The effects and D2 receptor-mediated mechanisms of dopaminergic system modulation in in-vivo and in-vitro experimental models of migraine

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Abstract

The dopaminergic system is implicated in the pathophysiology of migraine. However, the underlying mechanisms remain unclear. We explored the effects and mechanisms of dopaminergic system modulation in the in-vivo and in-vitro rat models of migraine. Dopaminergic agonist apomorphine, D2 receptor antagonists metoclopramide and haloperidol, and 5-HT3 receptor antagonist ondansetron alone and together were tested in nitroglycerin-induced migraine model, in vivo. Likewise, the combinations of drugs were also tested on basal CGRP release in-vitro hemiskull preparations. Mechanical allodynia was tested by von-Frey filaments. CGRP concentrations in trigeminovascular structures and in-vitro superfusates, and c-Fos levels in brainstem were determined by ELISA. Meningeal-mast cells were evaluated with toluidine-blue staining. Apomorphine further enhanced nitroglycerin-induced mechanical allodynia, brainstem c-fos expression, trigeminal ganglion and brainstem CGRP concentrations, and meningeal mast cell degranulation, in vivo. Haloperidol completely antagonised all apomorphine-induced effects and also alleviated changes induced by nitroglycerin without apomorphine. Metoclopramide and ondansetron partially attenuated apomorphine- or nitroglycerin-induced effects. A combination of haloperidol and ondansetron decreased basal CGRP release, in-vitro, while the other administrations were ineffective. Apomorphine-mediated dopaminergic activation exacerbated nitroglycerin-stimulated migraine pain by further enhancing c-fos expression, CGRP release and mast cell degranulation in strategic structures associated with migraine pain. Metoclopramide partially attenuated the effects of apomorphine, most likely because it is also a 5-HT3 receptor antagonist. Haloperidol with pure D2 receptor antagonism feature appears to be more effective than metoclopramide in reducing migraine-related parameters in dopaminergic activation- and/or NTG-induced migraine like conditions.

INTRODUCTION

Migraine is one of the most common and disabling neurovascular disorders characterized by the occurrence of dopaminergic symptoms such as yawning, drowsiness, nausea, vomiting, fatigue and mood changes (Barbanti et al., 2013 and 2020). The dopaminergic system plays key roles in a wide variety of nervous system functions such as cognition, synaptic plasticity, motor control, motivation, reward and psychic processes, and its dysregulation is involved in various pathophysiological conditions such as Parkinson’s disease, schizophrenia, Huntington’s disease, attention deficit and addiction (Vosberg et al., 2020; Smith and Villalba, 2008; Monzani et al., 2019; Kortleven et al., 2011).

In addition to its noted functions, accumulating clinical evidence indicates that the dopaminergic system is involved in the pathophysiology of migraine, and dopamine contributes substantially to the pathogenesis of migraine (Charbit et al., 2010; Barbanti and Fabbrini, 2002). It was reported that plasma concentrations of dopamine in chronic migraine patients are several times higher than in patients with tension-type headache and healthy subjects (D’Andrea et al., 2013). A recent cross-sectional study has revealed that migraineurs...
with dopaminergic symptoms exhibited longer lasting attacks, more frequent allodynia and unilateral cranial autonomic symptoms (Barbanti et al., 2020). Dopamine agonists are able to aggravate certain types of headache. For instance, a dopamine agonist apomorphine evokes head pain in 86% of migraine patients (del Bene et al., 1994) and leads to yawning in migraineurs (Blin et al., 1991). The role of dopamine in migraine pathophysiology is mostly attributed to D2 receptors rather than its other receptors because of a variety of D2 receptor antagonists exhibit great clinical efficacy in migraine (Akerman and Goadsby, 2007; Marmura, 2012). However, the mechanism of action of dopaminergic agonists in inducing migraine symptoms, and the mechanism of action of D2 antagonists in the treatment of migraine remain unclear.

