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EFFECT OF ANTI-CGRP ANTIBODY GALCANEZUMAB ON NEUROPEPTIDE-MEDIATED TRIGEMINOVASCULAR FUNCTION IN RATS

Summary of Ph.D. Thesis

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I. PUBLICATIONS

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II. INTRODUCTION

Migraine is a complex neurovascular disorder. Its pathogenesis involves multiple components of both the peripheral and the central nervous system. Although the exact mechanism underlying the generation of a migraine attack is not completely understood, it is widely accepted, that the headache pain is generated by the activation of the trigeminal nociceptive pathway.

In the pathophysiology of migraine, calcitonin gene-related peptide (CGRP) is one of the most important neuropeptides. In the trigeminal ganglion, about 50% of the neurons are CGRP-positive. The peripheral axons of these CGRP-positive nerve fibers are mainly located along the arteries from the circle of Willis and meningeal blood vessels. Besides the neuronal presence, CGRP is found in non-neuronal structures, including endothelials cells, macrophages and adipocytes. In the central nervous system, CGRP is primarily released during nociceptor activation and may contribute to the central sensitization by increasing glutamate transmission in the medulla and the dorsal horn of the spinal cord.

Clinical and experimental evidences indicate the role of CGRP in migraine pathophysiology. In migraine patients the plasma level of CGRP increased in the external jugular vein during migraine attacks. Furthermore, elevated levels of CGRP have been measured in tears and saliva during migraine attacks and in the plasma of chronic migraine patients between attacks. Another piece of evidence is that intravenous administration of CGRP in migrain patients triggers migraine-like headache. Last but not least, one of the most significant proofs for the role of CGRP in migraine pathophysiology is the succesful use of anti-CGRP therapy in clinical practice.

There is increasing evidence that pituitary adenylate cyclase-activating polypeptide (PACAP) may also play a role in the pathophysiology of migraine. The physiological effects of PACAP are similar to CGRP. PACAP has a potent vasodilator effect and induces dural mast cell degranulation in rats. Clinical trials have revealed that intravenous administration of PACAP induces headache in both control group and migraine patients, unlike CGRP, which only induces migraine-like headaches in migraine sufferers. It may be involved in the development of peripheral and central sensitization, could influence neurogenic inflammation, making it a potential target for therapeutic options.

Both CGRP and PACAP are considered as mediators of migraine pathophysiology. Both peptides induce vasodilatation in the innervated tissue and they can cause migraine-like headaches when infused into people and migraine-like symptoms when injected into rodents.

CGRP and PACAP are found in distinct, but overlapping areas of peripheral and central nervous system relevant to migraine. In rodents, under experimental conditions the two peptides share functions, including neurogenic inflammation of the innervated tissue and they also accelerate nociceptive reactions. Despite the functional similarities they bind to different specific receptors and act by independent mechanisms possibly by distinct intracellular signalling pathways.

Substance P (SP) is mainly expressed in the trigeminal sensory nerve fibers and along the dural vessels, similarly to CGRP. The SP-induced increased permeability of blood vessels mainly applies to postcapillary venules, thereby supporting neurogenic inflammation of the peripheral tissue. Additionally, SP plays a role in pain transmission. Its relationship with migraine is not entirely clear, but elevated SP levels have been measured in saliva during spontaneous migraine attacks without aura. Furthermore, increased SP levels have been observed during attack-free periods in both episodic and chronic migraine patients. However, during migraine attacks, plasma SP concentrations were not found to be elevated and clinically, NK receptor antagonists have shown no significant therapeutic effect in migraine patients.

Somatostatin (SOM) is a neuropeptide which has an analgesic effect in rodents and humans. In sensory nerves it inhibits nociceptive processes and release of other neuropeptides from sensory nerve endings. In migraine attacks the pain suppressing role of SOM was suggested. SOM concentration was low interictally in cerebrospinal fluid, and further reduction was measured during headache attacks in migraineurs.

