responses were partially co-localized with adenosine triphosphate-gated P2X or capsaicin-sensitive TRPV1 receptors. A potential crosstalk between serotonin and calcitonin gene-related peptide in peripheral trigeminal nociception was investigated. We found that calcitonin gene-related peptide increased responses to serotonin in adenosine triphosphate-sensitive fraction of cells, suggesting a potential crosstalk between serotonin and calcitonin gene-related peptide in peripheral trigeminal nociception. In summary, this project extends our knowledge on molecular
pathways of two major migraine modulators, calcitonin gene-related peptide and serotonin, important contributors to this common neurological disorder.
Introduction

Migraine is a common episodic neurological disorder since it occurs among 12–20% of adult population (Albury et al., 2017). Migraine has been estimated to be the most expensive neurological disorder for society (Stovner et al., 2007). Approximately, 8% of males and about 17% of females are experiencing migraine headache (Anttila et al., 2010). Nevertheless, the neurobiological mechanisms leading to migraine pain are not well understood.

The International Classification of Headache Disorders (3rd edition, beta version) identified two different common forms of migraine-without aura (MWA) and migraine with aura (MA) (Peter et al., 2002 and Gunjan, 2015). MA and MWA are determined based on the presence or absence of aura during the early stage of migraine attack (Anttila et al., 2010). However, it is not always clear that patient have symptoms of the MWA only, or MA only, or have combination of both types of migraine (MA plus MWA) (Russell 1995; 1996).

Trigemino-vascular system (TVS) is believed to play a crucial role in the pain generation manifested as migraine headache period (Lauritzen 1987; Spiri et al., 2014). The nociceptive fibers of the trigeminal nerve in the TVS generate action potential that is propagated through the brainstem and thalamus to the somatosensory cortex (Burstein and Jakubowski, 2009). Thus, the activation of the brainstem is the essential steps in the transmission of the migraine pain. This activation promotes also the release of various pro-nociceptive neuropeptides that leads to neurogenic inflammation and vasodilation in the dura mater. Specific neuropeptides, which play a major role in the meningeal nociception, are calcitonin gene-related peptide (CGRP), substance P, and PACAP (Schou et al., 2017).

Overall, migraine without aura (MWA) is the most common form of migraine (about 80% of cases) (Coppola et al., 2015). MWA is characterized by pulsating, unilateral headache, moderate or severe intensity aggravated by routine physical activity and often associated with nausea and/or photophobia and phonophobia (The International Classification of Headache Disorders 3rd edition, Beta version), (Coppola et al., 2015; Malik et al., 2015). The moderate or severe headache episodes can up to one or two days (Sinha, 2015). Pulsating and pounding headache usually associated with nausea, vomiting or hypersensitivity to sound and light (Steward et al., 1994). Typically, migraine attack is also associated by autonomic, cognitive, emotional, and motor disturbance (Nye and Thadani, 2015).
Migraine with aura (MA) is based on neuronal and glial depolarizations, called cortical spreading depression (CSD) that slowly spreads across the cerebral cortex with the rate of 3-5 mm/min (Somjen 2001; Lauritzen 1994). The development of CSD in cerebral cortex in MA is believed to provide by the activation of trigeminovascular system. CSD associated with a prolonged neuronal depolarization is accompanied by failure of ion homeostasis. CSD primarily is triggered by the activation glutamate N-methyl-D-aspartate receptor (NMDA) (Somjen 2001; Lauritzen 1994).

The symptoms of aura are very specific for the migraine attack. The most typical symptoms of the aura are visual disturbances, more rare there are other sensory disorders such as the numbness or tingling in the lips and, tongue or motor disturbances. These symptoms are
gradually progressing during aura within 20 minutes, and then, they are gradually regressing. The total duration of the aura does not typically exceed 60 minutes.

