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ACCELERATED COMMUNICATION

Differential Expression of Sumatriptan-Sensitive 5-Hydroxytryptamine Receptors in Human Trigeminal Ganglia and Cerebral Blood Vessels

ISABELLE BOUCHELET, ZVI COHEN, BRUCE CASE, PHILIPPE SÉGUÉLA, and EDITH HAMEL

Neurobiology Unit, Montreal Neurological Institute (I.B., Z.C., P.S., E.H.), and Department of Pathology, McGill University (B.C.), Montréal, Québec, Canada

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SUMMARY

The efficacy of sumatriptan in migraine relief has been attributed to its interaction with 5-hydroxytryptamine$_{1D}$ (5-HT$_{1D}$) receptors in cerebral blood vessels and/or on nerve endings of the trigeminovascular system in the dura mater. Using the high sensitivity of polymerase chain reaction (PCR) amplification, we investigated the expression of the sumatriptan-sensitive 5-HT receptors, namely, the 5-HT$_{1D\alpha}$, 5-HT$_{1D\beta}$, and 5-HT$_{1F}$ subtypes in human trigeminal ganglia (10 experiments) and cerebral blood vessels (seven experiments) obtained postmortem. Messages for the 5-HT$_{1D\alpha}$ and 5-HT$_{1D\beta}$ receptors were expressed in all except one of the 10 trigeminal ganglia studied. Expression of the 5-HT$_{1F}$ receptor was detected by gel electrophoresis of the PCR products in six ganglia and by Southern blot hybridization in two additional cases. In human brain vessels, message for the 5-HT$_{1D\beta}$ receptor was present in all samples, whereas specific PCR products corresponding to the 5-HT$_{1D\alpha}$ receptor could hardly be detected in only two preparations. PCR products indicative of the 5-HT$_{1F}$ receptor message were detected by gel electrophoresis in three brain vessel preparations and confirmed in the other four by Southern blot hybridization. Restriction mapping and sequence analysis of all PCR products identified the expected human 5-HT receptor DNA sequences. The data confirm that the 5-HT$_{1D\beta}$ receptor is the dominant species in human cerebral blood vessels and further show that this receptor and the 5-HT$_{1F}$ are expressed in both neural and vascular tissues. In contrast, the data point to a preferential expression of 5-HT$_{1D\alpha}$ receptors in neural versus vascular tissues and strongly reemphasize the need for selective 5-HT$_{1D\alpha}$ agonists in the identification of the target tissue(s) for antimigraine drugs. Moreover, the data stress the importance to better understand the role of 5-HT$_{1F}$ receptors in cerebrovascular functions and dural inflammation and further raise interest regarding their possible involvement in migraine therapy.

Sumatriptan, originally developed as a selective agonist at the 5-HT$_1$-like receptor mediating vasoconstriction (1), has proved to be highly effective in the acute treatment of migraine headache (2). Two mechanisms have been proposed to account for its antimigraine activity. The first one has been attributed to the ability of sumatriptan to induce contraction of cerebral blood vessels (3, 4), whereas the second relies on its effective blockade of dural inflammation mediated by trigeminovascular afferents (5).

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The nature of the receptor involved in these respective vascular and neurogenic responses to sumatriptan has been investigated, and evidence points to the 5-HT$_{1D}$ receptor (for recent reviews, see Refs. 6 and 7), which consists of two molecular variants identified as the 5-HT$_{1D\alpha}$ and 5-HT$_{1D\beta}$ receptor subtypes, in humans (8, 9). Using pharmacological correlates and Northern blot hybridization, we identified the 5-HT$_{1D}$ (10) and, more specifically, the 5-HT$_{1D\beta}$ subtype (11, 12) as the receptor mediating vasoconstriction of human cerebral blood vessels. Similarly, pharmacological studies in animal models of dural inflammation suggest that a presynaptic 5-HT$_{1D}$-like receptor (13, 14), and possibly another subtype not yet identified (15, 16), is involved in blockade of this neurogenic response. In humans, molecular biology data

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; RT-PCR, reverse transcriptase-polymerase chain reaction; SSC, standard saline citrate; SDS, sodium dodecyl sulfate; bp, base pair(s).