The prominent role of the trigeminovascular system in the pathophysiology of migraine pain is not only the activation of the trigeminal nociceptive pathway potentially leading to headache, but also the generation of neurogenic inflammation in the meningeal tissues. In the sensory system neurogenic inflammation induced by the release of vasoactive neuropeptides is considered as a key component of the nociceptor sensitization. Peptides released by activated nociceptors increase blood flow and vascular permeability in the innervated tissue, and by degranulation of mast cells or activation of other meningeal cells further vasoactive and nociceptor sensitizing mediators can be released.

A few years ago the concept of the trigeminal nocisensor complex as a morphological substrate of meningeal nociceptor sensitization was formulated. The nocisensor complex consists of the trigeminal nociceptive primary afferent neurons with their peripheral and central terminals innervating the dura mater and providing the sensory information to second order neurons, respectively, the meningeal blood vessels and mast cells. According to current theories

the combination of changes in the neural activity and vascular reactions may generate head pain during migraine attacks.

Stimulation of dural nociceptors by noxious agents results in the transmission of nociceptive signals by the release of vasoactive peptides such as CGRP and SP, which generate a sterile neurogenic inflammation in the meningeal tissues; produce vasodilatation and plasma extravasation, respectively. Tissue mediators may excite or sensitize the transient receptor potential vanilloid 1 (TRPV1) and transient receptor potential ankyrin 1 (TRPA1) channels expressed by meningeal nociceptors. Mast cells may be activated by CGRP and SP released from the nociceptors and also by mast cell tryptase. A mutual activation of mast cells through the activation of the proteinase-activated receptor-2 by triptase was reported, which provides a possibility for a self triggering mechanism of mast cells resulting in an augmentation of the release of mast cell mediators such as histamine. Histamine released by mast cells may amplify vascular reactions and may also sensitize nociceptive afferents. Although the role of vasodilatation and increased blood flow to the dura mater is probably not the activation of nociceptors, vascular reactions may accelerate the removal of noxious substances and restoring homeostasis within the meningeal tissue.

Forty years ago, the discovery of the vasoactive neuropeptide CGRP and its role in migraine pathophysiology opened a new era in migraine treatment and prophylaxis. Triptans target 5-HT_{1B} and 5-HT_{1D} serotonin receptors, and through receptor activation they reduce CGRP release. Gepants are small-molecule non-peptide competitive antagonists at the CGRP receptor.

Nowadays four monoclonal antibodies are available for prevention of episodic and chronic migraine. Three of them, eptinezumab, fremanezumab and galcanezumab, are anti-CGRP antibodies, while erenumab is an anti-CGRP receptor antibody. All these monoclonal antibodies are IgG isotypes, have a unique structure and affinity, and also a long serum half-life. The size of the anti-CGRP and anti-CGRP receptor antibodies are around 150 kilodalton, so they do not cross the blood-brain barrier and do not cause significant central nervous system side effects or toxicity.

Our laboratory has a long tradition in migraine research. In ex vivo dura mater preparation of rats we can measure basal and stimulated peptide/mediator release from different cellular components of the dura mater. Using immunohistochemical methods in whole mount preparation of the dura mater and cryostate sections of trigeminal ganglia we can study the morphological changes affecting the trigeminovascular functions under pathophysiological conditions relevant to migraine.

