

Fig. 1. (a) Immunoblotting showing the expression of 5-HT1B receptor, serotonin transporter, tyrosine hydroxylase, and aromatic L-amino acid decarboxylase in lymphoblasts. All of these molecules were seen in lymphoblasts from both MWA patients and control subjects. M1, M2: migraine with aura; C1, C2: control; 5-HT1B R: 5-HT1B receptor; SERT: serotonin transporter; AADC: aromatic L-amino acid decarboxylase; TH: tyrosine hydroxylase. (b) The expression level of AADC was examined using RT-PCR. The expression of non-neuronal type AADC was seen in lymphoblasts from both MWA patients and control subjects, whereas the expression of neuronal type AADC was not seen in both lymphoblasts. P: positive control (mouse brain).

and enzymes were seen between the MWA and control cells (Fig. 1(a)).

As for AADC, non-neuronal type was found in the human lymphoblasts employing RT-PCR (Fig. 1(b)).

3.2. 5-HTP and 5-HT levels in human lymphoblasts

On the other hand, the 5-HT level in the MWA lymphoblasts (1.77 ± 0.66 pg/ 10^4 cells) was significantly higher than that in the control cells (0.51 ± 0.34 pg/ 10^4 cells) (Fig. 2), whereas no

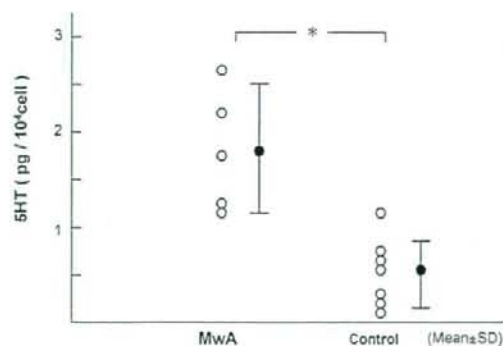


Fig. 2. 5-HT levels in lymphoblasts. The 5-HT concentration was significantly higher in lymphoblasts from MWA patients than in those from control subjects. * $p < 0.01$.

significant difference in the 5-HTP concentration was seen between the MWA lymphoblasts (0.79 ± 0.41 pg/ 10^6 cells) and the control cells (6.4 ± 9.3 pg/ 10^6 cells) (Fig. 3). However, there was no correlation between 5-HT level in the MWA lymphoblasts and any specific features of migraine attack in each migraine patient.

Moreover, no significant difference in the uptake of 5-HT into the lymphoblasts was seen between the MWA lymphoblasts (4.90 ± 0.87 DPM/ 10^4 cells) and the control cells (4.91 ± 0.97 DPM/ 10^4 cells) (Fig. 4).

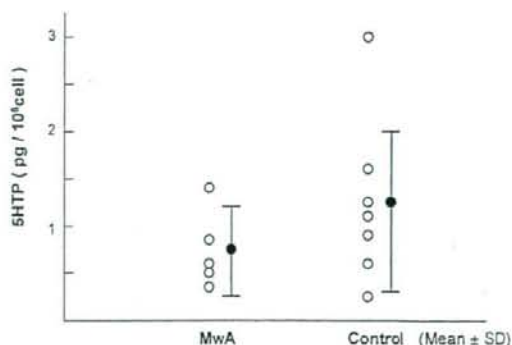


Fig. 3. 5-HTP levels in lymphoblasts. No notable difference was seen between the MWA patients and the control subjects.

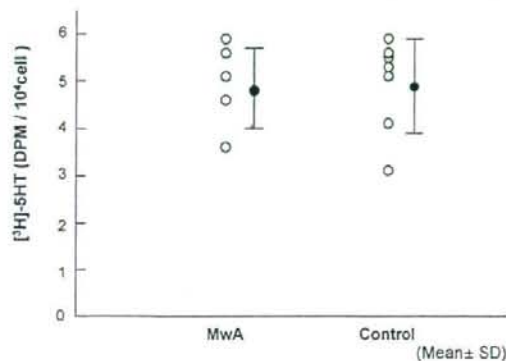


Fig. 4. $[^3\text{H}]$ 5-HT uptake in lymphoblasts. No significant difference in $[^3\text{H}]$ 5-HT uptake was observed between lymphoblasts from MWA patients and those from control subjects.

4. Discussion

Infection with Epstein-Barr virus *in vitro* easily transforms resting B cells obtained from human peripheral blood into actively proliferating lymphoblast cell lines. The percentage of B cells susceptible to transformation has been estimated to be 10–100%. In general, these cells become immortalized without becoming tumorigenic. They also have an unlimited life span with no other additional changes, although they usually have a normal diploid karyotype (Sugimoto et al., 2004; Miller, 1990). Previously, we used a gene array to examine expressions in Epstein-Barr virus-immortalized lymphoblast cell line and B cell from the same control subject. No significant differences in the expression of molecules related to 5-HT metabolism, such as 5-HT receptors and enzymes, were detected between the two groups (data were not shown).

To date, lymphoblast cell lines have been used for a variety of purposes, such as sources of DNA, cells to study various genetic disorders, and the study of cell biology. Moreover, we previously reported the use of lymphoblasts from patients with Huntington disease to reveal mitochondrial dysfunction and morphological alterations (Sawa et al., 1999, 2005; Nagata et al., 2004).

We prepared the human lymphoblast cell lines used in the present study using cells obtained from MWA patients and healthy controls. Careful attention was given to the cell culture conditions used for both cell lines: the duration of freezing was the same, the cells were thawed at the same time (within 4 weeks of the experiments), and the cells were maintained in the same medium (RPMI 1640 plus 10% fetal bovine serum). No differences in adventitious factors, dietary treatments, or drug treatment were likely to impact our experimental results.

Recent evidence has shown that 5-HT is closely related to migraine pathophysiology. 5-HT is synthesized from an essential amino acid, tryptophan, in a two-step process: hydroxylation of tryptophan, catalyzed by the enzyme TH, producing 5-HTP, and decarboxylation of 5-HTP, catalyzed by the enzyme AADC (Ichinose et al., 1992). TH is the rate limiting enzyme in the biosynthesis of 5-HT, catalyzing the

oxygenase reaction. On the other hand, SERTs are localized on presynaptic axon terminals and are widely known as the site of action of serotonin reuptake inhibitors (Lima and Urbina, 2002). A polymorphism of the SERT gene promoter might be involved in the polygenic etiology of MWA (Yilmaz et al., 2001; Marziniak et al., 2005).

Human lymphoblasts express receptors and enzymes relevant to 5-HT metabolism, like 5-HT_{1B/D} receptors, AADC, and SERT. Our data suggest that no significant differences in the expression levels of these molecules exist in lymphoblasts from MWA patients and control subjects.

We previously reported that the plasma 5-HT level was significantly lower in the MWA group than in the control group, whereas no significant difference in the plasma 5-HTP concentration was seen between the MWA group and the controls group (Nagata et al., 2006). However, the 5-HT level in the lymphoblasts of MWA patients was higher than that in the lymphoblasts of control patients. $[^3\text{H}]$ 5-HT uptake into the lymphoblasts was similar in the MWA patients and the control subjects. Moreover, the 5-HTP concentration was similar in the lymphoblasts from the MWA patients and the control subjects. Thus, the level of 5-HT synthesis was likely similar in the lymphoblasts from the MWA patients and the control subjects. Therefore, lymphoblasts in MWA patients have altered levels of serotonin metabolism. Those results support that several previous papers also have reported platelet dysfunction in patients with migraines (Rishpon et al., 1976; Malmgran et al., 1980; Gawel and Rose, 1982; Oxman et al., 1982).

However, platelet cell lines cannot be established. The lack of a model system is one reason why the pathophysiology of migraine has yet to be clarified. This study suggests that lymphoblasts could be used as a model system for studying the pathophysiology of migraine. That is, we can investigate the mechanisms of the migraine pathophysiology not only in brain, but also in peripheral blood. Moreover, lymphoblast cell lines could be used to customize treatments, since lymphoblasts carry an individual's genetic information. A notable advantage of our system is that it enables us to perform repeated genetic and biochemical analyses using only a single blood sample. Moreover, this method could be applied to a variety of diseases other than migraine.

We emphasize that this lymphoblast model for studying migraine could be very useful for the elucidation of the migraine pathophysiology.

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Research Report

Distribution and origin of TRPV1 receptor-containing nerve fibers in the dura mater of rat

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ABSTRACT

We examined the distribution and origin of the nerve fibers innervating the dura mater of the rat that show immunoreactivity for the TRPV1 receptor (TRPV1-IR). Nearly 70% of the nerve fibers showing TRPV1-IR in the dura mater also exhibited CGRP-IR. Using a combination of immunohistochemistry and a retrograde tracer technique, we detected tracer accumulation in 0.6% of the neurons in the trigeminal ganglion and a few neurons in the dorsal root ganglion; half of the neurons in the trigeminal ganglion were small- and medium-sized ($\leq 1000 \mu\text{m}^2$). Among the tracer-accumulated neurons in the trigeminal ganglion, approximately 25% exhibited TRPV1-IR. Furthermore, nearly 80% of the tracer-accumulated small- and medium-sized neurons in the trigeminal ganglion that exhibited TRPV1-IR also exhibited CGRP-IR. Our findings indicate that the TRPV1 receptor in the dura mater and sensory ganglia may contribute to the pathophysiology of migraine, providing an important clue for the development of therapeutic strategies for migraine.