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obtained through RT-PCR amplification showed the presence of 5-$HT_{1D\alpha}$, but not 5-$HT_{1D\beta}$, message in two of eight human trigeminal ganglia (17).

If true, such a tissue and function selectivity for the two 5-HT_{1D} receptor subtypes would offer tremendous potential in drug design and in identifying which, if any, of the two proposed mechanisms is important in aborting migraine attack. However, in addition to the 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptor subtypes for which it has a similar affinity (pK_i = 8.5 and 8.1, respectively; Ref. 6), sumatriptan exhibits relatively high affinity for the more recently cloned 5-HT_{1F} receptor (pK_i = 7.6; Ref. 18). It is thus possible that this receptor represents an additional therapeutic target for sumatriptan and other related antimigraine drugs.

We therefore examined the expression of mRNA transcripts for these three sumatriptan-sensitive 5-HT_1 receptors in human trigeminal ganglia and cerebral blood vessels by using RT-PCR amplification. Our results indicate that the three sumatriptan-sensitive receptors are expressed in human trigeminal ganglia, with only the 5-$HT_{1D\beta}$ and 5-HT_{1F} species being clearly present in cerebral blood vessels. These findings underscore the importance of obtaining selective pharmacological tools to functionally discriminate the trigeminal receptor from the 5-$HT_{1D\beta}$ receptor mediating cerebral vasoconstriction. These results have been presented in part in abstract form (19).

Materials and Methods

Tissue preparation. Tissue samples were obtained postmortem (6–24 hr) from male or female adults of varying ages who died from diseases not related to the central nervous system. Trigeminal ganglia were removed from the skull base of 10 subjects, cleansed of any contaminating dura or other tissues, and frozen at −80° until use. Cerebral blood vessels were obtained from seven subjects, four of them corresponding to those from whom we obtained the trigeminal ganglia (see ganglia 5, 10, 9, and 8 in Fig. 1 corresponding to vessels 1, 2, 3, and 6 in Fig. 3, respectively). In all cases, vessels corresponded to distal ramifications of major basal arteries and to small pial vessels overlying the cortical mantle of both cerebral hemispheres. They were kept in ice-cold phosphate-buffered saline (pH 7.4) while being isolated from the pia-arachnoid membrane. They were frozen in liquid nitrogen and turned into powder with a pestle before further processing.

RNA isolation. Tissues were homogenized in TRIzol Reagent (GIBCO-BRL, Gaithersburg, MD) with a polytron (Brinkmann, Westbury, NY), and total RNA was extracted according to the method of Chomczynski (20). The RNA samples (~100–150 μg/ganglion or vessel extraction) were treated with RQ1-DNase (Promega, Madison, WI) for 15–30 min at 37°, and the reactions were terminated through the use of phenol extraction. The RNA was then precipitated in ethanol and resuspended in water.

Oligonucleotide primers. For each receptor, specific oligonucleotide primers were designed according to published sequences (9, 18). They were flanked with the T7 (5′-GGTAATACGACTCACTATAGGGCGA-3′) and SP6 (5′-CTCGGATTTAGGTGACACTATAGAATAC-3′) RNA polymerase promoter sequences for use in other experiments. Oligonucleotides were synthesized in an Applied Biosystems synthesizer and purified using an OPC column (Applied Biosystems). The primers were as follows: 5-$HT_{1D\alpha}$ (1DαF, 5′-CACCATCTACTCCACCTGTG-3′ and 1DαR, 5′-CAGAAATCCTCTTGCGTTC-3′), defining a 340-bp fragment of DNA that included the region from the fifth transmembrane domain to the third intracellular loop of the receptor; 5-$HT_{1D\beta}$ (1DβF, 5′-AAGCCTTCTCCTCAAGCA-3′ and 1DβR, 5′-AGGTGATGAGCGCCAATA-3′), encompassing a 595-bp DNA fragment that included the region from the 5′-untranslated sequence to the first transmembrane domain of the receptor; and 5-HT_{1F} (1FF, 5′-CTTGAAGCCTTCTCTGAACTG-3′ and 1FR, 5′-AGAGATGCAAGATGGAGCAC-3′), providing amplification of a 482-bp DNA fragment that included the region from the 5′-untranslated region of the gene to the third transmembrane domain of the receptor.