Dopamine receptors are present in the trigeminovascular system, the activation of which is involved in the generation and maintenance of migraine pain (Bergerot et al., 2007; Lazarov and Pilgrim, 1997; Peterfreund et al., 1995). Thus, presence of dopamine receptors in the trigeminovascular system implies that the mechanism of action of dopaminergic agonists and antagonists may be related to the modulation of trigemino-vascular system activation. Therefore, the present study aimed to investigate the actions and possible mechanisms of dopaminergic system modulation by dopaminergic agonist apomorphine, and two prominent D2 receptor antagonists metoclopramide and haloperidol in the nitroglycerin-induced in vivo migraine model and in vitro hemiskull preparations in rats.

**MATERIALS AND METHODS**

The experimental procedures were allowed by Bolu Abant Izzet Baysal University Animal Experiments Local Ethics Committee (decision no: 2022/36). The male Wistar rats aged 8–12 weeks (190-250 g) acquired from the animal centre of University of Bolu Abant Izzet Baysal, Turkey were enrolled for the experiments. The animals were treated according to the National Institutes of Health guide for the care and use of Laboratory animals, and were kept back at with a 12 h light/dark cycle at 22 °C. They were allowed to access to unlimited rat feed pellets and drinking water.

**Drugs and reagents**

Nitroglycerin solution (Adeka, Pharm. Ind., Turkey) was diluted to a dose of 10 mg/kg. An equal volume of alcohol, propylene glycol and saline mixture to obtain the ultimate concentration of nitroglycerin was utilised as the nitroglycerin vehicle (6% propylene glycol, 6% alcohol, 0.9% saline). Apomorphine hydrochloride (Recipharm, England), metoclopramide hydrochloride (Tum-ekip Pharm. Ind., Turkey), haloperidol (Aris Pharm. Ind., Turkey) and ondansetron hydrochloride (Haver Pharm. Ind., Turkey) were diluted in 0.9% NaCl solution to achieve the ultimate concentrations. Rat C-fos ELISA kits were purchased from SunRed Biotechnology (Shanghai, China). Rat CGRP ELISA kits were purchased from Elabscience (Texas, USA). Aprotinin were purchased from Merck (Schnelldorf, Germany).

**Experimental groups and the establishment of in vivo migraine model**

The rats were randomly divided into 9 groups according to a randomization list (n=6 for each group). All drug and vehicle injections were performed intraperitoneally. The administration doses of the drugs were chosen according to the literature as follows: nitroglycerin (NTG) 10 mg/kg (Kilinc et al., 2022a), apomorphine (Apm) 0.5 mg/kg (Greco et al., 2008), metoclopramide (Met) 1 mg/kg (Doğanay Aydin et al., 2017), haloperidol (Hal) 1 mg/kg (Collins et al., 2014) and ondansetron (Ond) 0.5 mg/kg (Giorno et al., 2018). The migraine model was induced by a single administration of nitroglycerin. NTG (or its vehicle for control) was administered to the groups 30 min after administration of saline, apomorphine, metoclopramide, haloperidol, ondansetron or their combinations. Group-1 (saline+NTG vehicle) was administered saline and then NTG vehicle, group-2 (saline+NTG) was administered saline and then NTG, group-3 (Apm+NTG) was administered apomorphine and then NTG, group-4 (Apm+Met+NTG) was administered apomorphine plus metoclopramide and then NTG, group-5 (Apm+Hal+NTG) was administered apomorphine plus haloperidol and then NTG, group-6 (Apm+Ond+NTG) was administered apomorphine plus ondansetron and then NTG, group-7 (Met+NTG) was administered metoclopramide and then NTG, group-8 (Hal+NTG) was administered haloperidol and then NTG, group-9 (Ond+NTG) was administered ondansetron and then NTG. All rats were exposed to von Frey test 2 h after NTG administration and due to the nature of this
acute model, the experiments were terminated 4 h after the first drug administration, as in our previous studies (Kilinc et al., 2018; 2020; 2022a; 2022b). Under ketamine anaesthesia (90 mg/kg, i.p.), rats’ heads was perfused intracardially with 200 mL of phosphate-buffered saline (pH: 7.4) through a cannula inserted into the left ventricle. Immediately after, cranial dura mater, brainstem trigeminal nucleus caudalis and trigeminal ganglia were gently harvested. While trigeminal nucleus caudalis and trigeminal ganglia were instantly homogenized, the dura mater was fixed by incubation with 4% paraformaldehyde (pH, 7.4) overnight.