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1. Introduction

Headache can occur as a result of the activation of pain-sensitive cranial structures, such as cranial and cervical muscles, ligaments, vasculature and the dura mater. Among these, the dura mater has been recognized as one of the most important regions because of its ability to facilitate the conduction of nociceptive sensation and of its being a major site of neurogenic inflammation, a phenomenon putatively related to the development of migrainous headache (Moskowitz, 1984).

The dura mater is innervated by sensory nerve fibers containing neuropeptides like calcitonin gene-related peptide (CGRP)

or substance P; these nerve fibers originate mainly from the trigeminal ganglion (TG) (Andres et al., 1987; Messlinger et al., 1993; Reuss et al., 1992; Uddman et al., 1989). Immunohistochemical and molecular biological observations have revealed that, in addition to such neuropeptides, the transient receptor potential vanilloid subfamily member 1 (TRPV1) receptor is also expressed in the sensory ganglia (Helliwell et al., 1998). Moreover, the TRPV1 receptor is also reportedly expressed in the nerve fibers of peripheral tissues, such as the paw skin, the cornea and the bladder in several species (Hou et al., 2002; Ichikawa and Sugimoto, 2001; Murata and Masuko, 2006; Szallasi and Blumberg, 1999).

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The TRPV1 receptor is a capsaicin, proton- and heat-sensitive cation-selective channel and has been shown to have a role in conducting nociceptive signals to the central nervous system (Szallasi et al., 2006). Double labeling immunohistochemistry has demonstrated that several neurons in the sensory ganglia showing immunoreactivity for the TRPV1 receptor (TRPV1-IR) also exhibited CGRP-IR (Hou et al., 2002; Ichikawa and Sugimoto, 2001; Murata and Masuko, 2006). Furthermore, the results of a physiological experiment revealed that the

TRPV1 receptor might regulate vascular responses via controlling the release of CGRP (Jansen et al., 1990). The activation of the TRPV1 receptors has been reported to cause vasodilatation of the dural blood vessels by promoting the release of CGRP from the sensory nerve fibers (Akerman et al., 2003, 2004; Dux et al., 2003). Although the existence of the TRPV1 receptor in the dura mater has been recently reported, its precise origin remains obscure (Koizumi et al., 2005). In this study, we investigated the distribution of immunoreactivity for the TRPV1

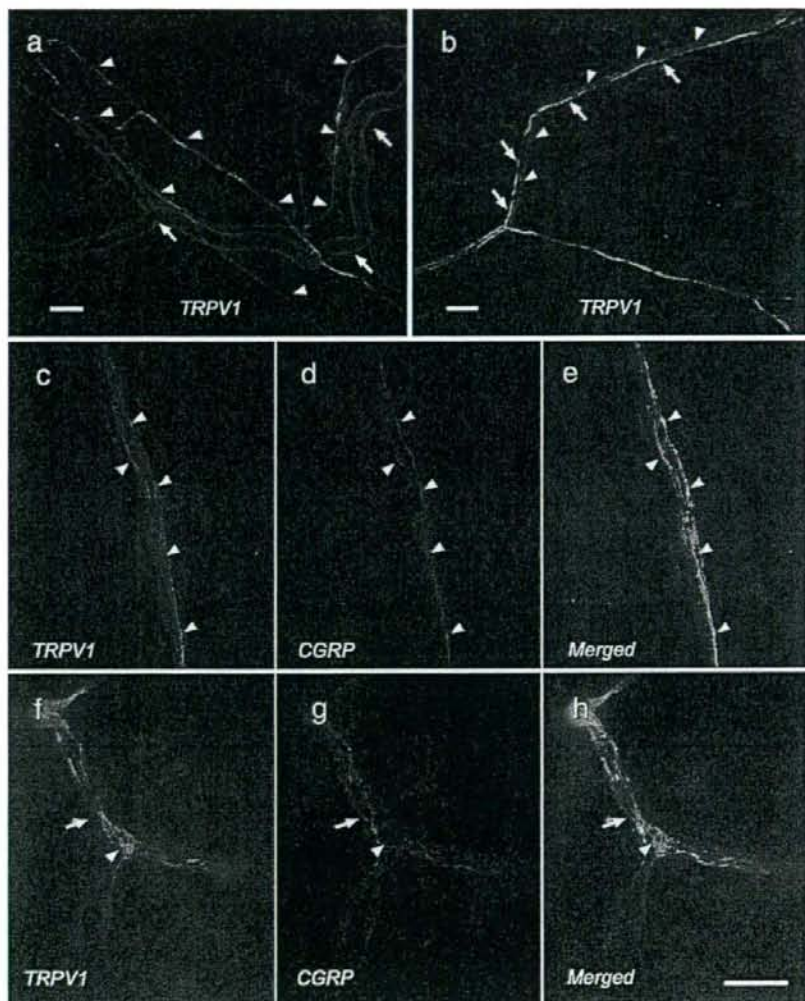


Fig. 1 - Immunohistochemical examination of the dura mater in whole-mount preparations. (a) Nerve fibers with varicosities expressing TRPV1-IR (arrowheads) were observed in the vicinity of the middle meningeal artery (arrows). Scale bar, 100 μ m. (b) Thick (arrows) and fine nerve fibers (arrowheads) showing TRPV1-IR were also found away from the blood vessels in the nerve bundle. Scale bar, 100 μ m. (c, d) Double labeling for TRPV1 (c; green) and CGRP (d; red) immunoreactivity. Nerve fibers with TRPV1-IR also exhibiting CGRP-IR (arrowheads). These nerve fibers are shown as yellow in the merged image (e) of panels c and d. (f, g) Double labeling for TRPV1 (f; green) and CGRP (g; red) immunoreactivity. Spiral nerve fibers showing TRPV1-IR did not contain CGRP-IR (arrowheads). Conversely, arrows indicate CGRP-IR nerve fibers without TRPV1-IR. (h) Merged image of panels f and g. Scale bar, 50 μ m for panels c-h. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

receptor in the dura mater and sensory ganglia. In addition, we used immunofluorescence histochemistry and a neural retrograde tracer technique to identify the origins of the TRPV1-IR-positive neurons that innervate the dura mater. We believe that our results provide clues to elucidate the role of the TRPV1 receptor in the pathogenesis of headache.

2. Results

2.1. Immunohistochemistry of the dura mater

The nerve fibers showing TRPV1-IR were mainly observed around the middle meningeal artery (Fig. 1a). Fibers exhibiting TRPV1-IR were also found away from the blood vessels (Fig. 1b). TRPV1-IR was mostly detected in fine fibrous nerve fibers with varicosity-like structures, although a few thick nerve fibers also exhibited TRPV1-IR (Fig. 1b). Double labeling immunohistochemistry with a combination of antibodies to CGRP revealed that approximately 70% of the TRPV1-IR nerve fibers showed co-localization of TRPV1-IR and CGRP-IR (Figs. 1c, d, e; range, 68–76%, $n=62$ fibers from three animals). However, we also found TRPV1-IR nerve fibers not showing CGRP-IR and nerve fibers expressing only CGRP-IR (Figs. 1f, g, h).

2.2. Subpopulations of the neurons showing TRPV1-IR in the TG and DRG

Neurons showing TRPV1-IR were observed in the TG (Figs. 2a, b, c) and dorsal root ganglia (DRG; C₅; Figs. 2d, e, f), but not in the sphenopalatine (SPG), otic (OTG), or superior cervical ganglia (SCG).

We counted a total of 1850 neurons in the TG and 579 neurons in the DRG of four ganglia (one ganglion per animal). In both the ganglia, about 80% of all the neurons were small- and medium-sized neurons (range, 79–93% in the TG, 77–85% in the DRG). Both in the TG and DRG, an analysis of the residuals showed a significantly higher proportion of small- and medium-sized neurons (TG, $Z=10.9$, $P<0.001$; DRG, $Z=4.8$, $P<0.001$).

In the TG, nearly 25% of the small- and medium-sized neurons (range, 19–32%) and 3% of the large-sized neurons (range, 1–5%) showed TRPV1-IR. After quantitative analyses of double labeling immunohistochemistry with antibodies to CGRP, we found that 40% of the neurons exhibiting TRPV1-IR also exhibited CGRP-IR (range, 27–62% of the small- and medium-sized neurons, and 50–80% of the large-sized neurons) (Figs. 3a, b).

In the DRG, about 30% of the small- and medium-sized neurons (range, 20–40%) and only a few large-sized neurons were positive for TRPV1-IR. Approximately one-third of the small- and medium-sized neurons showing TRPV1-IR also exhibited CGRP-IR. A statistical analysis showed no preferential association of the proportion of TRPV1-IR neurons with the cell size (TG, $Z=0.3$, $P>0.05$; DRG, $Z=2.2$, $P>0.05$).