RT-PCR amplification. cDNAs were synthesized (1 hr at 42°) from 5–30 μg of total RNA using random primers and avian myeloblastosis virus reverse transcriptase. A control reaction lacking the reverse transcriptase enzyme was also prepared for each RNA sample and used in PCR analysis as described below to monitor potential DNA contamination of the RNA samples. The cDNA was amplified with *Taq* DNA polymerase using primers selective for each receptor. The reaction mixture contained 1–5 μl of cDNA samples, 0.2 mM concentration of each deoxynucleotide triphosphate, 5% dimethylsulfoxide, 1–3 mM $MgCl_2$, 2–5 U of *Taq* DNA polymerase in 50 mM KCl, 10 mM Tris·HCl, 0.1% Triton X-100, and 0.5–1 μM concentration of each primer in a total volume of 50 μl. The PCR was performed in an MJ Research minicycler under the following conditions: 2 min at 95°, 5 min at 72°, 40 sec at 95°, 40 sec at 55°, and 40 sec at 72° for 40 cycles, followed by a 5-min final extension at 72°. All amplifications were run two or three times on different occasions, and they were found to be highly reproducible from experiment to experiment.

Analysis of PCR samples. All PCR-derived fragments were electrophoretically separated on a 1.7% Tris/borate/EDTA agarose gel containing ethidium bromide and photographed under UV light. When not visible by gel electrophoresis, PCR reaction products for the 5-HT_{1F} receptor fragment were further analyzed by Southern blot hybridization. The electrophoresed DNA was transferred to a N^+ nylon membrane (Amersham) by overnight capillarity blotting in 0.4 N NaOH. The membrane was prehybridized 1 hr at 42° in a buffer containing 50% formamide, 5× SSC (1× SSC = 0.15 M NaCl and 0.015 M citrate, pH 7.0), 10 mM Tris, 1× Denhardt's solution, 1% SDS, 10% dextran, and 100 μg/ml salmon sperm DNA. The hybridization was carried out at 42° in the same buffer with a ^{32}P-labeled (1–5 × 10^6 cpm/ml) cDNA probe synthesized from the full coding sequence of the 5-HT_{1F} receptor (kindly provided by Dr. R. Hen, Columbia University, New York, NY) generated by random priming (Multiprime kit; Pharmacia, Piscataway, NJ). This DNA fragment overlaps the expected 5-HT_{1F} PCR product over a region of 343 bp. After an overnight hybridization, the membranes were washed at low (25°, 2× SSC, 0.1% SDS) to high (65°, 0.1× SSC, 0.1% SDS) stringency and placed in cassettes for exposure to Kodak XAR film. This was not performed for the 5-$HT_{1D\alpha}$ PCR products because a significant difference between reversed transcribed and non-reverse-transcribed RNA samples could not be achieved in vascular samples not readily detectable on the gel (this is well illustrated in Fig. 3, *top lanes 1 and 5*).

Restriction mapping and sequence analysis. All PCR products were extracted from agarose using the Glassmax DNA extraction kit (GIBCO). Their identity was verified by restriction enzyme analysis. The PCR products were incubated (1 hr at 37°) with enzymes that each yielded two or three fragments of predicted size on gel electrophoresis. Each PCR product was digested separately as follows: 5-$HT_{1D\alpha}$, *Sau*3aI (two fragments, 91 and 249 bp) and *Hind*III (two fragments, 273 and 67 bp); 5-$HT_{1D\beta}$, *Pvu*II (two fragments, 242 and 353 bp) and *NCo*I (two fragments, 385 and 240 bp); and 5-HT_{1F}, *Sau*3aI (three fragments, 132, 110, and 240 bp) and *Dde*I (three fragments, 147, 262, and 73 bp). In addition, the PCR fragments were subcloned into the M13 mp19 vector, and their nucleotide sequence was determined using the Sanger dideoxynucleotide chain termination method and Sequenase (United States Biochemical, Cleveland, OH) in an automated sequencer (ALF, Pharmacia).