III. THE AIM OF THE STUDY

The aim of our study was to define functional changes in the trigeminovascular nocisensor complex of rats that may affect the nociceptor function after treatment with the anti-CGRP antibody galcanezumab. To visualize the distribution of the anti-CGRP antibody in the dura mater and the trigeminal ganglion, we used fluorophore-labelled galcanezumab. To define the localization of the anti-CGRP antibody in the meningeal tissue and the trigeminal ganglion coexpression of the fluorophore-labelled galcanezumab with the neuropeptide CGRP, an endothelial marker von Willebrand factor, smooth muscle actin and histamine was studied. Changes in the release of CGRP, SP and SOM from meningeal afferents following stimulation of meningeal afferents with the TRPV1 receptor agonist capsaicin or the depolarizing agent KCl was measured in an ex vivo dura mater preparation after galcanezumab treatment. Provided that CGRP and SP are mainly colocalized in trigeminal neurons, stored in the same vesicles and co-released upon activation, we also asked if treatment with a CGRP-binding antibody galcanezumab changes the release of SP similar to that of CGRP. We also studied the effect of galcanezumab on meningeal mast cell function; in an ex vivo rat dura mater preparation we examined the effect of anti-CGRP antibody treatment on CGRP and compound 48/80-induced histamine release from meningeal mast cells.

IV. MATERIALS AND METHODS

1. Experimental animals

Adult male (weighing 270-320 g) and female (weighing 230-260 g) Wistar rats were used in all of our experiments. The animals were housed under a 12-h light/dark cycle, at a temperature of 22 ± 2 °C, in 50 – 70 % relaive humidity with free access to food and water. All experimental procedures were approved by the Ethical Committee for Animal Care of the University of Szeged (approval ID: XIV./1800/2021 and XIV./2368/2023) and carried out in accordance with the Directive 2010/63/EU of the European Parliament. All efforts were made to minimize animal suffering. The number of experimental animals was kept as low as possible.

2. Administration of the antibodies

Rats were anaesthetized with isoflurane at an increasing concentration up to 4 % (Aerrane, Baxter Hungary Kft, Hungary). One group of animals received 30 mg/kg of the anti-CGRP antibody galcanezumab (Emgality, Eli Lilly Netherlands B.V., Utrecht, Nederland, in

10 mg/ml solution) via subcutaneous injection into the shaved area at the neck and shoulder region. Control rats received subcutaneous injection of the vehicle (0.9 % NaCl).

To visualize the presence and the distribution of galcanezumab in the dura mater and the trigeminal ganglion, a fluorophore-labelled antibody (Cy3-galcanezumab) was used. For comparison Cy3-bevacizumab (30 mg/kg in 10 mg/ml solution, Avastin, Roche, Switzerland), another fluorophore-labelled antibody acting independently of the CGRP pathway was used.

3. Immunohistochemical staining of the dura mater and the trigeminal ganglion

To visualize the exact localization of the antibody in the different cellular components of the dura mater and the trigeminal ganglion coexpression of the fluorophore-labelled antibodies with an endothelial marker von Willebrand factor, smooth muscle actin or histamine was studied with the indirect immunofluorescence method. Spatial relationship between structures containing the fluorophore-labelled antibody and CGRP-containing axons in the dura mater or neuronal cell bodies in the trigeminal ganglion expressing CGRP was also studied in histological preparations.

Rats injected with the fluorophore-labelled Cy3-galcanezumab or Cy3-bevacizumab 3 or 30 days prior to the experiment were deeply anaesthetized with thiopental sodium (200 mg/kg, intraperitoneally, Braun, Spain) and perfused transcardially with physiological saline followed by 4 % paraformaldehyde in phosphate buffer (pH 7.4). The animals were decapitated, skin and muscles were removed and the skull was divided into halves along the midline. The brain was removed, the parietal dura mater and the trigeminal ganglia were dissected and postfixed for 2 h in the same fixative. Then trigeminal ganglia were placed in 0.1 M phosphate buffered saline (pH 7.4) containing 30 % sucrose at 4 °C for 24 h and cut into 16 µm thick longitudinal sections with a cryostat.

Whole mount preparations of the dura mater and sections of trigeminal ganglia of rats treated with fluorophore-labelled antibody were processed for staining with the indirect immunofluorescence technique, and were examined with a confocal fluorescence microscope using the appropriate filter settings of the confocal scanner.