2.3. Retrograde tracer study

Tracer-accumulated neurons were observed in the TG, DRG, SPG and SCG on the side ipsilateral to the tracer application. While neurons showing TRPV1-IR were observed in both the TG and DRG, only a few of these neurons in the DRG exhibited tracer accumulation; therefore, we performed a quantitative analysis of the neural populations only in the TG.

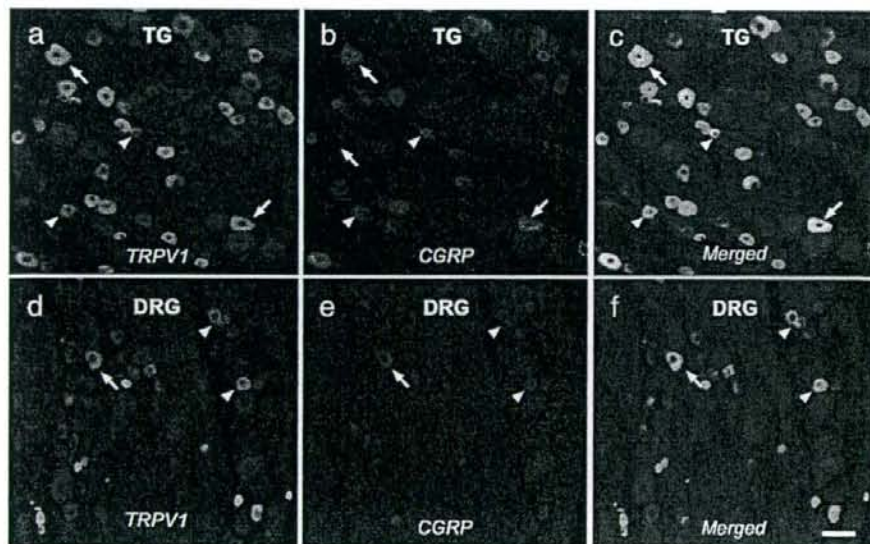


Fig. 2 – Double labeling for TRPV1 (green; a, d) and CGRP (red; b, e) immunoreactivity in the TG (a, b, c) and DRG (d, e, f). Arrowheads indicate small neurons showing both TRPV1-IR and CGRP-IR. Arrows show large neurons showing both TRPV1-IR and CGRP-IR. (c) Merged image of panels a and b. (f) Merged image of panels d and e. Neurons showing that both TRPV1-IR and CGRP-IR are shown in yellow in the merged image. Scale bar, 50 μm for all. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

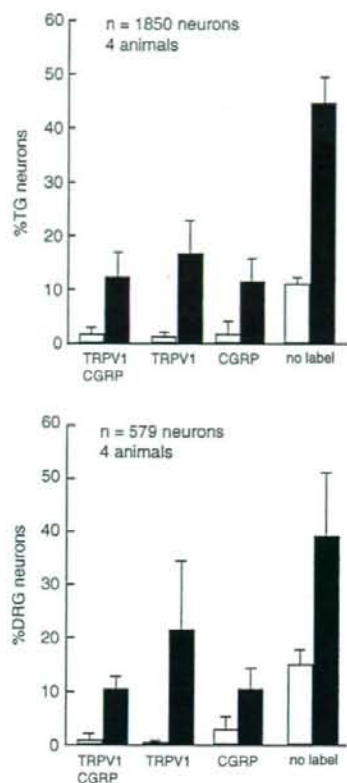


Fig. 3 - Histogram summarizing the quantitative data from double labeling for TRPV1 and CGRP immunoreactivity in the TG (a) and DRG (b). The open bars show large-sized neurons (>1000 μm^2), and the solid bars indicate small- and medium-sized neurons ($\leq 1000 \mu\text{m}^2$). The bars show the mean \pm SD.

Tracer accumulation was detected in 0.6% of the neurons in the TG ($n=30,845$ neurons from two animals). Among these neurons showing tracer accumulation, 57% were small- and medium-sized (range, 27-64%) and 43% were large-sized (Figs. 4a-d, 5). As shown in Fig. 5, among the small- and medium-sized tracer-accumulated neurons, 20% showed both TRPV1 and CGRP-IR and 4% exhibited only TRPV1-IR. On the other hand, 1% of large neurons showed both TRPV1 and CGRP-IR and 0.3% exhibited only TRPV1-IR.

Furthermore, about 80% of the tracer-accumulated small- and medium-sized neurons exhibiting TRPV1-IR also exhibited CGRP-IR. Meanwhile, only a few large-sized tracer-accumulated neurons exhibited TRPV1-IR in these cells, precluding an adequate assessment of co-localization. An analysis of the residuals showed the relative proportion of neurons exhibiting TRPV1-IR and CGRP-IR among the tracer-accumulated neurons with TRPV1-IR to be significantly higher than that exhibiting TRPV1-IR and CGRP-IR among the total TG neurons with TRPV1-IR ($Z=2.8$, $P<0.005$).

3. Discussion

Our immunohistochemical study revealed the existence of nerve fibers in the dura mater that were positive for TRPV1-IR. Our study also indicated that these TRPV1-IR-positive nerve fibers originated from the TG and DRG. Nearly half of the tracer-accumulated neurons in the TG exhibited TRPV1-IR positivity; as discussed below, most of these tracer-accumulated neurons were small- and medium-sized neurons.

3.1. Technical considerations

In our study, we utilized True Blue as a retrograde tracer to explore the origin of the neurons innervating the dura mater. Although the possibility of leakage to the surrounding tissues is a concern, this technique has previously been shown to be an effective method for investigating the innervation of small vessels (Suzuki et al., 1988). In addition, we built a small bank

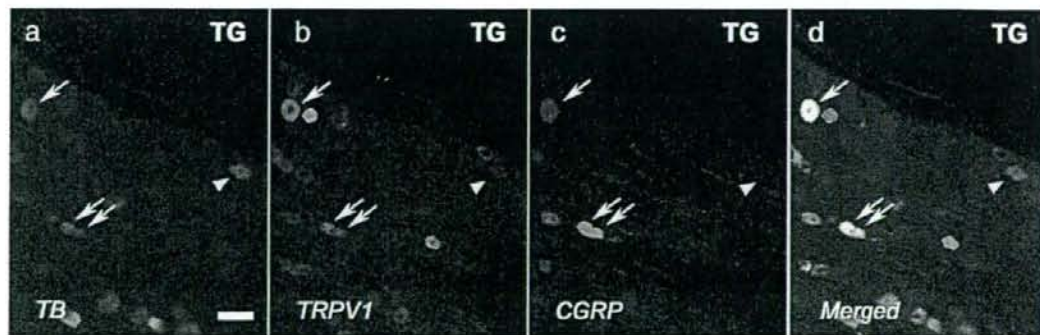


Fig. 4 - Sections of TG (a, b, c, d) containing neurons retrogradely labeled with True Blue (a), TRPV1-IR (b) and CGRP-IR (c). Application on the dura mater. Arrows indicate retrogradely labeled small- and medium-sized neurons expressing TRPV1-IR and CGRP-IR. Arrowheads indicate tracer accumulation in small neurons showing neither TRPV1-IR nor CGRP-IR. (d) Merged image of panels a, b and c. In the merged image, retrogradely labeled neurons showing both TRPV1-IR and CGRP-IR are shown in white. Scale bar, 50 μm for all.

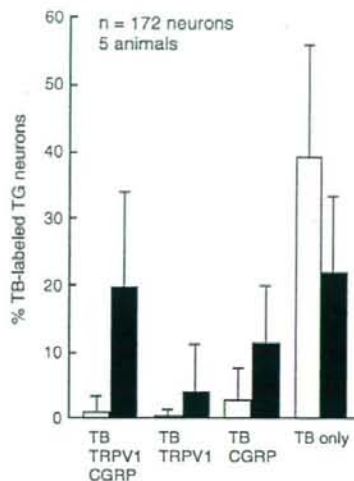


Fig. 5 – Histogram summarizing the neurochemical profiles of retrogradely labeled neurons with True Blue (TB) in relation to TRPV1-IR and CGRP-IR and their association with the neuronal size in the TG. Open bars show large-sized neurons ($>1000 \mu\text{m}^2$), and the solid bars indicate small- and medium-sized neurons ($\leq 1000 \mu\text{m}^2$). The bars show the mean \pm SD.

around the tracer application site using carboxylate cement to prevent the oozing of the tracer in this experiment. These procedures prevented the tracer from leaking to other peripheral sites.

In the TG, tracer-accumulated neurons were observed in 0.6% of the total neurons. To calculate this result, we counted the total number of TG neurons, examining 30,845 neurons from two animals. Other studies have reported that about 55,000 cells are present in the TG at the time of birth (Henderson et al., 1994) and that 33,000 axons were present in the adult infraorbital nerve (Jacquin et al., 1984). These previous findings suggest that the number of TG neurons may have actually been higher than what we counted. Although the ratio of tracer-accumulated neurons may actually be lower, the percentage of tracer-accumulated neurons seems to be appropriately labeled from a single dural site. Accordingly, we believe that our tracer experiment was properly conducted.