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Results

RNA isolated from each trigeminal ganglion was concurrently treated for PCR amplification of the three sumatriptan-sensitive 5-HT$_1$ receptors. PCR products of the expected size for the 5-HT$_{1D\alpha}$ or 5-HT$_{1D\beta}$ receptor subtypes (Fig. 1) were identified in all ganglia except one, which, interestingly, was not the same for the two receptor subtypes (Fig. 1, *lanes 1 and 6*). From the same pool of ganglia, six yielded PCR products corresponding to the 5-HT$_{1F}$ receptor visible by gel electrophoresis, whereas expression of this receptor in two of the remaining ganglia was confirmed by Southern blot hybridization (Fig. 2).

Cerebral blood vessels corresponded to arteries free of the arachnoid membrane of varying sizes. The three receptor messages were amplified from the individual reverse-transcribed RNA samples. A specific signal for the 5-HT$_{1D\alpha}$ subtype could only weakly be demonstrated by gel electrophoresis in two cerebral blood vessel extracts (Fig. 3, *lanes 2 and 3*), but all cerebrovascular preparations exhibited PCR products corresponding in size to the 5-HT$_{1D\beta}$ receptor (Fig. 3). With the exception of three vascular preparations (Fig. 3, *lanes 4, 5, and 7*), PCR products of the expected size for the 5-HT$_{1F}$ receptor were not detected by gel electrophoresis. Additional Southern blot hybridization with a ^{32}P-labeled cDNA probe complementary to the human 5-HT$_{1F}$ receptor gene confirmed the presence of 5-HT$_{1F}$ message in the remaining four human cerebrovascular preparations (Fig. 2).

Restriction mapping of the PCR products generated in human trigeminal ganglia and cerebral blood vessels for the three receptors yielded fragments of the expected molecular size for each receptor (for details, see Fig. 4). In addition, sequencing data from the PCR products fitted unambiguously the published sequences for their respective receptors; the identity was 98.6%, 96.7%, and 99.1% for the 5-HT$_{1D\alpha}$, 5-HT$_{1D\beta}$, and 5-HT$_{1F}$ receptors, respectively. Such slight variation is in the expected range of acceptability for single-strand sequencing analysis.

Discussion

The present data provide unequivocal evidence of the expression of 5-HT$_{1D\alpha}$, 5-HT$_{1D\beta}$, and 5-HT$_{1F}$ receptors in human trigeminal ganglia, thus supporting the presence of

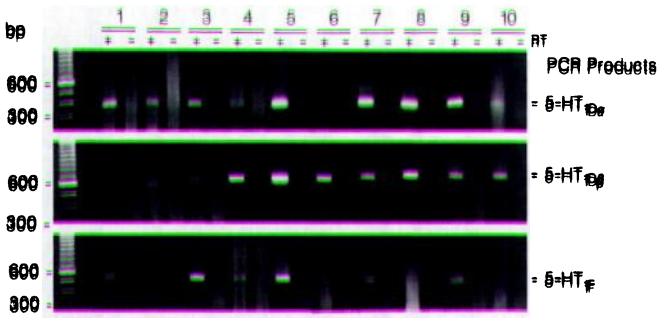

Fig. 1. Identification of sumatriptan-sensitive 5-HT$_1$ receptors in human trigeminal ganglia by RT-PCR. Agarose gel electrophoresis of PCR-amplified DNA from 10 ganglia using specific oligonucleotide primers for the 5-HT$_{1D\alpha}$ (*top*), 5-HT$_{1D\beta}$ (*middle*), and 5-HT$_{1F}$ (*bottom*) receptor genes. Samples without reverse transcriptase were included (−) to monitor for genomic and/or PCR contamination.

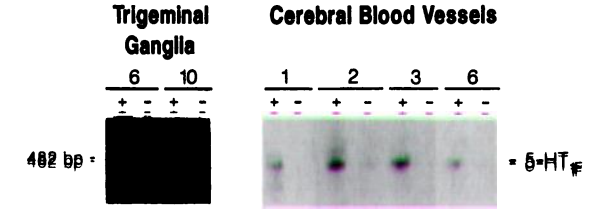

Fig. 2. Southern blot analysis of 5-HT$_{1F}$ PCR-amplified fragments not detectable under UV light after gel electrophoresis. PCR products from trigeminal ganglia and cerebral blood vessels were blotted and hybridized with a ^{32}P-labeled cDNA probe synthesized from the full coding sequence of the human 5-HT$_{1F}$ receptor (see Materials and Methods). Of the four trigeminal ganglion samples blotted, two did not yield any signal (not shown), whereas all four vessel samples exhibited good hybridization signals. Control samples prepared without reverse transcriptase were included (−) to monitor for signal specificity.