**Assessment of the withdrawal pain threshold by von Frey monofilaments**

Evaluation of hind paw withdrawal pain threshold was carried out with von Frey monofilaments (North Coast Medical, Morgan Hill, CA, USA) in compliance with the up and down procedure as described in our previous study (Kilinc et al., 2022b). In short, the middle plantar surface of the hind paw was applied with a series of von Frey monofilaments (twisting strength ranging from 0.008 to 300 g). The first monofilament applied was 2 g. A pain response was considered as raising or trembling of the hind paw of the rat following mechanical stimulation. Each monofilament was applied to the surface of the hind paw three times in succession until the rat drew away its paw or the monofilament folded. The withdrawal threshold was considered as the strength of the lighter monofilament that resulted in at least 2 withdrawal responses from 3 consecutive applications with the same monofilament. A 50% withdrawal threshold was computed utilising free online software at https://bioapps.shinyapps.io/von_frey_app/ by application of exact inter-filament steps.

**Homogenization of trigeminal ganglion and brain stem tissues**

After harvesting, trigeminal ganglia and trigeminal nucleus samples were homogenized in trizma-HCl solution (50 mmol/L, pH 7.4) containing 20 IU/mL of aprotinin and 0.2% bovine serum albumin in a constant volume (100 mg wet tissue/mL) utilising a light-duty Ultra-Turrax homogenizer (ISOLAB, Turkey). Following centrifugation (at 26,000 g for 30 min at 4°C), the supernatant samples were aliquoted and stored at -20°C until c-Fos and CGRP measurements are performed by ELISA.

**Whole-mount dura mater preparations for imaging mast cells**

Dura mater samples obtained from in vivo experiments were mounted on the poly-L-lysine coated slides as defined in our previous studies (Kilinc et al., 2018; 2022a; 2022b). Then the preparations were stained by toluidine-blue dye (0.1% in 2.5 pH sodium chloride) to observe mast cells. Dural mast cells were calculated bilaterally utilising the light microscope in five different areas along the middle meningeal artery branches and were classified as either intact or degranulated by an investigator blinded to the groups. Representative images of mast cells were taken with a camera (Nikon DS-Fi1, Nikon, Japan) mounted on the microscope.

**Isolated hemiskull preparations and CGRP release from trigeminal afferents**

These preparations allow direct administration of drugs to meningeal trigeminal nerve terminals harboring abundant CGRP-containing vesicles and are commonly used to directly measure CGRP release. The preparations were prepared as defined in our and other groups’ previous studies (Strecker et al., 2002; Labastida-Ramírez et al., 2020; Kilinc et al., 2017; Citak et al., 2022c). Shortly, two isolated hemisected skull preparations were prepared from one rat. The preparations were superfused for 30 min with carbogen-gassed (95% O2; 5% CO2) synthetic interstitial fluid (SIF). Afterwards, the preparations were fixed in a humid incubator at 37°C after being placed in caps filled with vaseline. Then, the preparations were treated with SIF (control) or apomorphine (10 μM, Himeno et al., 2011), metoclopramide (10 μM, Wolfs et al., 2021), haloperidol (10 μM, Romeo et al., 2021) and ondansetron (10 μM, Hur et al., 2014) or their combinations, respectively. 250 μL of incubation medium was collected for each treatment at 10 min intervals and was mixed with 20 μL of aprotinin to prevent the degradation of CGRP. The aliquots were stored at -20°C until CGRP measurement.