3.2. Populations of neurons exhibiting TRPV1-IR and the nature of nerve fibers exhibiting TRPV1-IR in the dura mater

The percentage of TRPV1 receptor containing neurons and the proportions of large and small neurons exhibiting TRPV1-IR, as well as the co-localization pattern of neurons exhibiting CGRP-IR, that were observed in the present study were reasonably consistent with the data reported by other studies (Ichikawa and Sugimoto, 2001; Murata and Masuko, 2006).

The tracer accumulation pattern in our study was similar to that in a previous study (Uddman et al., 1989). In terms of the DRG, most of the tracer studies exploring the origins of the nerves innervating the dura mater indicated that tracer-

labeled neurons were observed mainly in the C₂ of the DRG (Uddman et al., 1989; Liu et al., 2004). In addition, neurons in the C₂ dorsal horn have been reported to receive sensory input from supratentorial dura mater and the superior sagittal sinus (Goadsby and Zagami, 1991; Bartsch and Goadsby, 2002); therefore, we observed only the C₂ of the DRG in this study.

Our retrograde tracer study revealed that the majority of the TRPV1-IR neurons innervating the dura mater were small- and medium-sized neurons. Immunohistochemical data from the sensory ganglia were also consistent with the findings in the dura mater, and TRPV1-IR was mainly observed in fine fibers with varicosity-like structures. Although the neurons in the DRG that have small diameters are unmyelinated or have thinly myelinated axons are concerned with thermal sensation and nociception (Gardner et al., 2000), the nerve fibers innervating the dura mater are known to conduct only pain-invoked signals (Ray and Wolff, 1940). Therefore, our observations imply that the nerve fibers exhibiting TRPV1-IR in the dura mater may mainly conduct nociceptive signals.

Compared with the DRG, clear functional correlations based on neuronal size are not available in the TG, especially for the neurons innervating the dura mater. However, Levy and Strassman (2002) recently reported the existence of dural nerve fibers that responded to mechanical stimulation and originated in the TG. Although no established functional distinction based on the size of the neurons presently exists, our data on the relationship between neuronal size and TRPV1-IR may provide important insight as to the neuronal features in the TG innervating the dura mater.

3.3. Functional implications of nerve fibers exhibiting TRPV1-IR

Several studies have reported that the activation of TRPV1 receptors causes vasodilatation. Furthermore, based on the finding that this vasodilatory response is attenuated by the administration of a CGRP receptor antagonist, it seems likely that the activation of the TRPV1 receptor promotes the release of CGRP from sensory nerve endings that are co-localized with the TRPV1 receptors; this, in turn, induces vasodilation (Akerman et al., 2003, 2004; Dux et al., 2003; Jansen et al., 1990). Based on these previous findings, we expected to observe the co-localization of TRPV1-IR and CGRP-IR nerve fibers in the dura mater. The results of our experiments revealed that, although all of the nerve fibers exhibiting TRPV1-IR in the dura mater did not show CGRP-IR, many of the nerve fibers exhibited both TRPV1-IR and CGRP-IR. These dura mater observations are consistent with our data showing a significant proportion of neurons with tracer accumulation in our retrograde tracer study to exhibit both high TRPV1 and high CGRP-IR as compared to the total ganglion cell population. In addition, our data provide a strong anatomical basis for the contention that vasodilation in the dura mater can be caused by the TRPV1 receptor-mediated release of CGRP (Akerman et al., 2003). We also observed that TRPV1-IR nerve fibers did not exhibit CGRP-IR in the dura mater. When TRPV1 receptors in the dura mater are activated by inflammation, these nerve fibers presumably transmit

nociception to the central nervous system (Szallasi and Blumberg, 1999; Szallasi et al., 2006). Furthermore, it has been suggested that the excitation of TRPV1 receptors may directly activate mast cells (Biro et al., 1998). Mast cells are known to exist in the dura mater and to play a crucial role in neurogenic inflammation (Keller et al., 1991; Moskowitz, 1984). From these findings, it may be deduced that TRPV1 receptors in the dura mater may be closely involved in the pathogenesis of migraine. Moreover, clinical trials examining the efficacy of a small-molecule TRPV1 receptor antagonist (SB-705498) in patients with migraine are under way (Szallasi et al., 2006). We believe that our data provide important information for interpreting the outcome of these trials.

4. Experimental procedures

4.1. Animals

Experiments were performed on male Sprague–Dawley rats ($n=9$; body weight, 200–250 g), four of which were used for immunohistochemistry and the remaining five of which were used for the combined retrograde tracer and immunohistochemical experiments. These experimental procedures were approved by the Animal Welfare Committee of Keio University (No. 040073). Furthermore, all the procedures were undertaken with the utmost caution to minimize the suffering of the animals.

4.2. Tissue preparation

The animals were deeply anesthetized using an intraperitoneal injection of pentobarbital (30 mg/kg body weight). They were then transcardially perfused with 200 mL of 0.01 M phosphate-buffered saline (PBS, pH 7.2) followed by a mixture of 2% formaldehyde and 0.2% picric acid in 0.1 M phosphate buffer, pH 7.0. Immediately after the perfusion-fixation, the dura mater and the cranial ganglia innervating the dura mater, i.e., the TG, DRG (C₂), SPG, OTG and SCG, were dissected out and immersed overnight in phosphate buffer containing 20% sucrose at 4 °C. The dura mater was processed as a whole-mount preparation, while the other specimens were processed into sections of 10 μ m thickness using a cryostat (Reichert–Jung Cryocut 1800; Leica Instruments).

4.3. Immunohistochemical examination of the dura mater and the ganglia

Cryostat sections were preincubated with 10% normal donkey serum for 30 min. The sections and whole mounts were incubated with primary antibodies for 48–72 h at room temperature. The primary antibodies were then washed off with 0.01 M PBS, and the tissue specimens were incubated with species-specific secondary antibodies for 2 h at room temperature. The specimens were mounted in buffered glycerol (pH 8.6).

The slides were labeled with anti-TRPV1 receptor antibody corresponding to residues 824–838 (EDAEVFKDSMVPGEK) of rat TRPV1 (raised in rabbits; code, KM 018; TransGenic, Kumamoto, Japan; 1: 50 for whole mounts, 1: 200 for sections). To assess the specificity of the anti-TRPV1 receptor antibody, we performed the following adsorption test. After preincubation of the primary antiserum with 10 μ g/mL of the TRPV1 carboxy-terminal peptide (code, ACC-030; Alomone Labs, Jerusalem, Israel), immunoreactivity for the TRPV1 receptor was abolished, as shown in Fig. 6.

The slides of the dura mater were double labeled with anti-TRPV1 receptor antibody and anti-CGRP antibody (raised in guinea pigs; code, B-GP 640-1; Euro-Diagnostica; 1: 200 for whole mounts, 1: 1000 for sections). Immunoreactivities with the primary antibodies were visualized with species-specific secondary antibodies raised in donkeys and conjugated to Cy 3 or fluorescein isothiocyanate (FITC); all of the antibodies were obtained from Jackson ImmunoResearch Laboratories (West Grove, PA).

Every third section was used for immunohistochemistry, and we analyzed twelve sections per ganglion in the TG and four sections per ganglion in the DRG.

To analyze the proportion of the nerve fibers showing both TRPV1 and CGRP-IR to the nerve fibers with TRPV1-IR, the dura nerve fibers in the vicinity of the proximal part of the middle meningeal artery exhibiting TRPV1-IR were counted on whole-mount preparations made from three animals.

4.4. Retrograde tracer study

The animals were anesthetized by intraperitoneal injection of ketamine hydrochloride (100 mg/kg) and xylazine hydrochloride (15 mg/kg). The heads of the animals were fixed in a stereotaxic frame, and a small burr hole was made in the left parietal

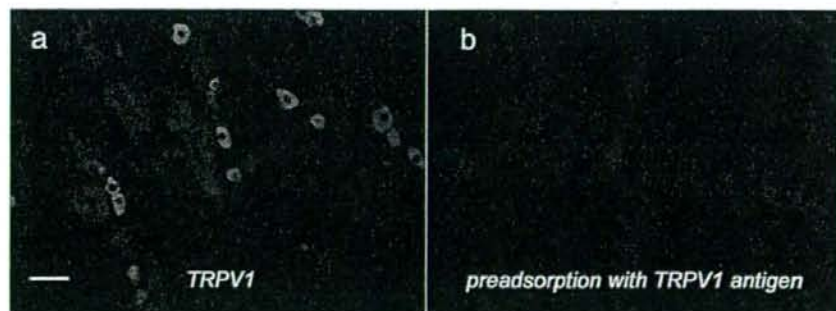


Fig. 6 – Sections of TG showing TRPV1-IR (a). After the adsorption test, no neurons with TRPV1-IR were observed (b). Scale bar, 50 μ m for all.

bone around the middle meningeal artery, while ensuring that the dura mater was kept intact. A small bank was built around the burr hole using carboxylate cement (Durelon®; ESPE, Norristown, PA) to prevent the spreading of the tracer. A retrograde axonal tracer, True Blue (trans-1, 2-bis (5-amidino-2-benzofuranyl) ethylene-2 HCl; Molecular Probes, Eugene, OR) crystal (0.5 mg), was applied to the dura mater. After the application of the tracer, the application site was covered with carboxylate cement.

