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Both 5-HT_{1B} and 5-HT_{1F} receptors modulate c-*fos* expression within rat trigeminal nucleus caudalis

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Abstract

A possible mechanism of action of antimigraine drugs such as sumatriptan is inhibition of the trigeminovascular pathway. Sumatriptan's effects might be mediated by 5-HT_{1B}, 5-HT_{1D} or 5-HT_{1F} receptors. To establish the relative importance of these subtypes, we compared the effects of sumatriptan with those of a selective 5-HT_{1F} receptor agonist (LY 344864) on c-*fos* protein expression in the trigeminal nucleus caudalis. c-*fos* expression was induced in urethane-anaesthetized rats by intracisternal capsaicin administration. Sumatriptan and LY 344864 decreased the number of capsaicin-induced c-*fos*-like immunoreactive cells within trigeminal nucleus caudalis ($ID_{50} = 0.04$ and 0.6 mg kg^{-1}). The effect of sumatriptan, but not of LY 344864, was prevented by pretreatment with the antagonist SDZ 21-009, which displays high affinity for rat 5-HT_{1B} receptors. LY 344864 appears to attenuate c-*fos*-like immunoreactivity via 5-HT_{1F} receptors, while sumatriptan acts via 5-HT_{1B} receptors. The fact that activation of 5-HT_{1F} receptors is sufficient to modulate the activity of the trigeminal system suggests that this receptor may be a target for antimigraine drugs with improved safety profile.

Keywords: C-*fos*; Nociception; 5-HT_{1B} receptor; 5-HT_{1F} receptor; Migraine; Sumatriptan

1. Introduction

Current antimigraine drugs, such as the triptans and ergot alkaloids, have been proposed to act by inhibiting trigemino-vascular neurons (Moskowitz, 1992). However both the anatomical localization and the molecular identity of their target receptors still need to be established. Sumatriptan binds with high affinity to three 5-HT receptor subtypes: 5-HT_{1B}, 5-HT_{1D} and 5-HT_{1F} (Hoyer et al., 1994). Binding sites for [^{3}H]sumatriptan have been detected by in vitro autoradiography within both the trigeminal ganglion and the brain stem trigeminal nucleus caudalis (Waeber and Moskowitz, 1995; Castro et al., 1997), while the messenger RNAs for these receptors have been detected within the trigeminal ganglion and trigeminal nucleus caudalis (Bruinvels et al., 1992; Rebeck et al., 1994; Adham et al., 1997). 5-HT_{1B} mRNA has also been found in cerebral blood vessels (Bouchelet et al., 1996). Sumatriptan has been shown to inhibit meningeal neurogenic inflammation induced by electrical stimulation of the trigeminal ganglion (Buzzi and Moskowitz, 1990; Shepheard et al., 1995). This effect is probably mediated by the inhibition of neuropeptide release from meningeal trigeminal nerve endings (Buzzi et al., 1991). Additionally, sumatriptan inhibits the activity of neurons within trigeminal nucleus caudalis, as it decreases the c-*fos*-like immunoreactivity within trigeminal nucleus caudalis following noxious meningeal stimulation (Nozaki et al., 1992a), as well as it reduces field potentials within trigeminal nucleus caudalis after electrical stimulation of the superior sagittal sinus (Kaube et al., 1993).

Sumatriptan also causes vasoconstriction of large blood vessels, including human isolated cerebral, meningeal, temporal and coronary arteries (Parsons et al., 1989; Kaumann et al., 1993; Lambert and Michalilcek, 1996). These findings support the clinical observations of infrequent coronary vasospasm reported in patients treated with sumatriptan (Perry and Markham, 1998). There is pharmaco-

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logical and anatomical evidence that the vascular effects of sumatriptan are mediated by 5-HT_{1B} receptors (Hamel et al., 1993; Bouchelet et al., 1996). Therefore, agents that modulate trigeminal pain through non-5-HT_{1B} receptors might retain their antimigraine efficacy while loosing their cardiovascular side-effects. Based on their predominant neuronal location in the trigemino-vascular system (Bouchelet et al., 1996; Longmore et al., 1997), 5-HT_{1D} receptors have been proposed as better targets for antimigraine drugs (Rebeck et al., 1994; Waeber et al., 1997). 5-HT_{1F} receptors may represent another target because they lack vasoactive properties (Johnson et al., 1997) and are expressed by neurons in the trigeminal ganglion and nucleus (Bouchelet et al., 1996; Adham et al., 1997). Interestingly, activation of 5-HT_{1F} receptors attenuate dural plasma protein extravasation induced by electrical stimulation of the trigeminal ganglion (Phebus et al., 1997; Johnson et al., 1997).