4. Ex vivo measurement of calcitonin gene-related peptide, substance P and somatostatin release from meningeal afferents

Measurement of CGRP, SP and SOM release from the rat dura mater was performed in ex vivo dura mater preparation. Control male and female rats treated with galcanezumab or vehicle 7 days prior to the experiment were deeply anaesthetized with thiopental sodium (200 mg/kg, intraperitoneally) and decapitated. After removal of the skin and muscles, the skull was divided into halves along the sagittal suture and the cerebral hemispheres were removed. The hemiskull preparations were washed with carbogen-gassed synthetic interstitial fluid (SIF) at room temperature for 30 min and then mounted in a humid chamber at 37 °C. The cranial fossa was filled with 300 µl of carbogen-gassed SIF solution. Samples of the superfusate were collected at periods of 10 min. Control samples were taken to determine basal peptide release in the presence of SIF, then the dura was stimulated for 10 min with the TRPV1 receptor agonist capsaicin at 100 nM concentration in case of CGRP and SP or with 60 mM KCl in case of SOM release. 200 µl of samples diluted with 50 µl of enzyme-linked immunoassay (EIA) buffer were placed into Eppendorf cups and immediately frozen at -70 °C for subsequent analysis.

5. Ex vivo measurement of histamine release from meningeal mast cells

Skull halves of control rats and animals treated with galcanezumab 7 days prior to the experiment were prepared as described above for the measurement of peptide release. Control samples were taken in the presence of SIF for 10 min to determine basal histamine release, then the dura mater was stimulated for 10 min by the application of 300 µl of CGRP at 10 µM or 2.5 µg/ml compound 48/80. The concentrations of CGRP and compound 48/80 used in the experiments were found effective in releasing histamine in previous experiments of our laboratory.

6. Analysis of the neuropeptide (CGRP, SP, SOM) content of samples and analysis of the histamine content of samples

The EIA method was used for the measurement of neuropeptide content of the defrosted samples. The EIA of CGRP is based on a double-antibody sandwich technique with capture and tracer antibodies binding the CGRP molecule. In the other half of the defrosted samples used for CGRP measurement, the SP concentration was measured. The SP-kit is based on sandwich enzyme-linked immunosorbent assay technology. The measurement of the SOM content of samples obtained before and after stimulation of the dura mater with 60 mM KCl

were measured with EIA method. Samples were processed according to the instructions of the manufacturer.

The EIA method used for measurement of the histamine concentration of samples is based on the competition between unlabelled histamine of samples and acetylcholin esterase linked to histamine for limited specific anti-histamine antibody sites in wells.

7. Statistics

Statistical analysis was performed using Statistica 13 software (StatSoft, USA). Following verification of the normal distribution of data, the Student's t-test and analysis of variance (factorial ANOVA or one-way ANOVA) extended by the unequal *N* honest significant difference (HSD) test were used as specified in the results. All values were expressed as mean ± standard error of the mean (SEM). A probability level of $p < 0.05$ was regarded as statistically significant.

V. RESULTS

1. Localization of fluorophore-labelled antibodies in the dura mater

In whole mount preparations of the dura mater from animals treated with Cy3-galcanezumab 3 days prior to fixation, the fluorescence signal was detected mainly in the arterioles of the middle meningeal artery. Axons of CGRP-positive afferent neurons were detected in close vicinity to arterioles showing the fluorescence signal. In addition, some cells in the connective tissue of the dura mater distant from visible blood vessels showed Cy3-fluorescence. To see whether mast cells augmenting the neurogenic inflammation in the meningeal tissue by the release of their mediators also accumulate the fluorophore-labelled antibody, histamine immunofluorescence in the dura mater whole mount preparations was identified. Mast cells were mainly located in close proximity to blood vessels labelled with Cy3-galcanezumab, but no colocalization of mast cell histamine with Cy3-galcanezumab labelling was observed in the meningeal tissue. Traces of Cy3-galcanezumab were still present in the dura mater 30 days after injection of the antibody.