Two weeks after the tracer application, the animals were reanesthetized using an intraperitoneal injection of pentobarbital and perfused as described above. The following structures were dissected out from both sides: TG, DRG (C₂), SPG, OTG and SCG. All specimens were processed for the preparation of cryostat sections, as described above. Sections were double labeled using a rabbit anti-TRPV1 receptor antibody and guinea pig anti-CGRP antibody. Secondary antibodies conjugated with FITC and Cy 3 were used to distinguish the immunolabeling from the labeling with True Blue.

To calculate the ratio of tracer-accumulated neurons, we counted the total number of TG neurons and the number of tracer-accumulated neurons in sections cut consecutively from the top to the bottom; cells were counted when their nuclei were visible. Two animals were used for the analysis.

To perform a quantitative analysis of the tracer experiment, we counted the number of tracer-accumulated neurons in every third section that contained nuclei. Four animals were used for the analysis.

4.5. Microscopy and data acquisition

The immunolabeled specimens were examined under an Olympus BX 50 microscope (Olympus, Tokyo, Japan) fitted with highly discriminating filters. Images from the BX 50 microscope were captured via a Sony CCD video camera (model XCD-SX 900; Sony, Tokyo, Japan) connected to an EPSON computer. After the images were stored, the area of the cell bodies that contained nuclei was measured using image analysis software (CHORI Imaging Corporation, Yokohama, Japan). According to a system reported in previous studies (Ichikawa and Sugimoto, 2001), we categorized the neurons into small- and medium-sized (area $\leq 1000 \mu\text{m}^2$) and large (area $> 1000 \mu\text{m}^2$) neurons. The frequency data were analyzed using log-linear analyses (SPSS for Windows, version 14; SPSS Inc., Chicago, IL). Standardized residuals (z values) outside the range of -2.58 to $+2.58$ were considered to represent significant deviations from the null hypothesis.

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治療薬

シリーズ(24)

片頭痛治療薬②

片頭痛の最新のトピックス

—薬物治療の問題点と今後望まれる薬剤

清水 利彦, 鈴木 則宏

要約: セロトニン 5-HT_{1B/1D} 受容体アゴニストであるトリプタン系薬剤は片頭痛急性期発作に用いられ、多くの患者で著効する。本邦ではスマトリプタン、ゾルミトリプタン、エレトリプタンおよびザトリプタンの4種類が使用可能なトリプタン系薬剤である。あるトリプタン系薬剤が無効な場合、別のトリプタン系薬剤が有効なこともあり、そのような症例では他のトリプタン系薬剤への変更を試みる価値がある。しかし片頭痛発作に対しすべてのトリプタン系薬剤が無効な症例も知られている。このため片頭痛の病態生理にもとづき新しい片頭痛治療薬として5-HT_{1F}受容体作動薬、5-HT_{1D}受容体作動薬、CGRP受容体作動薬、アデノシン A₁受容体作動薬、TRPV1受容体作動薬およびGABA受容体作動薬などが候補としてあげられ開発や臨床試験が現在行なわれている。

はじめに

片頭痛急性期治療に用いられるセロトニン 5-HT_{1B/1D} 受容体アゴニストであるトリプタン系薬剤の登場は頭痛診療を画期的に進歩させた。トリプタン系薬剤は指摘されているような心血管に対する副作用も明らかでなく、安全で多くの片頭痛患者に奏効している。このようなトリプタン系薬剤であるが残念ながら一部には有効性を示さない症例も認められておりトリプタン系薬剤以外の片頭痛治療薬も必要とされている。本稿では片頭痛の病態およびトリプタン系薬剤について概説するとともに今後の開発が望まれる薬剤について解説する。

1. 片頭痛の病態

片頭痛の病態は、①頭蓋血管の反応性の異常による

とする血管説 (vascular theory)、②拡張性抑制 (spreading depression) のような大脳皮質の神経細胞の過剰興奮がまず始めに生ずるとする神経説、③三叉神経と脳血管周囲や硬膜血管周囲の神経原性炎症の関与を考える三叉神経血管説の3つの機序により説明されていた。

これらの中で以前は、片頭痛の病態をもっとよく反映するものとして血管説が考えられていた。しかし最近では片頭痛は中枢神経系の活性化により生じる二次的な血管反応性の変化をとまなう脳の疾患であるという見解が受け入れられるようになってきている。

1) 血管説 (vascular theory)

片頭痛の病態が血管から始まるとする説で Wolff らにより唱えられた(1)。病態の中心を頭蓋内外の血管とし、前兆と拍動性の頭痛については生体内血管作動性物質に対する脳血管反応性の変化により生じるものと説明している。

2) 神経説 (neural theory または neuronal theory)

神経説は spreading depression のような神経細胞の活動性変化がまず片頭痛の1次的な要因として生ずるとする考え方である。Spreading depression は1944年 Leão によりウサギの大脳を用いた実験で報告された現象である(2)。脳局所における神経細胞やグリア細胞に生ずる脱分極とその後15分～30分の間、電気的活動が抑制された状態となる現象であり約2～3 mm/分の速さで周囲に伝播する。さらに spreading depression に伴い脳血流は一過性に上昇しその後数時間の血流低下を示すことが明らかにされている。

一方、片頭痛患者の脳血流動態を測定すると spreading oligemia と呼ばれる後頭葉に始まり、約2～3 mm/分の速さで前方に拡大していく血流の低下

キーワード: 片頭痛, トリプタン系薬剤, 新しい片頭痛治療薬

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が観察され、spreading oligemia と spreading depression との類似性が指摘された(3)。脳表における spreading oligemia の伝播が、血管の支配領域とは無関係であることから、spreading oligemia は大脳皮質神経細胞興奮性の変化を反映しているものと考えられた。このことは、片頭痛発作が単に反応性の血管拡張作用によるものではないことを示しており、片頭痛発生の機序として神経説が注目されるようになった。

3) 三叉神経血管説 (trigeminovascular theory)

硬膜血管や脳血管には三叉神経節由来の無髄神経線維が分布し頭蓋内の痛覚を中枢へ伝える働きをしている。また三叉神経節を実験的に刺激すると、これらの神経を介して逆行性に刺激が伝わり硬膜の血管拡張および神経原性炎症が生じることが知られている(4)。三叉神経血管説は、血管反応性の異常に加え、このような三叉神経を介した神経原性炎症が関与し頭痛を起しているとするもので、次に述べるような機序が考えられている。

何らかの刺激が硬膜の血管周囲に存在する三叉神経の軸索に作用すると、神経終末から substance P, calcitonin gene-related peptide (CGRP), neurokinin A

などの神経伝達物質でありかつ血管作動性物質の神経ペプチドが放出され硬膜周辺に存在する肥満細胞の脱顆粒や血管透過性の亢進、血漿タンパクの流出、血管拡張などが生じ神経原性炎症が惹起される。このような硬膜でおきた神経原性炎症は三叉神経を介し順行性伝導により中枢に疼痛として伝達され頭痛が生じるのではないかと三叉神経血管説では説明している(図1)。

なお三叉神経終末からの神経ペプチドの放出は三叉神経終末に存在するセロトニン受容体のうち5-HT_{1D}受容体を介し一部抑制を受ける。

2. トリプタン系薬剤

1) トリプタン系薬剤と片頭痛

トリプタン系薬剤はセロトニン類似の脳血管収縮作用をもつ薬剤として開発された。セロトニンが片頭痛の病態に関与している可能性は1961年に Sicuteri が片頭痛患者の尿中セロトニンの代謝産物である5-HIAAの高値を指摘したことに始まっている(5)。その後片頭痛の治療薬としてセロトニンを使用することが考えられたが、セロトニンの投与は全身血管に作用しさまざまな副作用を呈するため脳血管に選択的に