To investigate the importance of the 5-HT_{1B} and 5-HT_{1F} receptors in the trigeminal nociceptive pathway, we compared the effects of LY 344864 [(*R*)-(+)-*N*-[3-(*N*,*N*-dimethylamino)-1,2,3,4-tetrahydrocarbazol-6-yl]-4-fluorobenzamide], a novel selective 5-HT_{1F} receptor agonist (Phebus et al., 1997), with those of sumatriptan, on the expression of the immediate early gene c-*fos* within the rat trigeminal nucleus caudalis after meningeal irritation with capsaicin. We also studied the effect of selective 5-HT_{1B} receptor blockade using the β-adrenoceptor blocking drug SDZ 21-009 (Hoyer et al., 1985).

2. Materials and methods

2.1. Animal preparation and c-fos immunohistochemistry

A soft catheter (PE-10, 0.28 mm internal diameter; Intramedic, Clay Adams, Parsippany, NJ, USA) was introduced into the cisterna magna of male Sprague–Dawley rats (200–300 g, Charles River Laboratories, Wilmington, MA, USA), anaesthetized with urethane (1.2 g kg^{-1}, intraperitoneally). The animals were injected intraperitoneally with the drug-vehicle or drug 30 min later. One hour after catheter placement, a capsaicin solution (0.1 ml, 50 μM) was injected into the cisterna magna via the catheter. Animals were killed by an overdose of pentobarbital (100 mg kg^{-1}, intraperitoneally) 2 h after capsaicin administration and perfused immediately with 0.9% saline, followed by 4% formaldehyde in 0.1 M phosphate buffer. Brain stems with attached cervical cords were stored overnight in the same fixative and then placed in a cryoprotectant (20% sucrose, 30% ethylene glycol in 0.1 M phosphate buffer) for 48 h before sectioning in a freezing microtome (Reichert–Jung, 2000 Leica, Deerfield, IL, USA). 50 μm thick tissue sections (from the C2 level to 3 mm rostral to obex) were processed for immunohistochemistry, using the avidin–biotin method as previously described (Mitsikostas et al., 1998). The primary c-*fos* antibody (Ab-5; Oncogene Research Products, Cambridge, MA, USA) was diluted in 0.1 M phosphate buffer (1/8000). C-*fos* positive nuclei were counted by an observer naive to the treatment groups (D.D.M) and confirmed (in randomly selected sections) by another investigator (M.S.R.) under similar conditions. Cells displaying c-*fos*-like immunoreactivity were counted in laminae I, II of trigeminal nucleus caudalis using the weighted average method that reflects the total c-*fos* expression within the entire trigeminal nucleus caudalis, from obex to upper spinal cord (−6.45 mm) (Mitsikostas et al., 1998).

2.2. Drug treatment

Drug vehicle (normal saline, 0.2 ml, $n = 13$), LY 344864 0.01 mg kg^{-1} ($n = 5$), 0.1 mg kg^{-1} ($n = 5$), 0.3 mg kg^{-1} ($n = 8$), 1 mg kg^{-1} ($n = 7$), or 5 mg kg^{-1} ($n = 7$) and sumatriptan 0.01 mg kg^{-1} ($n = 7$), 0.1 mg kg^{-1} ($n = 7$), or 1 mg kg^{-1} ($n = 7$) were injected intraperitoneally. Drug-vehicle ($n = 5$) or the receptor antagonist SDZ 21-009 (1 mg kg^{-1}, $n = 5$) were injected intraperitoneally in a separate set of animals. In a third set of animals, drug-vehicle ($n = 5$), sumatriptan alone (0.1 mg kg^{-1}, $n = 5$), or sumatriptan plus SDZ 21-009 (0.1 and 1 mg kg^{-1}, respectively; $n = 5$), were administered. Drug-vehicle ($n = 6$), LY 344846 alone (0.3 mg kg^{-1}, $n = 5$), or LY 344846 plus SDZ 21-009 (0.3 and 1 mg kg^{-1}, respectively; $n = 6$) were administered in a fourth set of animals. Drug treatment was administered 30 min before the intracisternal capsaicin injection in all animal groups.