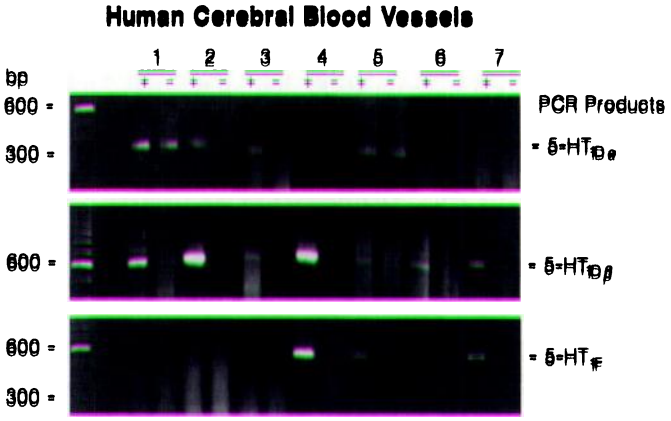

Fig. 3. Identification of sumatriptan-sensitive 5-HT$_1$ receptors in human cerebral blood vessels by RT-PCR. Agarose gel electrophoresis of PCR-amplified DNA from seven cerebrovascular preparations using specific oligonucleotide primers for the 5-HT$_{1D\alpha}$ (*top*), 5-HT$_{1D\beta}$ (*middle*), and 5-HT$_{1F}$ (*bottom*) receptor genes. Samples without reverse transcriptase were included (−) to monitor for genomic and/or PCR contamination.

prejunctional sumatriptan-sensitive 5-HT$_1$ receptors in the human trigeminovascular system, as documented in animal models (for a review, see Ref. 5). However, our results only partly agree with those of Rebeck *et al.* (17), who reported selective expression of 5-HT$_{1D\alpha}$ receptor message in a minor proportion (25%, two of eight ganglia) of human trigeminal ganglia. We found that virtually all human ganglia samples expressed both 5-HT$_{1D\alpha}$ and 5-HT$_{1D\beta}$ receptors, thus invalidating any selectivity of expression between these two subtypes. These discrepancies remain unexplained but are most likely related to experimental conditions rather than degradation of mRNA because the postmortem delays were comparable for the two studies. The presence of 5-HT$_{1D\beta}$ receptor message in human trigeminal ganglion, however, concurs well with previous *in situ* hybridization studies in the rat that showed message for the 5-HT$_{1B}$ receptor (the rodent homologue of human 5-HT$_{1D\beta}$ receptor) in trigeminal neurons (21). Furthermore, identification of message for the 5-HT$_{1F}$, a third sumatriptan-sensitive receptor (18), in human trigeminal ganglia strongly suggests that molecular biology data alone may not be sufficient to identify the functional 5-HT$_1$ receptor or receptors involved in the sumatriptan-mediated inhibition of trigeminovascular inflammation.

In animal models of dural inflammation, ergot alkaloids

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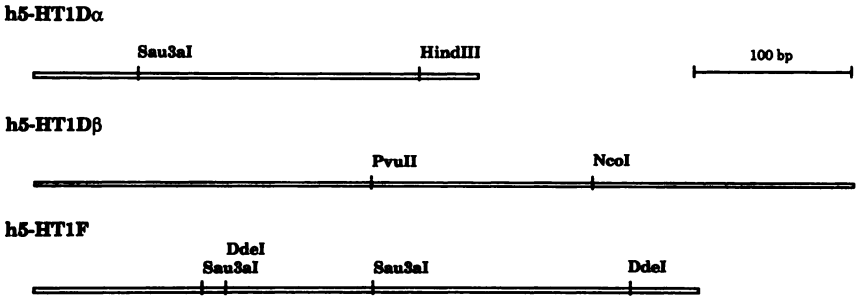