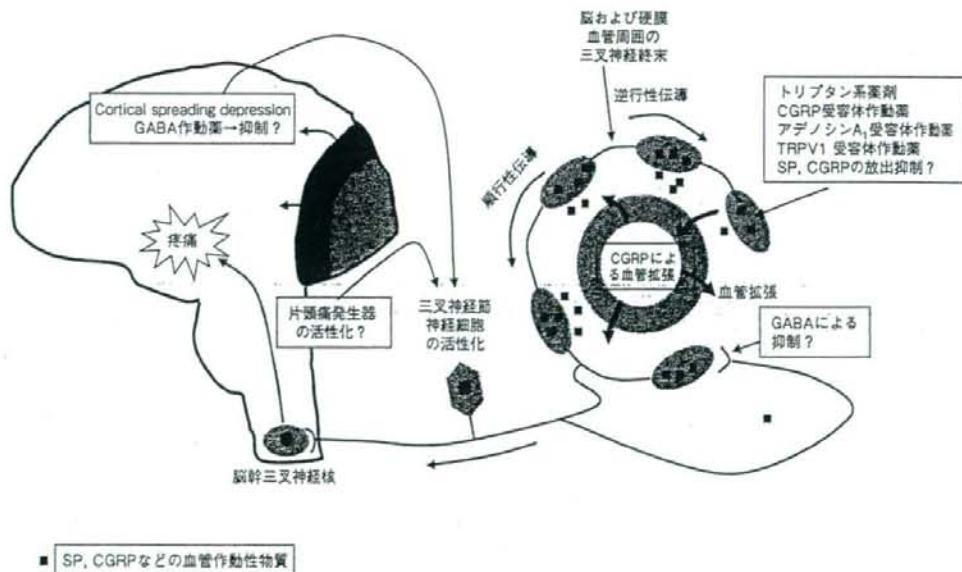


図1 三叉神経血管説を中心とした片頭痛の病態生理と片頭痛に対する有効性が示唆される薬剤の作用部位

三叉神経血管説では硬膜血管周囲の三叉神経の軸索に何らかの刺激(cortical spreading depressionを介するものや片頭痛発生器からの信号などの可能性があるが現在詳細は不明)が加わり、substance PやCGRPなどが遊離され、血管拡張、血漿蛋白の漏出および肥満細胞からのヒスタミンの遊離などにより神経原性炎症が生じる。三叉神経終末の刺激が順行性に伝えられると脳幹三叉神経核から大脳に至り痛みとして自覚される。

トリプタン系薬剤は、頭蓋内血管平滑筋に存在する5-HT_{1B}受容体を介し血管収縮作用を示し、三叉神経終末に存在するセロトニン受容体の中の5-HT_{1D}受容体により神経ペプチド放出を抑制し神経原性炎症を抑制し頭痛発作改善に効果を示すと考えられている。CGRP受容体作動薬、アデノシンA₂受容体作動薬、TRPV1受容体作動薬はともに三叉神経血管系に作用し片頭痛発作を鎮静させると考えられている。GABA受容体作動薬は大脳神経細胞に作用しspreading depressionなどを抑制することで片頭痛予防効果を示すほか三叉神経血管系に作用する可能性も考えられている。(文献4を一部改変)

作用するセロトニン作動薬の研究開発が検討された。さらにセロトニンには5HT₁から5HT₇までの7種類の受容体が存在し、脳血管の収縮は主に5HT₁受容体、中でもそのサブタイプ5HT_{1B/1D}受容体を介することが明らかにされ、アゴニストとしてスマトリプタンが開発された。以後スマトリプタンを改良した数種類のトリプタン系薬剤が出現し、最初に開発されたスマトリプタンに対し、第二世代のトリプタン系薬剤と呼ばれている。これらはゾルミトリプタン、エレトリプタン、リザトリプタンなどである。第二世代のトリプタン系薬剤はスマトリプタンに対し生物学的活性度が高く、血中濃度が治療域に達する時間が短く血中半減期が長い。さらに脂溶性が高いため、血液脳関門を通過して中枢神経内にも作用するといわれている。

トリプタン系薬剤の作用機序については不明な点が多いが、①頭蓋内血管平滑筋に存在するセロトニン5-HT_{1B}受容体を介する脳血管収縮、②脳血管を支配する三叉神経終末に存在する5-HT_{1D}受容体に作用し血管拡張性神経ペプチドの放出抑制、③脳幹の三叉神経核の5HT_{1B/1D/1F}受容体を介する疼痛伝導の抑制などが考えられている。

2) トリプタン系薬剤の使用法と問題点

今日、中等度以上の片頭痛発作では、トリプタン系薬剤の経口投与が第1選択となっている。本邦で使用可能なトリプタン系薬剤はスマトリプタン、ゾルミトリプタン、エレトリプタンおよびリザトリプタンの4種類である(6)。トリプタン系薬剤を使用する場合はコントロールされていない高血圧、重度の肝機能障害、心筋梗塞の既往、虚血性心疾患、脳血管障害の既往などの禁忌事項に加え併用禁止薬や併用注意薬もあり注意を要する(6)。

どのトリプタン系薬剤を第1選択とするかについて十分な根拠は現在得られていない。実際の臨床現場では、処方可能な4種類のトリプタン系薬剤の中から1種類をランダムに抽出し最少量から投与を開始し、片頭痛に対する効果の経過観察が行われている。

ある1種類のトリプタン系薬剤が無効な場合、別のトリプタン系薬剤が有効なことがあるため、初回投与で効果が無い場合は他のトリプタン系薬剤への変更を試みる価値があると思われる。またトリプタン系薬剤を連用していると効果が減弱するケースがあるが、他のトリプタン系薬剤に変更すると改善を認めることがある。なお、ノンレスポンスと称されるトリプタン系薬剤に反応しないケースもあり、このような症例では治療に難渋する。

3. 今後開発が期待される薬剤

トリプタン系薬剤はセロトニンと類似の性質である脳血管収縮作用におもな焦点をおき開発された薬剤である。しかし、最近では片頭痛はspreading depressionのような中枢神経系の活性化のあとに起こる二次的な血管反応性の変化をとともなう疾患ではないかとする考えが広く受け入れられるようになり、神経細胞の興奮性を調整する薬剤が片頭痛治療薬の候補としてあげられている(7)。また三叉神経血管説に見られるような三叉神経系の活動性の上昇とそれに伴う脳血流や脳硬膜血流の増加は片頭痛の病態を反映していると考えられており、三叉神経の活動性を抑制する薬剤も急性期の片頭痛治療薬のターゲットとなってきた。

1) 5-HT受容体作動薬

トリプタン系薬剤は脳血管での存在が知られている5-HT_{1B}受容体に加え、三叉神経に認められるが脳血管では存在が報告されていない5-HT_{1D}および5-HT_{1F}受容体に対するアゴニストの作用を有していることから片頭痛治療のターゲットとしてそれぞれの受容体に個々に親和性をもつアゴニストの開発が行われている。5-HT_{1D}および5-HT_{1F}受容体とも三叉神経系に対する抑制作用を示すが機能的な違いについては現在のところ明らかにされていない(8)。

① 5-HT_{1F}受容体作動薬

5-HT_{1F}受容体は脳幹三叉神経核および三叉神経節に存在し、この受容体の刺激はラットおよびネコにおいて神経細胞に対する抑制的な作用を示すことが知られている。LY-334370は強力な5-HT_{1F}受容体アゴニストとして開発された薬剤である。動物実験では脳硬膜の神経原性炎症によるplasma proteinの滲出を抑制するが血管反応性に対しては作用を示さないとされている(9)。片頭痛発作急性期にLY-334370は有効であったが、中枢神経系に対する副作用が認められ、さらに動物実験での毒性の問題で開発が中止された(7)。しかし、最近、脳幹三叉神経核のシナプス前終末にも5-HT_{1F}受容体の存在が報告されており、5-HT_{1F}受容体が片頭痛の治療薬として期待されている(10)。

② 5-HT_{1D}受容体作動薬

5-HT_{1D}受容体アゴニストは動物実験において神経原性炎症により脳硬膜に生じたplasma proteinの滲出を抑制するが5-HT_{1F}受容体アゴニストと同様に血管反応性に関しては作用も示さないことが知られている(10)。5-HT_{1D}受容体アゴニストのうちPNU-142633について片頭痛発作に対する臨床試験が行われたが有効性は認められなかった(11)。PNU-142633は、in vi-

troにおいてスマトリプタンと比べ5-HT_{1D}受容体アゴニストとしての作用は弱く脳血液関門の透過性も乏しいとされている(10)。このため5-HT_{1D}受容体アゴニストとして片頭痛発作改善作用を十分に示さなかったと考えられ、今後さらに強力なアゴニストの開発が行われている。

2) CGRP 受容体作動薬

血管拡張作用をもつ神経ペプチドであるCGRPは脳血管を支配する神経線維のなかで三叉神経節を主な起源とする感覚系の神経線維に認められている(12)。三叉神経節の刺激は血中CGRP濃度およびsubstance P濃度を上昇させるが、片頭痛および群発頭痛の発作期には血中CGRP濃度のみが上昇しsubstance Pは変化しないことが報告されている(13)。さらに片頭痛および群発頭痛の発作がトリプタン系薬剤で鎮静されると血中CGRPレベルが正常化される。またネコ上矢状静脈洞の刺激はCGRPの血中への放出を促進し、この現象がトリプタン系薬剤で抑制される(7)。血中CGRPレベルはこのように三叉神経の興奮と関連し変動するため片頭痛の治療薬としてCGRP受容体作動薬が注目されている。CGRP受容体アンタゴニストであるBIBN4096BSは脳幹三叉神経核の活動性を抑制し、急性期の片頭痛発作の治療に有効であることが知られておりトリプタン系薬剤にかわる新しい治療薬の1つとして期待されている(14)。