2.3. Systemic physiological parameters

Physiological monitoring was carried out in 6 randomly selected animals. After anaesthesia with urethane and placement of the intracisternal catheter, a catheter (PE-50, internal diameter 0.58 mm; Becton Dickinson, Sparks, MD 2152, USA) was placed in the left femoral artery. The effect of intracisternal capsaicin injection on arterial blood pH, $PaCO_2$ and PaO_2, mean arterial blood pressure and heart rate were measured for 60 min in animals pretreated with 5 mg kg^{-1} LY 344864 ($n = 2$), 1 mg kg^{-1} SDZ 21-009 ($n = 2$) and drug vehicle ($n = 2$). Blood gases and pH measurements were performed three times in each animal (before and after drug treatment and after intracisternal capsaicin injection) using a blood gas/pH analyzer (Corning 178; Ciba-Corning Diagnostics, Medford, MA, USA). Mean arterial blood pressure and heart rate were monitored using Mac/Lab8 data acquisition system (AD Instruments, Medford, MA, USA) equipped with an ETH 400 transducer/amplifier. Core temperature was maintained at 36–37°C by a homeothermic blanket (Harvard Apparatus No. 551, South Natick, MA, USA).

2.4. Drugs

A fresh capsaicin solution (8-methyl-*N*-vanillyl-6-nonenamide, Sigma) was made 2 h before each experiment by dissolving 1.55 mg capsaicin in 1 ml of saline: ethanol:Tween 80 (8:1:1) and sonicating for 30 min. The solution was further diluted into artificial cerebrospinal fluid (132 mM NaCl, 3 mM KCl, 0.6 mM $MgCl_2$, 1.5 mM $CaCl_2$, 49 mM $NaHCO_3$, 6.6 mM urea, 7.4 mM D(+)-glucose, 5 mM HEPES, pH adjusted to 7.4) to a final concentration of 50 μM. LY 344864 [(*R*)-(+)-*N*-[3-(*N*,*N*-dimethylamino)-1,2,3,4-tetrahydrocarbazol-6-yl]-4-fluorobenzamide] (Eli Lilly, Indianapolis, IN, USA) and SDZ 21-009 [4(ter-butyl-amino-2-hydroxypropoxy] indol-2-carbonic acid-isopropyl-ester] (Novartis, Basle, Switzerland) were dissolved in dimethyl sulfoxide (Sigma) and normal saline (1:3) and further diluted in normal saline. Sumatriptan (Glaxo-Wellcome, Greenford, UK) was diluted in normal saline. Sodium pentobarbital was obtained from Abbott Laboratories (Chicago, IL, USA).

2.5. Statistics

Data are expressed as a weighted average ± standard error of the number of immunoreactive cells per 50 μm section. The weighted averages were compared by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Student's two-tailed *t*-test was used when appropriate for simple comparisons between means. *P* values of 0.05 or less were considered significant. Dose response curves were fitted by non linear regression analysis using the software GraFit 3.0 (Erithacus Software, Staines, UK)

3. Results

3.1. Physiology

Baseline values of mean arterial blood pressure, heart rate and blood gas concentrations did not differ between groups (94 ± 3 mmHg and 379 ± 90 bpm, respectively); LY 344864 did not significantly change mean arterial blood pressure or heart rate. SDZ 21-009 caused a increase in mean arterial blood pressure (7%) and decrease in heart rate (5%). Intracisternal capsaicin injection (5 nmol) caused transient (10 min) mean arterial blood pressure and heart rate changes, similar to that previously reported (Mitsikostas et al., 1998). No significant changes in $PaCO_2$ and PaO_2 were seen, either before or after drug treatment ($PaCO_2$ varied from 30 to 40 mmHg and PaO_2 from 80 to 100 mmHg in both vehicle- and drug-treated animals). The blood pH values were stable (from 7.40 to 7.49) and did not change after intraperitoneal drug administration and intracisternal capsaicin treatment.