Migraine is a disease that depends both from genetic and environmental factors such as stress, irritability, weather, absence or excess of sleep, fatigue (Burstein et al., 2009), intense sunlight, menstrual cycle and depression that can induce and determine the frequency of migraine attacks (Robbins, 1994). Figure 2 shows that migraine can be triggered by different stimulus such as hormonal instability, emotional changes along with genetic predisposition, various mutations and a variety of epigenetic combinations (Burstein et al., 2009). Moreover, headache can be based on deficiency of serotonin (Ferrari and Saxena, 1993), mitochondrial abnormalities (Barbirolli et al., 1992), low level of magnesium (Welch and Ramadan, 1995), and defects of the ion transporters and pumps (Ophoff et al., 1996, 1997).
Current evidence suggest that migraine can be controlled by genetic heritability and associated genes. Interestingly, common form of migraine is likely polygenetic, with heritability of about 50% (Larsson et al., 1995; Mulder et al., 2003). Current genetic studies are supposed to identify implicated genes and the key proteins that are involved to the migraine pathways for better understanding of the molecular mechanisms of this disorder. Genetic variants were established only for Mendelian forms of familial migraine, but there was no well established susceptibility variants associated with common forms of the migraine (Nyholt et. al., 2008). However, recently, heritability of migraine genes and susceptibility of gene loci started to be actively explored (Wessman et al., 2004).

For familial type, migraine genes involved in the development of migraine development are already established. They are: CACNA1A gene (encoding P/Q type calcium channels of in neurons CACNA1A), gene encoding glial sodium/potassium ATP1A2 pump, and SCN1A encoding sodium channels of in neurons (Mulder et al., 2003). Techniques for testing candidate genes are continuously developing such as genome wide association studies (GWAS), full exome sequencing and next generation sequencing (NGS) studies. Finally, these novel tools helped to find out at least 13 independent loci associated with migraine. Interestingly, each identified variant only partly contributes to the genetic risk of the migraine, suggesting a genetic heterogeneity (Gormley et al., 2016). Further genetic investigations are currently ongoing based mainly on GWAS and NGS technique in larger cohorts for patients and respective controls (Ducros, 2013).

Finally, 44 independent single nucleotide polymorphisms (SNPs) were identified correlating with migraine risk, which are mapped to the 38 different genetic loci. This analysis pointed to genes expressed in vascular and smooth muscle tissues consistent with vascular theory of migraine (Gormley et al., 2016). Moreover, the minor allele of rs1835740 on chromosome 8q22.1 was also associated with the migraine headache (Anttila et al., 2010).

Migraine pain is originating from the activation of trigeminal nerve terminals in meninges followed by neuronal sensitization via release of migraine mediators such as CGRP (Goadsby, 2007; Bolay et al., 2009; Levy, 2010; Aggarwal et al, 2012; Zakharov et al., 2015). The main migraine mediator CGRP released during the migraine attack is a neuropeptide that consists of 37-amino-acids (Amara, 1982). Notably, CGRP is expressed both in peripheral and central neurons (Pludda, 2017). Thus, CGRP was found in the C and Aδ sensory fibers located from
the trigeminal ganglia of nociceptive nervous system, as well as the central nervous system (Iyengar et al., 2017). CGRP receptors are expressed in 35-50% of trigeminal ganglia neurons, critical component of migraine related pain pathways (Schueler et al., 2014; Stevens and De Souza, 2017).

Fig 3. Molecular pathways of the regulation P2X3 receptors from trigeminal neurons (TG) in physiological conditions (A) and in a migraine pain model (B–D). Pain mediators such as CGRP (B, C) or NGF (D) are released (Giniatullin et al., 2008).