3) アデノシン A₁ 受容体作動薬

侵害刺激に対しアデノシンのアンタゴニストであるmethylxanthine adenosineがラットにおいて侵害刺激の閾値を低下させたことから侵害刺激にアデノシンの関与が示唆されるようになった(15)。さらにアデノシン受容体に対する選択的アゴニストやアンタゴニストを用いた研究からアデノシンの侵害刺激に対する作用はアデノシン A₁ 受容体を介していると考えられている(16)。アデノシン A₁ 受容体は三叉神経節および三叉神経のシナプス前終末に存在し神経トランスマッターの放出抑制に関与している。これは2つの選択的なアデノシン A₁ 受容体アゴニストであるGR79236およびGR190178は脳幹三叉神経核の活動性を抑制するのみならず、三叉神経血管系の活性抑制および血中へのCGRPの放出を抑制することとも一致する(16)。このようにアデノシン A₁ 受容体作動薬では三叉神経系の抑制効果を有していることから急性期の片頭痛治療薬として有効性が期待されている。

4) TRPV1 受容体作動薬

TRPV1受容体は唐辛子の辛み成分として知られている「カプサイシン」に対する感受性を示す受容体で

ある。カプサイシンはその構造にvanillyl基を有することからvanilloidと総称されているため、クローニングされた受容体ははじめvanilloid receptor subtype 1 (VR1)と命名された。その後、VR1は構造上transient receptor potential (TRP)のサブファミリーの1つであるTRPVに属することが明らかにされたため、現在ではTRPV1と呼ばれている(17)。

TRPV1受容体はCa²⁺に高い透過性をもつ非選択性陽イオンチャネルであり、感覚神経系の後根神経節や三叉神経節の小型から中型の神経細胞とC線維およびA δ 線維に特異的に発現している。さらにこれら神経細胞の投射先である脊髄後角の表層(第I, II層)や三叉神経脊髄路核にも認められている。さらに我々は最近、脳硬膜にも三叉神経節を起源とするTRPV1受容体陽性の神経線維が存在することを報告している(18)。

TRPV1受容体は侵害受容のほか神経伝達物質の放出にも関与している。カプサイシンの静脈内投与は炎症性の神経ペプチドであるsubstance P, neurokinin AおよびCGRPなどを三叉神経節から放出させ硬膜血管からの血漿滲出や血管拡張を促すことが知られている(19)。このことはTRPV1受容体が三叉神経節および脳硬膜支配神経においてCGRPと共存していることとも一致する(18)。さらにカプサイシンによる硬膜血管の拡張はTRPV1受容体のアンタゴニストであるcapsazepineで抑制されることも明らかにされている。なお最近分子量の小さいTRPV1受容体アゴニストSB-705498が開発され、カプサイシンおよび紫外線照射による皮膚炎に対する有効性が発表され、さらに片頭痛急性期の臨床試験としてphase II試験がイギリス、米国を中心におこなわれておりその結果が待たれるところである(20, 21)。

5) GABA 受容体作動薬

γ -Aminobutyric acid (GABA)は、神経細胞に対し抑制性の機能を有する神経伝達物質である。GABAは大脳皮質や小脳のPurkinje cellなどのほかに脊髄後角や三叉神経脊髄路核など多くの部位に分布している(22)。GABA類似の作用を示すバルプロ酸が片頭痛発作の予防効果を示すことは以前から知られているが、このほかトピラメートや最近本邦で使用可能となったガバペンチン(ガバペン®)も片頭痛発作に対する予防効果を有している。本邦において片頭痛に対する臨床試験が最近開始されたトピラメートは神経終末にあるGABA_A受容体に作用し神経細胞の抑制機能を増強するとともにグルタミン酸のAMPA/kainate受容体に働き興奮機能を抑制する作用も機序として考えられて

いる。ガバペンチンは神経終末の電位依存性カルシウムチャネルの $\alpha_2\delta$ サブユニットに結合し Ca^{2+} 流入を抑制しグルタミン酸の遊離抑制や GABA トランスポーターの活性化により興奮性アミノ酸抑制と抑制性アミノ酸の増強作用を示すことで効果を示すと考えられている (23)。GABA は中枢神経系のほかに三叉神経節や硬膜など末梢の侵害受容神経内での存在も報告されている (24)。三叉神経の刺激により生じる神経原性炎症が GABA_A 受容体のアンタゴニスト投与で抑制されることから脳硬膜における GABA_A 受容体の存在が示唆され片頭痛治療における GABA 受容体作動薬の新たな作用部位となる可能性も考えられている (25)。

おわりに

最後にこれまで述べてきた片頭痛発作への有効性が示唆される薬剤の作用部位と片頭痛の病態生理の関係について図 1 に記す。

片頭痛の病態を考える上での重要なポイントは、まず発作のトリガーが生じた時に発作が増強されやすくなるような特性をもともと片頭痛患者が有している可能性である。これは一部の遺伝性片頭痛患者において Ca^{2+} チャネル異常による神経細胞機能の変調が存在することや片頭痛患者において発作間欠期に交感神経系の機能低下の存在することと一致する。このような片頭痛を増強しやすいと考えられる母体に何らかの原因で脳幹に存在していると考えられている片頭痛発生器 (migraine generator) の活性化や神経説が唱える spreading depression が起こり三叉神経血管系が異常に活性化されることで血管説や三叉神経血管説が説明するような病態が起こり頭痛が生じていくと考えられている。

片頭痛発生器とは、片頭痛発作のトリガーとなる可能性が考えられている部位で脳幹の縫線核、青斑核および中脳水道周囲灰白質 (Periaqueductal Gray Matter: PAG) などが候補としてあげられている。特に、PAG は、前頭葉や視床下部から入力を受け、三叉神経脊髄路核や脊髄後角へ投射する下行性痛覚抑制系の神経回路を形成する一部であり、高解像度 MRI により片頭痛患者で PAG における鉄含有量の増加が認められたことと合わせ注目されている (26)。また中脳

PAG の血管奇形からの出血で慢性片頭痛となった症例が報告されており、この系の障害により疼痛抑制が生じ、頭痛発作が助長された可能性が考えられている (27)。

トリプタン系薬剤は、頭蓋内血管平滑筋に存在する 5-HT_{1B} 受容体を介し血管収縮作用を示すとともに、三叉神経終末に存在するセロトニン受容体の中の 5-HT_{1D} 受容体により神経ペプチド放出を抑制し神経原性炎症を抑制し頭痛発作改善に効果を示すと考えられている。

また CGRP 受容体作動薬、アデノシン A₁ 受容体作動薬、TRPV1 受容体作動薬はおもに三叉神経血管系、GABA 受容体作動薬は大脳神経細胞に作用し片頭痛の発作改善および予防に働くと考えられている。

以上片頭痛治療薬について現在開発中の薬剤を含め概説した。今後トリプタン系薬剤に加え片頭痛発作および予防に有効な薬剤の登場が期待される。

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最新の頭痛診療
—頭痛の全てが分かる—

Key Words

頭痛診療をめぐる最近のトピックス

片頭痛と脳梗塞

片頭痛
脳梗塞
危険因子
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はじめに

急性期治療薬トリプタンの出現と一般化に始まり、病態として脳血管疾患から脱却して中枢神経疾患としての概念への移行など、片頭痛にまつわる近年の話題には枚挙にいとまがない。さらにこの数年、それらに輪をかけてこの領域の話題をさらったのは、「片頭痛が将来発症する可能性のある脳梗塞の危険因子である」という衝撃的な疫学研究結果報告が相次いだことであろう。本稿ではこの片頭痛と関連性について、その研究の流れと内容の吟味、および問題点などを冷静かつ批判的な立場で眺めてみたい。

片頭痛と脳梗塞

片頭痛と脳梗塞は、片頭痛の病態としての血管収縮との関係で片頭痛患者に脳梗塞が合併することは十分可能性のあることと考えられていた。しかし、客観的証左は乏しく両者の因果関係は結論に達するに至っていなかった。

国際頭痛分類第2版では片頭痛と関連する脳血管障害は「1.5.4 片頭痛性脳梗塞」として

片頭痛の亜型として分類され、一つ以上の片頭痛前兆があり神経画像検査によって責任領域に虚血性梗塞巣が証明されるものとされている。頭痛自体は「前兆のある片頭痛」をもつ片頭痛患者に起こる頭痛発作であり、一つ以上の前兆が60分を超えて続くこと以外は、今まであった頭痛発作と同じであると定義されている。すなわち、片頭痛の前兆がそのまま脳血管障害の局所症状に移行してしまう、というものである。実際の臨床で片頭痛患者における脳梗塞は、①前兆のある典型的片頭痛の前兆がそのまま改善せずに残存し脳梗塞に移行するもの、②片頭痛と共存する（あるいは片頭痛を既往とする）その他の原因による脳梗塞、そして③前兆のある片頭痛に類似した症状を呈するその他の原因による脳梗塞、の3つに分類される。これらのうち①のみが国際頭痛分類の基準を満たし「1.5.4 片頭痛性脳梗塞」と診断されることになり、これは極めてまれな病態といえる。