In our experiments, Cy3-bevacizumab targeting the vascular endothelial growth factor was used as a control antibody. Although the localization of Cy3-bevacizumab not targeting CGRP was similar to Cy3-galcanezumab in the blood vessels of the dura mater, deposition of the fluorophore-labelled bevacizumab was overall visibly less intense.

2. Localization of fluorophore-labelled antibodies in the trigeminal ganglion

Three days after treating the animals with Cy3-galcanezumab in sections of the trigeminal ganglia fluorescence signal was detected in the wall of blood vessels, similar to the dura mater. Labelled blood vessels were localized close to CGRP-immunopositive trigeminal neurons. The fluorescence signal was also detected around the soma of some trigeminal ganglion cells. To clarify the exact localization of the fluorescence signal in the blood vessels of the trigeminal ganglia, immunohistochemistry identifying the endothelial marker von Willebrand factor and smooth muscle actin was carried out. Cy3-galcanezumab was not colocalized with the fluorescence marker for smooth muscle actin, but in almost all labelled blood vessels with the endothelial marker for von Willebrand factor. Capillaries positive for von Willebrand factor did not show Cy3-fluorescence for galcanezumab. Traces of Cy3-galcanezumab were still present in the wall of some blood vessels in the trigeminal ganglion 30 days after injection of the antibody.

Localization of Cy3-bevacizumab in the blood vessels of the trigeminal ganglia was similar to that of Cy3-galcanezumab but deposition of the antibody around the soma of neurons in the ganglion was absent and the labelling was overall less intense.

3. Altered calcitonin gene-related peptide and substance P release from the dura mater after galcanezumab treatment

Using ex vivo hemisected rat skull preparations, we measured the concentrations of CGRP and SP released spontaneously by the meningeal afferents in the presence of SIF. In control animals, basal CGRP release was lower in males than in females, while SP concentrations measured in the same samples tended to be higher in males.

In galcanezumab-treated animals, the stimulated increase in CGRP release was significantly lower than in the vehicle group. There was no significant difference between the sexes but post-hoc testing using the unequal *N* HSD test showed that the difference between the vehicle and the galcanezumab group was solely due to the female animals.

The capsaicin-stimulated SP release was below the basal release in vehicle-treated animals but not different from the basal release in galcanezumab-treated animals, which was indicated as significant between the groups. According to the unequal *N* HSD post-hoc test, the difference between the control and the galcanezumab group was solely due to the female animals.

4. Basal and stimulated release of somatostatin from the dura mater

In other hemisected skull preparations we measured the SOM release from the dura mater stimulated with 60 mM KCl. Since we did not want to exclude the transient receptor potential receptor negative but SOM positive neurons from the measurement, in this series of experiments we stimulated the meningeal afferents with 60 mM KCl instead of capsaicin. In vehicle-treated control animals both basal and KCl stimulated SOM release tended to be higher in females. In control animals stimulation with KCl induced no significant changes in SOM release. Seven days after galcanezumab treatment of the animals no significant change was observed in basal or stimulated SOM release.

5. Histamine releasing effect of different mast cell degranulating agents

In other hemisected skull preparations of male rats, we measured histamine release from the meningeal tissue. The basal histamine release was significantly higher in galcanezumab-treated animals. The histamine-releasing effects of mast cell degranulating agents were differentially influenced by galcanezumab treatment. In vehicle-treated control animals, CGRP (10 μM) increased the histamine release stronger, than in the galcanezumab-treated group. We also tested the histamine-releasing effect of the widely used mast cell degranulating agent compound 48/80 at 2.5 μg/ml that was not different between vehicle and galcanezumab-treated animals.

VI. DISCUSSION

Our study was initiated in an attempt to examine changes in the trigeminovascular system after the administration of the migraine preventing drug humanized monoclonal anti-CGRP antibody galcanezumab. Since the trigeminal nocisensor complex includes the peptidergic sensory neurons, the meningeal vascular elements and mast cells involved in the generation of the neurogenic inflammatory reaction of the dura mater and thereby playing significant role in the peripheral and central sensitization of the trigeminal nociceptive pathway, we measured functional changes in this system such as release of neuropeptides as well as histamine, regarded as relevant for headache generation in migraineurs.