**Determination of c-Fos and CGRP levels by ELISA**

c-Fos and CGRP levels in the samples from in-vivo and ex-vivo experiments were detected by enzyme-linked immunosorbsent assay kits. The detection sensitivity was 0.159 ng/mL for c-Fos and was 9.38 pg/mL for CGRP.
The ELISA protocols were performed in compliance with the manufacturers’ instructions for each kit and were applied as duplicates. Samples, and c-Fos or CGRP standards were incubated at 37°C for certain times with reagents specified in the instructions of the ELISA kits. Then, the optical density of each well in the 96-well plate was determined at 450 nm with a microplate reader (Epoch BioTek Instruments Inc., Winooski, VT, USA).

**Statistical analysis**

Data were expressed as mean ± SEM. SPSS software (V.22.0) was employed for statistical analyses (IBM Corp., NY, USA). The Shapiro-Wilk test was employed to determine whether the data exhibited to the normal distribution. Data acquired from in vivo groups were analysed with one-way ANOVA followed by LSD post-hoc. Data acquired from the repeated measures of CGRP release in the isolated preparations were analysed with one-way repeated measures ANOVA followed by Bonferroni post-hoc. A p value <0.05 was considered statistically significant.

**RESULTS**

**The effects of dopaminergic modulation and 5-HT3 receptor antagonist ondansetron on NTG-evoked mechanical allodynia**

NTG administration evoked mechanical allodynia through reducing hind paw withdrawal threshold in comparison of the vehicle control (p<0.001, Figure 1A). In addition, apomorphine potentiated NTG-evoked mechanical allodynia via further reducing the withdrawal threshold (p= 0.003, saline+NTG vs Apm+NTG, Figure 1A). However, while D2 receptor antagonist metoclopramide decreased the effect of apomorphine (p= 0.012, Apm+NTG vs Apm+Met+NTG), another D2 receptor antagonist haloperidol fully reversed it through inhibiting apomorphine-induced diminish in the withdrawal threshold (p<0.001, Apm+NTG vs Apm+Hal+NTG, Figure 1A). We also tested 5-HT3 receptor antagonist ondansetron to distinguish whether metoclopramide exerts its effect solely through D2 receptor antagonism because metoclopramide also is a 5-HT3 receptor antagonist (Walkembach et al., 2005). Similar to metoclopramide, ondansetron slightly alleviated the effect of apomorphine, but not fully (p= 0.049, Apm+NTG vs Apm+Ond+NTG, Figure 1A).

To evaluate the effects of metoclopramide, haloperidol and ondansetron independent of apomorphine, we also administered them without apomorphine. Haloperidol alleviated the mechanical allodynia stimulated by NTG alone (p= 0.003, saline+NTG vs Hal+NTG, Figure 1A), but metoclopramide and ondansetron were ineffective (p>0.05 for both, Figure 1A).

**The effects of dopaminergic modulation and 5-HT3 receptor antagonist ondansetron on NTG-evoked C-fos and CGRP expressions, in vivo**

NTG treatment evoked an increase in the c-fos expression in the trigeminal nucleus caudalis (TNC) in the brainstem in comparison of the vehicle control (p<0.001, Figure 1B). c-fos is a neuronal activation biomarker and is a good indicator of pain in this NTG-induced migraine model in addition to mechanical allodynia. Both of them therefore confirmed the success of the model. On the other hand, apomorphine further increased NTG-induced c-fos expression, consistent with behavioral results (p= 0.002, saline+NTG vs Apm+NTG, Figure 1B).