Interestingly, stimulation of the extracranial nerves in the temporal muscle caused a distant CGRP release in the dura mater and even elevated meningeal blood flow (Russo, 2015). However, in humans a direct activation of nociceptive fibers mediated by CGRP neuropeptide is unlikely (Pedersen-Bjergaard et al., 1991). Nevertheless, CGRP causes blood vessels dilatation and sensitization of pain transducing P2X3 receptors (Fig 3) which supposed to be involved in pain transmission in migraine (Giniatullin et al., 2008; Gunjan, 2015). The action
of CGRP on second- and third-order nociceptive neurons might prove the modulatory role also in central pain mechanisms.

CGRP is a member of the peptide family which includes also amylin, adrenomedullin and calcitonin. There are two major forms of CGRP: alpha-CGRP (best-known form), beta-CGRP, and they both can be found in the vascular system, peripheral nervous system and CNS. Whereas both peptides have similar amino-acid sequence in humans, few functional differences were identified in the action of these peptides (Hay, 2017).

In summary, the role of CGRP in migraine is well established. Thus, there is a release of CGRP into the cranial blood vessel during the acute migraine or cluster headache attacks (Edvinsson, 2017, Fig. 1). The intravenous injection of CGRP provokes migraine-like symptoms in migraine patients. Finally, CGRP receptor antagonists gepants and monoclonal CGRP neutralizing antibodies are efficient in migraine treatments (Edvinsson, 2017).

The role of serotonin or 5-hydroxytryptamine (5-HT) in migraine was discussed in many studies during last 40 years (Kowalska et al., 2016; Segelcke and Messlinger, 2017; Hamel, 2007). Serotonin is a major component of the inflammatory and biochemical processes and involved to the transformation of the pain via tissue injury providing an activation of different receptor subtypes (Zeitz et al., 2002). The pain caused by 5-HT is probably mediated via perivascular nociceptive fibers and activates trigeminal nociceptors that supply intracranial blood vessels and meninges (Zhang et al., 2007). This mechanism can explain how 5-HT contributes to the migraine process (Hamel, 2007). However, the effects of 5-HT could be mediated by different subtypes of 5-HT receptors and the role of these receptor subtypes in the trigeminal nociceptive system implicated in migraine is poorly understood.

During migraine attack, the 5-hydroxy-indole acetic acid metabolite of serotonin, 5-hydroxyindoleacetic acid, is excreted in increased amounts in the urine suggesting massive release of 5-HT in acute period of migraine. Thus, it is likely that patients suffering of the migraine, have chronically low 5-HT levels, predisposing to initiate migraine attacks.

Interestingly, most specific anti-migraine agents known as triptans are agonists of 5-HT1B/1D receptors. They have multiple targets: firstly, producing cranial vasoconstriction, secondly, evoking peripheral neuronal inhibition, and, thirdly, inhibiting transmission to the second order
neurons of the trigeminocervical complex (Ferrari et al., 2002). Currently, the 5-HT1B/1D agonists triptans are very effective anti-migraine drugs (Kowalska et al., 2016).

![Diagram of 5-HT3 receptor](image)

Fig 4. Schematic presentation of the structure of 5-HT3 receptor (Walstab et al., 2010).

5-HT3 is the only one ligand-gated ionotropic serotonin receptor (Hicks et al., 2002). 5-HT3 receptors mediate responses of nociceptive spinal neurons during neuroinflammation (Green et al., 2000). Fig 4 shows the typical structure of 5-HT3 receptor, which is composed by five subunits that surround the central cation-permeable channel.

As the molecular mechanisms of nociceptive signalling of 5-HT in trigeminal nerves are little understood, the aim of this project was to study the role of 5-HT receptors in migraine mechanisms. First, it was important to characterize 5-HT receptors in rat trigeminal neurons along with other classical pain transducers. Second, to analyse the effect of the migraine mediator neuropeptide CGRP on serotonin-induced currents in rat trigeminal neurons to investigate the contribution of 5-HT3 receptors in migraine pathology. This can help to identify the neurochemical pathways sensitized during the migraine attack after CGRP release. Finally,
we will explore the effect of its agonists/antagonists to provide pharmacological characterization of 5-HT receptors in trigeminal nociceptive system.
Aims of the study