3.2. Capsaicin-induced c-fos expression within rat brain stem

After capsaicin injection (5 nmol) and in the absence of 5-HT receptor agonist, c-*fos*-like immunoreactivity was observed bilaterally and most prominently within laminae I and II of trigeminal nucleus caudalis. The distribution and density of capsaicin-induced c-*fos*-like immunoreactive cells was similar to that previously reported (Mitsikostas et al., 1998), with a peak density at the −2.05 mm level (420 ± 34 cells per section). Capsaicin also induced c-*fos*-like immunoreactivity within the solitary tract, area postrema, medullary and lateral reticular nuclei, as well as within the leptomeninges. The number of c-*fos*-like immunoreactive cells was not determined in these regions.

3.3. Drug treatment

Both LY 344864 and sumatriptan dose-dependently reduced the weighted average of c-*fos*-like immunoreactive cells within laminae I and II of trigeminal nucleus caudalis with ID_{50} values of 0.6 ± 0.3 and 0.04 ± 0.02 mg kg^{-1} (or 1.5 ± 0.7 and 0.1 ± 0.05 μmol kg^{-1}), respectively (Fig. 1). Thus, sumatriptan was approximately 15-fold more potent than LY 344864. The maximum reduction of c-*fos*-like immunoreactivity was 53% (LY 344864) and 56% (sumatriptan). The 5-HT_{1B} receptor antagonist SDZ 21-009, when given alone at 1 mg kg^{-1} did not alter the number of c-*fos*-like immunoreactive cells within trigeminal nucleus caudalis (Fig. 2A). In rats pretreated with this dose of SDZ 21-009, 0.1 mg kg^{-1} sumatriptan (a dose found to inhibit

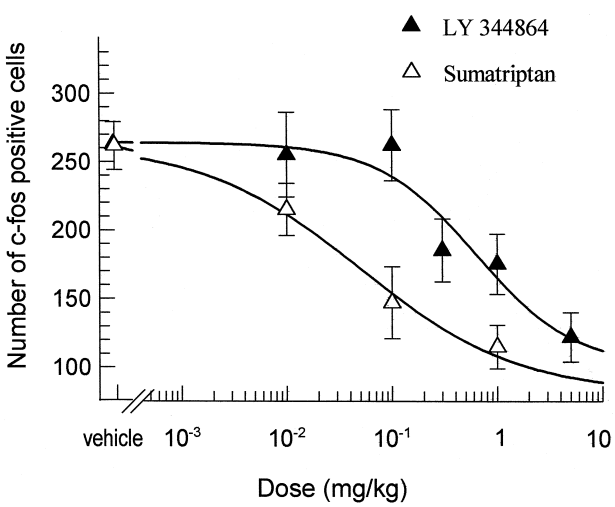

Fig. 1. Sumatriptan and LY 344864 dose-dependently decreased the capsaicin-induced c-*fos* response within trigeminal nucleus caudalis with an ID_{50} of 0.04 mg kg^{-1} (0.1 μmol kg^{-1}) and 0.6 mg kg^{-1} (1.5 μmol kg^{-1}) respectively. Data are presented as number of c-*fos* immunoreactive cells per section reflecting the total c-*fos* expression within the entire trigeminal nucleus caudalis (weighted average method). Error bars represent standard error.