Haloperidol and ondansetron reduced the apomorphine-stimulated additional increases in the c-fos expression, respectively (p= 0.014 for Apm+NTG vs Apm+Hal+NTG, and p= 0.027 for Apm+NTG vs Apm+Ond+NTG) while metoclopramide did not change it (p= 0.074, Apm+NTG vs Apm+Met+NTG, Figure 1B). Furthermore, haloperidol decreased NTG-induced c-fos expression (p= 0.002, saline+NTG vs Hal+NTG), but neither metoclopramide nor ondansetron altered it (p= 0.068 for saline+NTG vs Met+NTG, and p= 0.318 for saline+NTG vs Ond+NTG, Figure 1B).

On the other hand, NTG administration enhanced the CGRP concentration in both trigeminal ganglion and the TNC in the brainstem in comparison of the vehicle control (p<0.001 for both comparisons, Figures 2A and B). However, apomorphine further raised NTG-induced the CGRP concentration in both trigeminal ganglion and the TNC (p<0.001, saline+NTG vs Apm+NTG, Figures 2A and B). On the one hand, both
metoclopramide and haloperidol attenuated the apomorphine-induced additional increases in CGRP concentration in trigeminal ganglion (p = 0.001 for Apm+NTG vs Apm+Met+NTG, and p < 0.001 for Apm+NTG vs Apm+Hal+NTG), while ondansetron did not affect (p = 0.123, Apm+NTG vs Apm+Ond+NTG, Figure 2A). Interestingly, however, of these three antagonists, interestingly only haloperidol reversed the effect of apomorphine on CGRP concentration in the TNC (p < 0.001, Apm+NTG vs Apm+Hal+NTG), while the others failed to do it (p > 0.05 for both comparisons, Figure 2B).

In addition, both metoclopramide and haloperidol diminished NTG-stimulated CGRP level in trigeminal ganglion (p < 0.001 for both comparisons), but ondansetron did not change (p = 0.833, saline+NTG vs Ond+NTG, Figure 2A). However, in the TNC, only haloperidol decreased NTG-evoked CGRP level (p = 0.001, saline+NTG vs Hal+NTG), while the other antagonists were ineffective (p > 0.05 for both comparisons, Figure 2B).

The effects of dopaminergic modulation and 5-HT3 receptor antagonist ondansetron on NTG-evoked degranulation/activation and number of meningeal mast cells, in vivo

NTG administration evoked degranulation of meningeal (dural) mast cells in comparison of the vehicle control (p < 0.001, Figure 3A). Apomorphine further promoted NTG-induced mast cell degranulation (p = 0.001, saline+NTG vs Apm+NTG, Figure 3A), but not the number (p > 0.05). However, haloperidol and ondansetron alleviated the apomorphine-stimulated additional increases in mast cell degranulation (p = 0.033 for Apm+NTG vs Apm+Hal+NTG, p = 0.003 for Apm+NTG vs Apm+Ond+NTG, Figure 3A), while metoclopramide did not change (p > 0.05). Additionally, ondansetron also reduced NTG-induced mast cell degranulation (p = 0.034, saline+NTG vs Ond+NTG, Figure 3A), while metoclopramide and haloperidol did not change (p > 0.05). Representative images of the meningeal mast cells are shown in Figure 4.

On the other hand, NTG induced a significant increase in the number of mast cells in comparison of the vehicle control (p = 0.003, Figure 3B), but none of apomorphine, metoclopramide, haloperidol and ondansetron affected the NTG-induced number of mast cells (p > 0.05 for all comparisons).

The effects of dopaminergic modulation and 5-HT3 receptor antagonist ondansetron on the CGRP release from ex-vivo hemiskull preparations

This isolated preparation is a well-established tool for directly studying the release of the migraine mediator CGRP from the peripheral ends of the trigeminal nerve, a component of the migraine pain pathway. We therefore tested the effects of apomorphine, metoclopramide, haloperidol and ondansetron alone and in combination on basal release of CGRP from meningeal trigeminal nerve terminals. Combined administration of haloperidol and ondansetron reduced significantly basal release of CGRP compared to control (p = 0.003, Figure 5B), however none of the other administrations altered it (p > 0.05 for all comparisons, Figure 5A and B).