1. Distribution of Cy3-galcanezumab in the dura mater and the trigeminal ganglion

Cy3-labelled galcanezumab was identified in the dura mater and the trigeminal ganglion already 3 days after the treatment of the animals. Cy3-galcanezumab could be detected in the trigeminovascular system for up to 30 days after the administration. In both tissues mainly arterioles were labelled by the antibody. Cy3-galcanezumab was coexpressed to a great extent with the endothelial marker von Willebrand factor but not with smooth muscle actin. We assume that the strong affinity of some components of the luminal glycocalyx of endothelial cells in dural blood vessels to the circulating antibody may form a local depot that releases antibody in the dura mater and also in the trigeminal ganglion for at least 30 days after the injection.

In the dura mater and the trigeminal ganglion fluorophore-labelled blood vessels were in close proximity to CGRP-immunoreactive nerve fibers or cell bodies and in the dura mater also to mast cells, providing an antibody pool for the nocisensor complex for at least 30 days.

Although the presence of the anti-CGRP antibody was robust both in the dura mater and the trigeminal ganglion already 3 days after the Cy3-galcanezumab injection, we performed the functional tests a few days later (7 days after the injection) to allow the development of possibly complete functional changes in the nocisensor complex.

2. Sex differences observed in calcitonin gene-related peptide and substance P release from the dura mater

CGRP, the central pathophysiological factor of migraine headache can be colocalized with other peptides in nociceptive neurons. Based on earlier observations indicating that SP is present in a subpopulation of CGRP-containing sensory neurons, we asked if treatment with an anti-CGRP antibody changes the SP release from meningeal afferents in the same way as it changes the CGRP release. Corresponding to the earlier observations in our experiments systemic galcanezumab treatment reduced TRPV1 stimulation-induced CGRP release from meningeal afferents.

Our results revealed a sex difference in peptide release. Not only the basal but also the capsaicin-stimulated CGRP concentrations were higher in female rats. Migraine is more prevalent in the female population. The higher susceptibility of the female trigeminal system to release CGRP is reflected by our results. The higher sensitivity of trigeminal neurons towards factors modulating peptide release is indicated also by the stronger inhibition of CGRP release in female animals after galcanezumab treatment.

In control animals CGRP release was combined with a much lower SP release in the same sample corresponding to the lower number of SP containing neurons in the trigeminal system. Compared to male animals higher CGRP concentrations were combined with lower SP concentrations in vehicle-treated female rats after capsaicin application. While galcanezumab treatment decreased the capsaicin-induced CGRP release, SP content of the samples was increased in both sexes. The difference was significant in females.

While the clinical relevance of SP in migraine pathophysiology is not clear, we can not explain the function of increased SP production or release concomitant with a decreased CGRP production or release after galcanezumab treatment. Anyway, our results raises the notion that less CGRP production in the neuron may set the protein producing machinery free that leads to an increased SP production or transport to the vesicles. According to our hypothesis galcanezumab treatment may affect not only the targeted CGRP production and function but other neuropeptides may be also affected by the treatment.

3. Somatostatin release from meningeal afferents

Immunohistochemical studies have demonstrated the presence of SOM within trigeminal neurons. SOM inhibits not only the acute inflammation and nociception in the innervated tissue, but may also exert a "sensocrine" function with systemic antiinflammatory and analgesic effects.

Since we realized that galcanezumab treatment modifies not only the release of the targeted CGRP upon stimulation but concomitantly SP release is increased, we tested whether the migraine-preventing effect of the anti-CGRP antibodies is not only due to reduced expression and effectivity of CGRP but at least partially the result of an increased SOM release from the stimulated terminals. Thus it appeared possible that enhanced expression and release of SOM with its systemic pain inhibiting effect is also involved.