To study the role of 5-HT in peripheral nociception we will:

1. Study the responses to 5-HT in rat trigeminal neurons.

2. Investigate co-expression of 5-HT3 receptors with other pain receptors.

3. Explore a potential crosstalk between 5-HT and CGRP signalling in peripheral trigeminal nociception.

4. Compare kinetics of currents activated by 5-HT and mCGBP on 5-HT3 receptor.

5. Explore the action of specific and common antagonists on 5-HT3 receptors.

In order to explore these mechanisms in more detail, we will perform a patch clamp study of 5-HT-induced membrane currents in trigeminal neurons in control conditions and after modelling migraine-like conditions with the neuropeptide CGRP.
Materials and methods

Wistar rats were obtained from the Animal Facilities of the University of Eastern Finland (UEF). All experiments follow the Helsinki Declaration and guidelines set by the European Commission. The experiments were done under the licenses EKS-004-2014 and EKS-002-2017 approved by the Committee for the Welfare of Laboratory Animals of the University of Eastern Finland. All efforts were made to minimize the number of animals used and their suffering.

All reagents required for the tissue culture were obtained from Gibco Invitrogen (Carlsbad, CA, USA) while all reagents for experiments were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Cell culture

Trigeminal cell culture was prepared from Wistar rats of postnatal day 10–12 of both sexes. Immediately after rats decapitation the trigeminal ganglia were isolated and enzymatically dissociated in enzyme cocktail containing 0.5 mg/ml trypsin, 1 mg/ml collagenase, and 0.2 mg/ml DNase for 20 minutes at 37°C in thermomixer comfort (Eppendorf). The tissue was triturated by passing through a 1 mL pipette until complete dissociation. Trypsin inhibitor was added to mix to stop digestion; cells were spin for 5 min at 1000 rpm. The supernatant was removed and cells were re-suspended in pre-warmed Ham's F-12 Nutrient Mix medium (Gibco) with 10% FBS. The isolated cells were plated on glass coverslips coated with poly-l-lysine (0.2 mg/ml) and incubated for 24 -48 hours at 37 °C in an atmosphere containing 5% CO2. (Hautaniemi et al., 2012).

Cells treatment in migraine model

Primary cell culture were used for patch – clump experiments 24-48 h after cells plating. To explore the migraine processes condition on neurons, primary cell culture was pre-treated with 1 μm CGRP for 2 h at 37°C in F-12 medium and 10% fetal bovine serum. Cells without CGRP pre-treatment were used as control.
**Electrophysiological recording**

All substances (Sigma-Aldrich), were be applied through a rapid (exchange time ~20–40 ms) superfusion system (RSC-200; BioLogic, Grenoble, France). The interpulse interval between the applications in a single record was 2 sec and the application interval of different substances of a single cell was at least 5 min to minimize receptor desensitization.

Fig 5. A scheme presenting patch-clamp experiments with a single cell (neuron) that was patch clamped in the whole-cell configuration in order to record membrane currents in response to 5-HT, ATP or capsaicin.

During the registration cells were continuously perfused (3 mL/min) with physiological solution containing (in mM): 152 NaCl, 5 KCl, 1 MgCl2, 2 CaCl2, 10 glucose and 10 HEPES (pH adjusted to 7.4 with NaOH, osmolarity ~300 mOsm). Transmembrane currents were recorded in the whole-cell configuration using the HEKA PC-10 amplifier (HEKA Elektronik).
Microelectrodes (4-5 MΩ) were filled with intracellular solution containing (in mM): 130 CsCl, 10 HEPES, 5 EGTA, 0.5 CaCl2, 5 MgCl2, 5 KATP, 0.5 NaGTP. Currents were recorded from cells voltage clamped at -70 mV. All substances were applied through a rapid superfusion system (RSC-200; BioLogic, Grenoble, France).