DISCUSSION

By investigating the effects of dopaminergic modulation on behavioral and molecular parameters associated with migraine pain, we attempted to elucidate the possible mechanisms of dopaminergic activation and antagonism in migraine. The current study revealed that dopaminergic agonist apomorphine aggravated NTG-induced mechanical allodynia and c-fos expression, in vivo. It is well known that c-fos expression is increased during transmission of pain impulses to second-order neurons in the TNC in the brainstem. These findings therefore affirmed the pain-inducing effect of apomorphine-mediated dopaminergic activation in NTG-evoked rat model of migraine. Our findings are supported by a previous preclinical study showing that the loss of nigrostriatal dopaminergic neurons induced by 6-hydroxydopamine inhibited NTG-induced hyperalgesia and c-fos expression (Greco et al., 2008). In addition to this, clinical studies reported that apomorphine and/or the other dopamine agonists exacerbated head pain in patients with migraine or prolactinoma-related headache (del Bene et al., 1994; Levy et al., 2003). Our findings are compatible with these previous studies and provide further evidence for the relevance of dopaminergic activation in migraine pathobiology. Activation of the trigeminovascular system and the resulting meningeal neurogenic inflammation are the main phenomena
held responsible for the pathophysiology of migraine. CGRP release from trigeminal afferents, vasodilatation
and meningeal mast cell degranulation are the main contributors leading to neurogenic inflammation. Thus,
CGRP release from peripheral (meningeal trigeminal afferents and trigeminal ganglion) and central (TNC)
structures of the trigeminovascular system and meningeal mast cells are important targets in the mechanistic
investigations of migraine.

We found that apomorphine further increased NTG-stimulated CGRP release from both trigeminal gan-
glion and TNC in the in vivo. In addition, apomorphine further enhanced NTG-evoked meningeal mast
cell degranulation without affecting their number. Thus, our findings demonstrate for the first time that
apomorphine-mediated dopaminergic activation contributes to migraine pathophysiology by inducing CGRP
release from strategic structures of the trigeminovascular system and also by inducing meningeal mast cell
activation. These findings are consistent with the presence of dopamine receptors in the trigeminovascular
system (Bergerot et al., 2007; Lazarov and Pilgrim, 1997; Peterfreund et al., 1995). Moreover, our findings
provide mechanistic evidence for link between dopaminergic activation and migraine symptoms. Because it
is known that most symptoms in prodromal and postdromal phases of migraine headache such as yawning,
nausea, vomiting, hypotension and mood changes can be stimulated by dopaminergic activation (Peroutka,
1997; Levy et al., 2003; Barbanti et al., 2020; Blin et al., 1991; Barbanti et al., 2013). However, apomorphine
did not affect basal release of CGRP from meningeal trigeminal nerve terminals in the ex-vivo. This may be
due to the fact that dopamine receptors may have less distribution in meningeal trigeminal nerve terminals
than in other regions of trigeminovascular system. In addition, we did not stimulate CGRP release with
any agent in the ex-vivo. Thus, as in the in-vivo experiments, apomorphine may only affect the stimulated
release of CGRP instead of its basal release.