Capsaicin has been shown to induce SOM release in different tissues but only about 8% of trigeminal ganglion neurons express this neuropeptide. Therefore, to ascertain a measurable effect in SOM release, we used KCl instead of capsaicin as a potent depolarizing stimulus activating not only the capsaicin sensitive neuron population. Our results indicated sex difference for basal SOM release in control animals with a higher release in females compared to males. However, basal as well as stimulated SOM concentrations were very similar in both vehicle and galcanezumab-treated animals suggesting that it is unlikely that an increased SOM release may contribute to a pain reducing effect of galcanezumab treatment.

4. Histamine release from rat dura mater

In the wholemount preparations of the dura mater we could not find any colocalization between Cy3-galcanezumab and immunohistochemically detected histamine indicating mast cells, but the close proximity of fluorophore-labelled blood vessels, CGRP immmunoreactive afferents and mast cells may provide a morphological basis for a mast cell function modifying effect of anti-CGRP antibody. In our experiments histamine release was measured as an indicator of mast cell activation. The differential effects of mast cell degranulating agents on histamine release suggest that galcanezumab treatment modifies the mast cell-mediated amplifying of signals, but it does not alter the histamine content of mast cells.

Our present results indicate that changes in the CGRP receptor components induced by the antibody treatment may affect not only neurons but also other components of the trigeminal nocisensor complex such as mast cells expressing the receptor.

We do not know the reason for the difference between the basal histamine release in control (vehicle-treated) and galcanezumab-treated animals. The higher concentration of spontaneously released histamine measured in galcanezumab-treated rats may be due to changes in the number and/or function of mast cell receptors stimulated by tissue metabolites under basal conditions.

5. Clinical relevance

An important question regarding the clinical relevance of our study is: where these anti-CGRP antibodies in the trigeminal nociceptive pathway act? The migraine-preventing effect of CGRP-targeting antibodies is considered mainly as a peripheral effect in the trigeminovascular system as penetration of the antibody through the blood brain barrier into the central nervous system is limited. The trigeminal ganglion and the peripheral axons of the trigeminal nociceptors innervating the dura mater are not protected by the blood brain barrier and thus are likely targets of antibody treatment.

Experimental results suggest that the action of the anti-CGRP antibody is complex. It is not limited to the neutralization of released CGRP but it might also modify the production and/or release of other neuropeptides coexpressed with CGRP. Altered expression or function of CGRP receptors on trigeminal neurons and other components of the trigeminal nocisensor complex may prevent the sensitization of trigeminal neurons and the exaggeration of the initial nociceptive and vascular responses.

VII. SUMMARY

Migraine is a primary headache predominantly affecting women. Monoclonal antibodies binding CGRP are approved in the prophylaxis of migraine. The precise mechanism of their effect is yet unclear but they appear to protect trigeminal afferents from sensitization.

Our experiments detected a long-lasting multi-faceted change in the function of the trigeminovascular nocisensor complex after treatment of rats with the anti-CGRP antibody galcanezumab. Fluorophore-labelled galcanezumab was mainly accumulated in the arterioles of the dura mater and the trigeminal ganglion, in structures of the trigeminovascular system that are not protected by the blood brain barrier. Anti-CGRP antibody treatment modulated CGRP and SP release from activated nociceptors inversely. CGRP release was reduced while SP release increased upon activation of TRPV1 receptors of trigeminal nociceptors. SOM exerting systemic antinociceptive effect was not influenced by the antibody treatment.

Activating effects of CGRP on non-neural elements of the trigeminal nocisensor complex, notably the dural mast cells, seem to be mitigated by the antibody treatment. A shift in the equilibrium between pronociceptive and antinociceptive mediators released upon trigeminal activation may contribute to the beneficial effect of anti-CGRP antibody treatment in migraine.

Activity and susceptibility of the trigeminovascular system to anti-CGRP antibody treatment was higher in female animals.

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