Data analysis

The data were presented as mean±S.E.M. (n=the number of cells), with statistical significance accessed with paired t-test (for parametric data from electrophysiological experiments). To analyse data we used the following software: pClamp 10.0 (Molecular Devices), Origin v.8.0 (Microcal, Northampton, MA), FitMaster (HEKA Elektronik) and CorelDraw (Graphics Suite X6). A value of P≤0.05 was accepted as indicative of statistically significant differences.
Results

Functional properties of serotonin 5-HT3 receptors in trigeminal neurons

To characterize 5-HT induced currents in rat trigeminal neurons we applied different concentrations of 5-HT on these cells and recorded responses to the fast (2 s) application of this agonist using patch-clamp technique. To characterize responses to 5-HT we primarily measured the amplitude of currents and the density of the membrane currents (as the size of neurons was different). Notably, 5-HT can open only one type of ligand-gated channel, which belongs to 5-HT3 subtype.

![Fig 6. Membrane currents induced by fast application 5-HT, ATP and capsaicin in rat trigeminal neurons.](image)

In control conditions, 37 % (39 from 97) cells responded to 5-HT with mean amplitude 150.2±19.2 pA and current density 3.31±0.41 pA/pF (n=38). Fig 6 (left) shows a typical current thought 5-HT3 receptors induced by 2 s application of 20 µM 5-HT to trigeminal neurons. Notice that the 5-HT3 current quickly decayed in the presence of 5-HT indicating fast development of desensitization.

To obtain kinetic parameters of responses we measured the decay time, which is characterizing the onset of desensitization. The decay time of 5-HT induced currents was 1.8±0.16 s, a quantitative indicator of desensitization.

Co-expression of 5-HT3 receptors with other pain receptors

For testing the co-expression of 5-HT3 receptors with other pain transducers such as P2X and TRPV1 receptors, apart from application of 20 µM 5-HT, cells were additionally activated with ATP (10 µM) and capsaicin (1 µM). Notably, in many cases, applications of ATP and capsaicin induced membrane currents that were partially overlapping with 5-HT induced responses.
Thus, we compared the transient responses induced by serotonin and those induced by ATP or capsaicin in trigeminal neurons.

Obtained data indicated the simultaneous presence of ATP-gated P2X2 and P2X3 receptors as well as the functional co-expression of capsaicin-gated TRPV1 receptors in different fractions of trigeminal neurons (Fig 7).

![Fig 7. Profile of trigeminal neurons activated 5-HT and co-expression of 5-Ht3 receptors with receptors sensitive to ATP and capsaicin.](image)

According to these data, 37 % of the trigeminal neurons were sensitive to 5-HT. The highest co-expression (36%) was for combinations: serotonin + ATP + capsaicin and for serotonin + capsaicin. Thus, cells responding to 5-HT, could be separated into different populations with different set of pain transducers: responding only to 5-HT, to 5-HT and ATP, to 5-HT and capsaicin and the other fraction of neurons was sensitive to all three agents (5-HT, capsaicin and ATP).

**Effect of CGRP on 5-HT3 receptors**

The action of CGRP on 5-HT3 receptors was tested in different neuronal populations of cell activated by 5-HT alone or in combination with ATP and capsaicin receptors. To model migraine conditions, trigeminal neurons were exposed to 1 µM CGRP for 2 h and then neurons were tested with 20 µM 5-HT, 10 µM ATP and 1 µM capsaicin.
Fig 8. Sensitizing effect of CGRP in different populations of trigeminal neurons expressing 5-HT3 receptors. Responses to 5-HT and other agonists are presented as current density (pA/pF) in each 5-HT sensitive population. Mean ± SEM, *p=0.026 by t-test cells were incubated in 1 μM CGRP for 2h to model migraine before testing.