On the other hand, apomorphine-stimulated additional rises in the mechanical allodynia, c-fos expression,
trigeminal ganglion and brainstem CGRP concentrations and mast cell degranulation in the in-vivo expe-
riments were reversed by haloperidol. However, another D2 receptor antagonist metoclopramide reduced
apomorphine-stimulated mechanical allodynia and trigeminal ganglion CGRP level, but it did not affect
apomorphine-stimulated c-fos expression, brainstem CGRP level and mast cell degranulation. Metoclopra-
mide also did not alter NTG-induced c-fos expression, brainstem CGRP level and mast cell degranulation.
On the contrary, a preclinical study stated that metoclopramide decreased c-fos expression in the TNC in a
rat model of migraine induced by cortical spreading depression (Doğanay Aydin et al., 2017). The difference
in these results may be due to the different migraine models used. Metoclopramide is also a 5-HT3 receptor
antagonist in addition to D2 receptor (Walkemback et al., 2005). We therefore also tested 5-HT3 receptor an-
tagont ondansetron to distinguish whether the possible effects of metoclopramide would be mediated by D2
or 5-HT3 receptor antagonism. Ondansetron decreased the apomorphine-stimulated mechanical allodynia,
c-fos expression and mast cell degranulation without altering the apomorphine-stimulated CGRP concen-
trations in both trigeminal ganglion and brainstem. Thus, unlike metoclopramide, ondansetron modulated
c-fos expression and mast cell degranulation. Our findings therefore suggest that the antimigraine effect
of metoclopramide may be a common result of co-antagonism of D2 and 5-HT3 receptors. The 5-HT3 recep-
tors are widely expressed in trigeminovascular system (Kilinc et al., 2017; Barnes et al., 2021; Giniatullin,
2022). Moreover, the controversial role of 5-HT3 receptors in pathophysiology of migraine was previously
reported by us and the other groups (Kilinc et al., 2017; Sokolov et al., 2018; Giniatullin, 2022). This may
explain different effects of metoclopramide and ondansetron on the parameters associated with migraine in
the current study. However, haloperidol reversed all apomorphine-stimulated changes, suggesting that pure
D2 receptor antagonism alleviated migraine pain through modulation of CGRP release and meningeal mast
 cell activation. In addition to its antagonizing effects against apomorphine-induced changes, haloperidol also
alleviated the mechanical allodynia, c-fos expression and CGRP levels in trigeminal ganglion and brainstem
induced by NTG without apomorphine. It is well-established that the D2 receptor antagonists are first-line
medications in the emergency room due to their well-clinical efficacy, and some of them including metoclo-
pramide and haloperidol are often used in the acute treatment of migraine (Colman et al., 2004; Richer et al.,
2007; Schulman and Dermott, 2003; LaPorta, 2007; Levy et al., 2005). Taken together, our findings provide,
for the first time, mechanistic evidence for effective acute attack treatment of D2 receptor antagonism in
migraine.

On the other hand, none of metoclopramide, haloperidol and ondansetron alone did not change basal release of CGRP from meningeal trigeminal nerve terminals in the ex-vivo. However, these results do not guarantee that these antagonists will not alter stimulated release of CGRP from meningeal trigeminal nerve terminals because we did not test the effects of them on the induced CGRP release. This can be considered as a limitation of the present study and it would be interesting to explore the effects of them on the capsaicin- or nitroglycerin-induced CGRP release in the ex-vivo. On the other hand, interestingly, combined administration of haloperidol and ondansetron, but not the others, decreased basal release of CGRP in the ex-vivo. This implies a synergistic effect of haloperidol and ondansetron on basal CGRP release from peripheral terminals of trigeminal nerve.

CONCLUSION

In conclusion, apomorphine-mediated dopaminergic activation exacerbated NTG-stimulated migraine pain by further enhancing c-fos expression, CGRP release and mast cell degranulation in strategical structures associated with migraine pain. Haloperidol fully antagonised these apomorphine-induced effects and also improved changes evoked by NTG without apomorphine. However, another D2 receptor antagonist metoclopramide partially attenuated the effects of apomorphine, most likely because it is also a 5-HT3 receptor antagonist. Haloperidol with pure D2 receptor antagonism feature appears to be more effective than metoclopramide in reducing migraine-related parameters in dopaminergic activation- and/or NTG-induced migraine like conditions.

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Competing interest

The authors declare that there is no conflict of interest in this study.

Data availability statement

The data appeared during the current study are available at figshare Software: https://doi.org/10.6084/m9.figshare.22731461.v1

Authors’ contribution

YBK hypothesized and designed the study, analyzed and interpreted data, wrote and revised the manuscript. IET performed the experiments, collected data, and wrote the manuscript. EK supervised the experiments, analyzed and interpreted data, and wrote the manuscript.