Fig. 4. Restriction site analysis of the PCR products obtained after amplification with selective primers for the 5-$HT_{1D\alpha}$, 5-$HT_{1D\beta}$, and 5-HT_{1F} receptor genes. PCR products were separately incubated with two restriction enzymes, and each resulted in two or three fragments of the expected molecular sizes as illustrated (for details, see Materials and Methods).

and 5-HT_{1D} receptor agonists such as sumatriptan and 311C90 (13, 22) inhibit dural plasma extravasation elicited by stimulation of the trigeminal ganglia. However, the structural analog of sumatriptan, CP122,288, blocks dural extravasation in rats and guinea pigs (14, 16, 23) at concentrations several thousandfold lower than those needed to elicit vasoconstriction and unrelated to its affinity at either the 5-$HT_{1D\alpha}$ or 5-$HT_{1D\beta}$ receptor subtype (16). Furthermore, the 5-HT_{1D} antagonist GR127935, which successfully blocks the sumatriptan-induced inhibition of dural inflammation, is ineffective against CP122,288 (15). Combined, these observations suggest that in addition to the 5-HT_{1D}, other 5-HT receptors might be able to modulate dural extravasation. Our present finding that 5-HT_{1F} receptors are expressed in human trigeminal ganglia emphasizes the need for further investigation of their role in trigeminovascular inflammation.

Pharmacological correlates (6, 11, 12, 24) and the present and previous (11) molecular biology data indicate that smooth muscle 5-$HT_{1D\beta}$ receptors mediate the sumatriptan-induced vasoconstriction in human brain vessels. This conclusion also supports recent findings in peripheral blood vessels, in which mRNA expression for 5-$HT_{1D\beta}$, but not 5-$HT_{1D\alpha}$, receptor subtype was detected in several species, including humans (25). The very low and rare specific signal for the 5-$HT_{1D\alpha}$ message observed in the present PCR study is consistent with our failure to detect 5-$HT_{1D\alpha}$ mRNA in human brain vessels by Northern blot hybridization (11) and suggests that this receptor is expressed at very low levels, if any, in some cerebrovascular preparations. A low expression would be compatible with the recent observation that human brain endothelial cells, which contribute only a small proportion in RNA extracts of human cerebral arteries compared with smooth muscle cells, express 5-$HT_{1D\alpha}$ receptor message (26). Our data further revealed the expression of sumatriptan-sensitive 5-HT_{1F} receptors in cerebrovascular preparations. Previous pharmacological studies in human cerebral arteries (12) excluded the involvement of 5-HT_{1F} receptors in the sumatriptan-induced vasoconstriction; however, a definite exclusion awaits the availability of selective 5-HT_{1F} receptor agonists and antagonists. It thus seems that the expression of 5-$HT_{1D\beta}$ receptor message in human coronary arteries (19) or vascular cells (25), strongly implies that these arteries could constrict when exposed to sumatriptan. This functional activation, if present in patients with suspected or proven coronary disease, could account for some of the side effects associated with sumatriptan and fully justifies its cautious use in such patients.

In conclusion, the present data confirm that the 5-$HT_{1D\beta}$ receptor subtype is the vascular contractile target for sumatriptan in human brain vessels. In contrast, they show that messages for both 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptors are expressed in human trigeminal ganglia. This differential expression of the 5-$HT_{1D\alpha}$ receptor mRNA in neuronal rather than vascular tissues further supports the urgent need for selective 5-$HT_{1D\alpha}$ agonists. The availability of such compounds seems to be instrumental to establish which, if any, of the two proposed mechanisms (cranial vasoconstriction or blockade of neurogenic inflammation) is involved in the antimigraine effect of sumatriptan. Our findings further underline the need to determine the respective distribution of 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptors in human trigeminal ganglion cells. This could help clarify their role in the physiology and pathophysiology of the trigeminovascular system. More importantly, our results point to the need of identifying the role(s) and distribution of 5-HT_{1F} receptors in trigeminal ganglia and cerebral blood vessels. They raise the possibility that a new generation of compounds that are not related to 5-HT_{1D} receptors might be of potential use in migraine therapy.

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Send reprint requests to: Dr. Edith Hamel, Laboratory of Cerebrovascular Research, Montreal Neurological Institute, 3801 University Street, Montreal, Quebec, Canada H3A 2B4. E-mail: mceh@musica.mcgill.ca

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