Fig 8 shows that from all sub-populations, only ‘purinergic’ fraction of cells (neurons responding both to 5-HT and ATP) showed enhanced responsiveness to 5-HT after exposure to CGRP. Thus, the current density of 5-HT responses was 1.79±0.39 pA/pF in control (n=13) but this parameter was significantly increased after the 2 h exposure to CGRP to 5.01±1.39 pA/pF (p=0.026, n=11).

These findings indicated a selective sensitivity of certain fractions of trigeminal nociceptors to CGRP and suggested the interaction between the serotonin receptors and the signalling pathways activated by the migraine mediator CGRP. Finally, our data suggested that serotonin 5-HT3 receptors represent one of potential targets for the anti-nociceptive drugs in migraine treatment.

**Kinetics of currents activated by 5-HT and the selective 5-HT3 agonist mCPBG**

In order to characterize the properties of 5-HT3 receptors in rat trigeminal neurons we compared current responses induced by two agonists such as 5-HT and 1-(m-chlorophenyl)-biguanide (mCPBG). The latter is known as the specific agonist of 5-HT3 receptors (Kilpatrick
et al., 1990). To compare responses to two these agonists we applied them in the standard concentration 20 µM for 2 sec to trigeminal neurons. Fig 9 shows typical current responses separated by the interval of 5 s.

![Figure 9](image.png)

**Fig 9.** Membrane currents activated in trigeminal neurons by paired (interval 5s) applications of 20 µM 5-HT and 20µM mCPBG

Both agonists induced currents with the following currents: 146.3±17.3 pA, n=53 for 5-HT and 79.0±14.9 pA, n=13 for mCPBG (Figure 10). Notably, there was a strong desensitization in both cases, which was not significantly different between 5-HT and mCPBG (Fig. 12). Nevertheless, the recovery time after desensitization for these agonists was dramatically different.
Fig 10. Comparison of desensitization kinetics of currents activated by 20 µM mCPBG (A) and 20 µM 5-HT (B) in rat trigeminal neurons. Desensitization onset was measured as a decay time of membrane currents. C presents averaged data for decay times of mCPBG- and 5-HT-induced currents.
Fig 1. Recovery rate from desensitization induced by 20 µM 5-HT and 20 µM mCPBG in rat trigeminal neurons. Notice fast recovery in the case of 5-HT and extremely slow recovery after mCPBG. Mean±SEM

Thus, after serotonin application a full recovery was approximately 1 min, whereas after mCPBG application it took about 10 min (Figure 12). In summary, the recovery of 5-HT3 receptors from desensitization was determined by the nature of the agonist.

**The action of serotonin antagonists on 5-HT3 receptors**

In order to further characterize the pharmacological properties of trigeminal 5-HT3 receptors we tested the ability of two 5-HT antagonists, MDL-72222 and GR-127935 to block 5-HT-induced currents in rat trigeminal neurons. Figure 13 A,B shows that the specifically blocker of 5-HT3 receptors MDL-72222 (30 µM) almost completely prevented the activation of these receptors (n=5). As 5-HT1B/D receptors are implicated in the migraine (Kilinca et al., 2017) and references therein, we also tested the inhibitory activity of the 5-HT1B/D antagonist GR127935. Figure 12 C,D,E shows the effect of GR127935 on 5-HT response on rat trigeminal neurons. Interestingly, that application of for 2 min partially blocked 5-HT induced currents from 183.7±16.6pA in control to 87.3±23.2 pA (n=4 neurons). These findings suggested the sensitivity of 5-HT3 to this widely used receptor blocker.
Fig 12. Testing the action of 5-HT receptor antagonists on 5-HT3 receptor mediated currents. Membrane currents induced by 20 μM 5-HT (A) and the blocking effect of the 5-HT3 receptor antagonist MDL-72222 (B). Membrane currents induced by 20 μM 5-HT (C) and the blocking effect of the 5-HT1 receptor antagonist GR-127935 (D). Histograms
Discussion