Abbreviations

CGRP, calcitonin gene-related peptide; NTG, nitroglycerin; TNC, trigeminal nucleus caudalis; Apm, apomorphine; Met, metoclopramide; Hal, haloperidol; Ond, ondansetron.

References


Figure Legends

Figure 1. The effects of dopaminergic modulation and 5-HT3 receptor antagonist ondansetron on the nitroglycerin-evoked mechanical allodynia and c-fos expression, in vivo

The effects of apomorphine, metoclopramide, haloperidol and ondansetron on the nitroglycerin-evoked mechanical allodynia (A) and brainstem c-fos expression (B).

n.s. non-significance. *p<0.05, **P<0.01 and ***P<0.001. $P<0.01$ versus saline+nitroglycerin. Veh, vehicle; Met, metoclopramide; Hal, haloperidol; Ond, ondansetron.

Figure 2. The effects of dopaminergic modulation and 5-HT3 receptor antagonist ondansetron on the nitroglycerin-evoked calcitonin gene-related peptide concentrations, in vivo

The effects of apomorphine, metoclopramide, haloperidol and ondansetron on the nitroglycerin-evoked calcitonin gene-related peptide concentrations in the trigeminal ganglion (A) and brainstem trigeminal nucleus caudalis (B).

n.s. non-significance. **P<0.01 and ***P<0.001. $P<0.001$ versus saline+nitroglycerin. Veh, vehicle; Met, metoclopramide; Hal, haloperidol; Ond, ondansetron.

Figure 3. The effects of dopaminergic modulation and 5-HT3 receptor antagonist ondansetron on the nitroglycerin-induced the degranulation/activation and number of meningeal mast cells, in vivo

The effects of apomorphine, metoclopramide, haloperidol and ondansetron on the nitroglycerin-induced meningeal mast cells degranulation (A) and number (B).

n.s. non-significance. *p<0.05, **P<0.01 and ***P<0.001. $P<0.05$ versus saline+nitroglycerin. Veh, vehicle; Met, metoclopramide; Hal, haloperidol; Ond, ondansetron.

Figure 4. Representative images of meningeal mast cells stained by toluidine blue

Microscopic pictures of meningeal mast cells were captured at a magnification of x40. Scale-bars are shown on the pictures. Intact mast cells in control group (Saline+Vehicle) (A), nitroglycerin-induced degranulated mast cells in Saline+Nitroglycerin group (B), apomorphine further enhanced nitroglycerin-induced degranulation of mast cells in Apomorphine+Nitroglycerin group (C), metoclopramide did not alter the effects of apomorphine in Metoclopramide+Apomorphine+Nitroglycerin group (D), haloperidol and ondansetron alleviated the effects of apomorphine in Haloperidol+Apomorphine+Nitroglycerin group (E) and in Ondantrons+Apomorphine+Nitroglycerin group (F), metoclopramide and haloperidol did not change nitroglycerin-induced degranulation of mast cells in Metoclopramide+Nitroglycerin group (G) and in Haloperidol+Nitroglycerin group (H) unlike ondansetron attenuated it in Ondantrons+Nitroglycerin group (I). Intact (J) and degranulated (K) mast cells with a magnification of x100 from the control and Saline+Nitroglycerin groups, respectively. Open arrowheads display intact mast cells in the meninges/cranial dura mater, while thick-straight arrows indicate degranulated ones.

Figure 5. The effects of dopaminergic modulation and 5-HT3 receptor antagonist ondansetron on the CGRP release from meningeal trigeminal afferents

None of apomorphine, metoclopramide, haloperidol and ondansetron alone and in combination (A), except for haloperidol plus ondansetron (B), did not affect basal release of CGRP from meningeal trigeminal nerve terminals.
n.s. non-significance. **P<0.01. Ctrl, control; Apm, apomorphine; Met, metoclopramide; Hal, haloperidol; Ond, ondansetron.