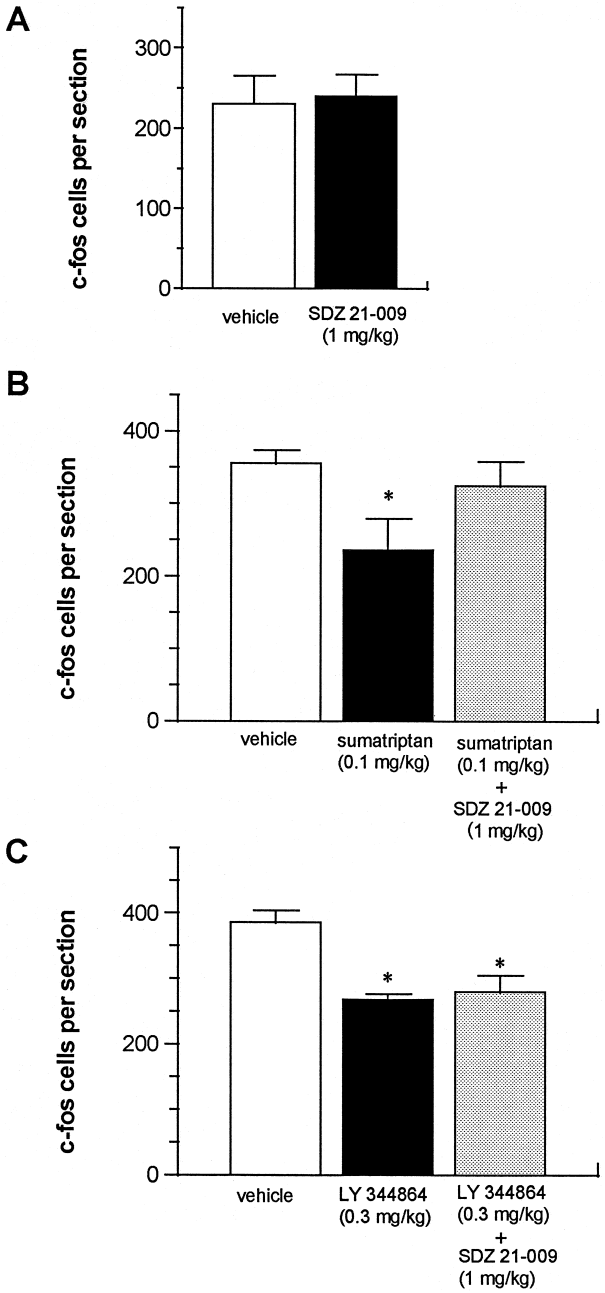

Fig. 2. SDZ 21-009, a β-adrenoceptor blocker that also displays high affinity for 5-$HT_{1A/1B}$ receptors did not change the number of cells showing positive c-*fos* immunoreactivity within trigeminal nucleus caudalis induced by capsaicin (panel A). Sumatriptan and LY 344864 inhibit the c-*fos* response within trigeminal nucleus caudalis. The effect of sumatriptan, but not LY 344864, was attenuated by SDZ 21-009 (panel B and C, respectively). Data are presented as weighted average of c-*fos*-like immunoreactive cells per section. Error bars represent standard error; $^{*}p < 0.05$ (compared to vehicle).

c-*fos* expression by 44%), did not significantly reduce the number of c-*fos*-like immunoreactive neurons (Fig. 2B). In contrast, pretreatment with SDZ 21-009 had no significant effect on the inhibitory response to 0.3 mg kg^{-1} LY 344864 (a dose found to attenuate c-*fos* expression by 27%).

4. Discussion

We found that both sumatriptan and LY 344864 decrease capsaicin-induced c-*fos*-like immunoreactivity within laminae I and II of trigeminal nucleus caudalis. Sumatriptan's efficacy in migraine has been established, as has its activity in various in vitro and in vivo animal models (Buzzi and Moskowitz, 1990; Nozaki et al., 1992a). Sumatriptan (720 nmol kg^{-1}, twice) reduced by 31% the number of c-*fos*-like immunoreactive cells in trigeminal nucleus caudalis 2 h after subarachnoid blood administration (Nozaki et al., 1992a). The decrease is similar to what we observe at the ID_{50} dose of sumatriptan (i.e., 100 nmol kg^{-1}) in the present study, suggesting that sumatriptan is slightly more potent inhibitor when c-*fos* expression is evoked by capsaicin rather than blood. It is possible that blood activates a larger population of axons as neonatal capsaicin treatment only suppressed 54% of the response to blood in a prior study (Nozaki et al., 1992b).