前述のように、最近話題になっているのは、②のカテゴリーに属する病態である。すなわち片頭痛がその個体に将来発症する可能性のある脳梗塞の危険因子であるか否かの議論で

ある。

これまで系統的な調査が行われていなかったこの領域で、Kruitらにより30～60歳の片頭痛患者を対象とした頭部MRIを用いた無症候性を含む脳病変（梗塞）の横断的有病率調査が行われた¹⁾。その結果、全脳病変の有病率は片頭痛群と対照群に差はなかったが、脳後部循環領域では片頭痛群が対照群に比し有意に有病率が高く、特に前兆のある片頭痛群で高かったと報告された（表1）。さらにこの所見は特に小脳、しかも各灌流動脈の分水嶺領域に顕著であること²⁾また脳幹、特に橋に目立つことが報告された³⁾。これらは、非器質性疾患であるはずの片頭痛が、血管イベントの遠隔的な危険因子になるとの極めて衝撃的な報告であり、その信憑性について議論が沸いた。

このような報告を受けてさらに正確を期す

ために、Etminanらにより片頭痛患者の脳梗塞の発生危険度を系統的レビューとメタアナリシスが行われた⁴⁾。この分析では脳梗塞発症の相対危険度は片頭痛全体で2.16、前兆のある片頭痛で2.27、前兆のない片頭痛で1.83、さらに片頭痛で経口避妊薬使用者は8.72と報告された（表2）。本報告についても大きな議論が起こり、データや分析方法に対する批判が相次ぎ、最初の報告の翌年にオンラインバージョンで最終改訂版が報告された⁵⁾。

このように、片頭痛患者に虚血性脳病変の発生危険度が高いのであれば、その原因をどこに帰せばよいのであろうか。このような疑問に対し、その後片頭痛患者における全身の心血管イベントの危険因子に関する研究が行われている。

一つはScherらによる20～60歳を対象とした調査で、そこでは血圧、総コレステロー

表1 片頭痛の既往、病型、発作頻度による後方循環領域脳梗塞の発症危険度¹⁾

Risk of PCT Infarcts by Migraine Diagnosis, Subtype, Attack Frequency, and Combination			
Migraine Characteristic	Total No. of Participants	No. of Participants With PCT Infarct	OR (95% CI) Model 2
Migraine History			
No (controls)	140	1	1.0
Yes	295	16	7.1 (0.9 ~ 55)
Diagnostic groups			
Migraine without aura	134	3	2.3 (0.2 ~ 23)
Migraine with aura	161	13	13.7 (1.7 ~ 112)
Migraine attacks			
< 1 attack per month	159	6	5.1 (0.6 ~ 44)
≥ 1 attack per month	136	10	9.3 (1.1 ~ 76)
Migraine attacks and subtype			
Migraine without aura			
< 1 attack per month	67	0	...
≥ 1 attack per month	67	3	4.4 (0.4 ~ 45)
Migraine with aura			
< 1 attack per month	92	6	11.3 (1.3 ~ 102)
≥ 1 attack per month	69	7	15.8 (1.8 ~ 140)

Abbreviations: CI, confidence interval; PCT, Posterior circulation territory; OR, odds ratio

ル, HDL コレステロール, 喫煙, 経口避妊薬などを片頭痛群と対照群とで比較している⁵⁾. その結果, 片頭痛群特に前兆のある片頭痛群では, 高コレステロール, 高血圧, 冠動脈疾患および脳血管障害の早期発症率が有意に高く, 心血管イベントの発生危険度が片頭痛を有さない対照群よりも高いことが明らかになっている. さらに, Kurt らにより片頭痛が脳血管障害発症の危険因子となっているかどうかを検証するための45歳以上の米国人女性対象の脳血管障害発症についての前向きコホート研究が行われている⁶⁾. 観察期間は9年間で, 脳血管障害は発症については自己申告であるがそれに対して医学的確認を条件に検討されている. この結果, 片頭痛は全脳血管障害(虚血性, 出血性)の発症とは関連しないが, サブグループ解析(45歳以上, 55歳未満)では前兆のある片頭痛群で全脳血管障害のリスクの増加がみられた. ただし, こ

のリスクの絶対増加は極めて低く, 3.8人/年/10,000人・女性であったと報告されている.

以上のように, 近年施行された多くの調査が, 片頭痛, 特に前兆のある片頭痛が後年, 虚血性脳血管障害を発症する危険度が高いと結論を下している. しかし, これらの調査・研究には, 片頭痛の既往は自己申告である, 片頭痛治療の詳細は不明である, 脳梗塞の診断が一定していないなどの, 情報としていくつかの不備があることは否めない. また, メタアナリシス報告⁴⁾については分析の対象となった試験の大部分が症例対照試験である, 各患者において薬剤内服状況がコントロールされていない, 抗リン脂質抗体などは両者の発症に関与している可能性がある, 脳血管障害の診断に画像上の裏付けなど客観性が乏しい, 片頭痛と脳血管障害発症の経時的関係は分析困難であるなどの, 問題点が横たわっている. また, 危険因子に関する調査では⁵⁾⁶⁾,

表2 片頭痛の病型, 経口避妊薬使用および年齢により増強される脳梗塞発症の相対危険度⁴⁾

Pooled relative risks of ischemic stroke stratified by migrakne type, oral contraceptive use, and age				
	No. of studies	Relative risk (95% CI)	Ri*	P value [†]
Migraine (any)				
All studies	14	2.16 (1.89 to 2.48)	0.00	0.77
Case-control studies	11	2.18 (1.86 to 2.56)	0.00	0.51
Cohort studies	3	2.10 (1.61 to 2.75)	0.00	0.96
Migraine with aura				
Case-control studies	7	2.27 (1.16 to 3.19)	0.49	0.06
Migraine without aura				
Case-control studies	6	1.83 (1.06 to 3.15)	0.60	0.04
Migraine among oral contraceptive users				
Case-control studies	3	8.72 (5.15 to 15.05)	0.26	0.28
Migraine among men and women < 45 years				
Case-control studies	9	2.36 (1.92 to 2.90)	0.07	0.38
Migraine among women < 45 years				
Case-control studies	7	2.76 (2.17 to 3.52)	0.00	0.82

* Proportion of the total variance due to between study variance. Large values (> 0.75) indicate large heterogeneity between studies; small values (< 0.4) indicate lack of heterogeneity[†] DerSimonian and Laird Q static

表3 Woman's Health Study 参加女性における片頭痛の種類と脳梗塞発症イベントの多変量補正ハザード比 (N = 27,840)^{a)}

	Hazard Ratio (95% Confidence Interval)								
	No Migraine History (n = 22715) n = 458		Active Migraine With Aura (n = 1434) n = 46		Active Migraine Without Aura (n = 2176) n = 37				
Ischemic Vascular Event	1.00	1.42 (1.16 ~ 1.74)	.001	2.15 (1.58 ~ 2.92)	<.001	1.23 (0.88 ~ 1.73)	.23	1.14 (0.82 ~ 1.58)	.45
Major cardiovascular event	n = 2.4	n = 47	n = 18	n = 17	n = 12	n = 17	n = 12	n = 12	n = 12
Ischemic stroke	1.00	1.22 (0.88 ~ 1.68)	.23	1.91 (1.17 ~ 3.10)	.01	1.27 (0.77 ~ 2.09)	.36	0.77 (0.43 ~ 1.38)	.38
Multivariable-adjusted	n = 196	n = 53	n = 20	n = 16	n = 17	n = 16	n = 17	n = 17	n = 17
Myocardial infarction	1.00	1.41 (1.03 ~ 1.91)	.03	2.08 (1.30 ~ 3.31)	.002	1.22 (0.73 ~ 2.05)	.45	1.14 (0.69 ~ 1.88)	.60
Multivariable-adjusted	n = 404	n = 110	n = 36	n = 30	n = 44	n = 30	n = 44	n = 44	n = 44
Coronary revascularization	1.00	1.35 (1.09 ~ 1.67)	.006	1.74 (1.23 ~ 2.46)	.002	0.98 (0.67 ~ 1.42)	.90	1.46 (1.07 ~ 2.00)	.02
Multivariable-adjusted	n = 314	n = 94	n = 28	n = 27	n = 39	n = 27	n = 39	n = 39	n = 39
Angina	1.00	1.47 (1.17 ~ 1.86)	.001	1.71 (1.16 ~ 2.53)	.007	1.12 (0.75 ~ 1.66)	.58	1.66 (1.19 ~ 2.32)	.003
Multivariable-adjusted	n = 102	n = 28	n = 10	n = 6	n = 12	n = 6	n = 12	n = 12	n = 12
Death due to cardiovascular disease	1.00	1.63 (1.07 ~ 2.50)	.02	2.33 (1.21 ~ 4.51)	.01	1.06 (0.46 ~ 2.45)	.89	1.65 (0.90 ~ 3.01)	.10
Multivariable-adjusted									