Our data indicate that 5-HT induces membrane currents in a large number of trigeminal neurons suggesting the essential expression and important functional role of 5-HT3 receptors in the peripheral part of the trigeminal nociceptive system. 5-HT3 receptor mediated current responses were characterized by the fast onset of desensitization and relatively fast recovery process, in sharp contrast to slowly recovering responses to the specific 5-HT3 agonist mCPBG. 5-HT3 receptors were partially co-localized with either ATP-gated P2X or capsaicin-sensitive TRPV1 channels. The neuropeptide CGRP selectively sensitized responses to 5-HT in a fraction of neurons expressing also ATP-gated P2X receptors suggesting a potential crosstalk between 5-HT and CGRP in peripheral trigeminal nociception.

ATP-gated P2X2 and P2X3 receptors and capsaicin-activated TRPV1 receptors represent ‘classical’ pain transducers in nociceptive sensory neurons. The function of these receptors could be enhanced in various pathological pain states and likely, in migraine. Release of the migraine mediator CGRP can be a trigger of migraine attack in patients (Edvinsson 2017; Holle-Lee et al., 2017), and CGRP receptor antagonists can reduce the migraine attack (Shelukhina, 2017; Walker, 2017). Previously it has been shown that the main migraine mediator CGRP up-regulates P2X3 receptors in trigeminal neurons via enhanced trafficking and elevated transcription, suggesting a purinergic hypothesis of migraine pain (Giniatullin et al., 2008). Unlike P2X and TRPV1 receptors, the action of CGRP on serotonin pathways, which also important for migraine pathology, was not studied so far. In this study we first characterized the functional activity of 5-HT in rat trigeminal ganglia neurons and found that 37% of neurons express 5-HT3 receptor subtype which is even higher than the functional expression of TRPV1 receptors (Simonetti et al., 2006; Zakharov et al., 2015). These data suggest that serotonin receptors could represent one of targets for the pro-nociceptive action of the migraine mediator CGRP. Indeed, we found that CGRP pre-treatment which is widely used a model of migraine-like conditions, significantly enhanced responses to 5-HT in trigeminal neurons (Guerrero-Toro et al., 2016). Interestingly, CGRP sensitized only those trigeminal neurons which co-express 5-HT3 and P2X3 receptors. This is consistent with co-expression of CGRP and P2X3 receptors in trigeminal neurons (Fabbretti et al., 2006; Yegutkin, 2016). Different treatments might have its anti-migraine effects through the actions on the intracranial vasculature (Ho et al., 2010). However, our data suggest that the ability of
CGRP to stimulate migraine attack possibly involves more than only the vasodilatatory effect proposed by others (Puledda, 2017).

As CGRP is considered as the main mediator of migraine (Edvinsson, 2017), these results provide new clinical implications along with targeting CGRP antagonism. This could contribute to better approaches in treatment of migraine and, probably, other types of chronic pain. It is possible to suggest that CGRP release in to the response of activation of the peripheral nociceptive system during the neurogenic neuroinflammation can largely enhance the pro-nociceptive stimulation of nerve terminals by ATP and 5-HT released from mast cells or platelets (Hamel, 2007; Giniatullin et al., 2008; Kilinca et al., 2017). These findings advance our knowledge on migraine mechanisms and can contribute to development of anti-migraine implications that based on the CGRP action (Edvinsson, 2017).

New novel drugs are brashly started to be available for migraine suffering patients. A better understanding of pathophysiological pathways of migraine gives us more deep knowledge of this common brain disorder (Puledda, 2017).

**Conclusion**

In conclusion, in the current study, we present experimental data showing the functional expression of serotonin 5-HT3 in a large population of trigeminal neurons. We also provide data suggesting that CGRP promotes the pro-nociceptive effect of serotonin in rat trigeminal neurons via 5-HT3 receptors.
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Implementation

There are communications related to this project:


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References