Shepheard et al. (1995) showed that pretreatment with sumatriptan (1 mg kg^{-1}) non significantly reduced c-*fos* mRNA expression in trigeminal nucleus caudalis by 26 ± 8%, whereas the same dose reduced the level of c-*fos* hybridization signal by 65% when the blood brain barrier was disrupted by injection of hyperosmolar mannitol. These results suggest that the anti-migraine action of sumatriptan is most likely mediated by an action outside the blood brain barrier (e.g., inhibition of neurotransmission, dural neurogenic inflammation or vasoconstriction). To our knowledge there is little evidence that the blood brain barrier is disrupted in patients during a migraine attack.

While sumatriptan binds with subnanomolar affinity to 5-HT_{1B} ($pK_i = 8.1$), 5-HT_{1D} ($pK_i = 8.5$) and 5-HT_{1F} ($pK_i = 7.6$) receptors (Hamel, 1996), LY 344864 shows considerable selectivity for 5-HT_{1F} ($pK_i = 8.2$) versus 5-HT_{1B} ($pK_i = 6.3$) and 5-HT_{1D} sites ($pK_i = 6.2$) (Phebus et al., 1997). LY 344864 inhibits neurogenic dural inflammation in rats with an ID_{50} of 0.6 ng kg^{-1} (2 pmol kg^{-1}) (Phebus et al., 1997). Sumatriptan was not tested in the same study, but it is worth mentioning that the same group published an ID_{50} value of 44 pmol kg^{-1} for this agonist in the guinea pig dural extravasation model (Johnson et al., 1997). Although possible species differences make direct comparisons unreliable, it seems that sumatriptan is at least one order of magnitude less potent than LY 344864 at inhibiting dural neurogenic inflammation. This contrasts with the 15-fold higher potency of sumatriptan at attenuating c-*fos*-like immunoreactivity in rat trigeminal nucleus caudalis.

The relatively low potency of LY 344864 in the present study could suggest the importance of 5-HT_{1B} rather than 5-HT_{1F} receptor mediated mechanisms within the inhibition of c-*fos* response following meningeal stimulation. To test the importance of the 5-HT_{1B} receptors, we administered the selective antagonist SDZ 21-009. We previously reported that GR 127,935 reduces the effect of sumatriptan on dural plasma protein extravasation and concluded that

5-$HT_{1B/1D}$ receptors are important in this paradigm (Yu et al., 1997). However, this antagonist possesses a moderate binding affinity for human 5-HT_{1F} receptors (K_i of 39.6 ± 9.5 nM) (Wainscott et al., 1998).

SDZ 21-009 displays nanomolar affinity for 5-HT_{1A} receptors and rat 5-HT_{1B} receptors (Hoyer et al., 1994), but only micromolar affinity for human and guinea pig 5-HT_{1B} binding sites (Waeber et al., 1990). In vivo administration of SDZ 21-009 blocks 5-HT_{1B} receptor mediated responses in rat (Chojnacka-Wojcik and Klodzinska, 1992) and mouse (Berthold et al., 1989). SDZ 21-009 is the 2-carbonic acid-isopropyl-ester derivative of the beta-adrenoceptor blocker pindolol, which does not displace [^{3}H]5-HT at human 5-HT_{1F} sites when added at 10 μM (Adham et al., 1993). An asparagine residue in the seventh transmembrane domain is required for the nanomolar affinity binding of these β-adrenoceptor blockers to 5-HT_{1A} and murine 5-HT_{1B} recognition sites (Adham et al., 1994). The presence of an alanine residue at this site in the 5-HT_{1F} receptor explains pindolol's low affinity for 5-HT_{1F} sites in ligand binding studies (Adham et al., 1993, 1997; Lovenberg et al., 1993). Although the binding constants of SDZ 21-009 for rat 5-HT_{1D} has not been published, the affinity is likely to be low because threonine is present in the seventh transmembrane domain of the rat 5-HT_{1D} (Wurch et al., 1997).

Pretreatment with 1 mg kg^{-1} SDZ 21-009 attenuates sumatriptan's but not LY 344864's effects on the c-*fos* response after intracisternal capsaicin. This finding indicates that 5-HT_{1B} receptors are the most likely mediators of sumatriptan's action, whereas LY 344864 probably blocks c-*fos* expression by a 5-HT_{1F} receptor mediated mechanism. The results obtained with each of these agonists agree with findings obtained previously in the dural plasma extravasation paradigm. Using 5-HT_{1B} receptor knockout mice as well as the receptor antagonist GR 127935, we have shown that sumatriptan blocks plasma protein leakage by 5-HT_{1B} receptor mediated mechanisms (Yu et al., 1996, 1997). However, the correlation between potency at blocking neurogenic dural inflammation was greatest for the 5-HT_{1F} receptor (Johnson et al., 1997). It now appears that in the rat both 5-HT_{1B} and 5-HT_{1F} receptors block the trigemino-vascular system.

Despite comparable affinities for 5-HT_{1B} ($pK_i = 8.02$) and 5-HT_{1F} ($pK_i = 7.59$) binding sites, sumatriptan does not seem to block c-*fos* expression by activating trigeminal 5-HT_{1F} receptors in the mouse (Yu et al., 1996, 1997; present report). This may reflect weak coupling properties between 5-HT_{1F} receptors and effector pathways. Indeed sumatriptan, its structural analog CP-122,288 as well as LY 344864 stimulated [^{35}S]guanosine-5′-*O*-(3-thio)triphosphate binding to guinea pig brain sections by 5-HT_{1A} and 5-HT_{1B}, but not by 5-HT_{1F} receptor mediated mechanisms, confirming a possible less efficient coupling of the latter sites (Waeber and Moskowitz, 1997; unpublished results for LY 344864). It is also possible that the relevant 5-HT_{1F} sites reside within the blood–brain barrier on intrinsic trigeminal nucleus caudalis neurons, whereas 5-HT_{1B} sites are located predominantly outside the blood–brain barrier (on the peripheral terminals of trigemino-vascular neurons in the meninges). While Bonaventure et al. (1998) recently reported that 5-HT_{1B} receptor mRNA is localized in both substance P and calcitonin gene related peptide-containing neurons in guinea pig trigeminal ganglia, the neurochemical identity of trigeminal ganglion cells synthesizing 5-HT_{1F} mRNA is unknown (Bouchelet et al., 1996; Adham et al., 1997). Sumatriptan crosses the intact blood–brain barrier only poorly (Saxena and Tfelt-Hansen, 1993) and would not be able to activate 5-HT_{1F} receptors on trigeminal nucleus caudalis neurons (Bruinvels et al., 1994), whereas LY 344864 is a more brain permeant agent and can access these sites (Phebus et al., 1997). Access to central neurons has been shown to influence the inhibitory properties of 5-HT_1 receptor agonists on c-*fos* expression in the trigeminal nucleus caudalis (Shepheard et al., 1995; Hoskin and Goadsby, 1998). It is unclear whether the ability to enter brain provides a beneficial effect in a clinical setting. The selective 5-$HT_{1B/1D}$ receptor agonist IS-159 (serotonyl(-oxymethylenecarbonyl)-tyrosyl-glycinamide) is effective in phase II clinical trials, but it is not significantly metabolized and does not access brain tissue indicating the importance of the peripheral binding sites or, less likely, that the blood–brain barrier opens during migraine attacks (Chauveau and Delaage, 1997).

The precise receptor sites of sumatriptan's anti-migraine activity in humans is still unclear, although high affinity binding to 5-$HT_{1B/1D}$ receptors appears predominant. Alniditan and IS-159 are effective clinically but show negligible affinity at 5-HT_{1F} receptors (Hamel, 1996; Chauveau and Delaage, 1997). There is some agreement however that 5-HT_{1B} receptors produce cardiovascular side-effects (Bouchelet et al., 1996; Perry and Markham, 1998). Considering the lack of 5-HT_{1D} and 5-HT_{1F} mRNAs in anatomical elements associated with vasoconstriction, and their presence on trigeminal ganglion cells and trigeminal nucleus caudalis neurons (Rebeck et al., 1994; Bouchelet et al., 1996), it is possible that drugs acting specifically at these sites might retain their anti-migraine activity without producing cardiovascular side-effects. The present report suggests that a selective 5-HT_{1F} receptor agonist inhibits the transmission of nociceptive information from the meninges to the central nervous system. 5-HT_{1F} receptor agonists might represent a new generation of migraine-aborting drugs devoid of vasoconstrictor activity.

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