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Lasmiditan inhibits calcitonin gene-related peptide release in the rodent trigeminovascular system

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Abstract

Migraine headache pathophysiology involves trigeminovascular system activation, calcitonin gene-related peptide (CGRP) release, and dysfunctional nociceptive transmission. Triptans are 5-HT$_{1B/1D/(1F)}$ receptor agonists that prejunctionally inhibit trigeminal CGRP release, but their vasoconstrictor properties limit their use in migraine patients with cardiovascular disease. By contrast, lasmiditan is a novel antimigraine and selective 5-HT$_{1F}$ receptor agonist devoid of vasoconstrictor properties. On this basis, this study has investigated the modulation of trigeminal CGRP release by lasmiditan. For this purpose, we have comparatively analysed the inhibition of several components of the trigeminovascular system induced by lasmiditan and sumatriptan through: ex vivo KCl-induced CGRP release from isolated dura mater, trigeminal ganglion, and trigeminal nucleus caudalis of mice; and in vivo dural vasodilation in the rat closed-cranial window model induced by endogenous (electrical stimulation and capsaicin) and exogenous CGRP. The ex vivo release of CGRP was similarly inhibited by sumatriptan and lasmiditan in all trigeminovascular system components. In vivo, intravenous (i.v.) lasmiditan or higher doses of sumatriptan significantly attenuated the vasodilatory responses to endogenous CGRP release, but not exogenous CGRP effects. These data suggest that lasmiditan prejunctionally inhibits CGRP release in peripheral and central trigeminal nerve terminals. Because lasmiditan is a lipophilic drug that crosses the blood–brain barrier, additional central sites of action remain to be determined.

Keywords: 5-HT$_{1F}$ receptors, CGRP, Lasmiditan, Migraine, Trigeminovascular system

1. Introduction

Migraine is a chronic neurovascular disorder characterized by recurrent severe attacks of throbbing headache, which can be accompanied by nausea, vomiting, photophobia, and phonophobia.[30] Although the pathophysiology of migraine has not been elucidated completely, it has been proposed that the headache phase results from activation and sensitization of trigeminal afferents from meningeal nociceptors, neuropeptide release, and dysfunctional nociceptive transmission.[31]

Calcitonin gene-related peptide (CGRP) is a key neuropeptide, widely expressed in the peripheral and central components of the trigeminovascular system, involved in craniofacial nociception modulation.[11,25,48] Infusion of CGRP triggers immediate headaches in healthy volunteers and immediate headaches as well as delayed migraine-like attacks in migraine patients.[30] Moreover, during spontaneous migraine attacks, this peptide is released in the extracerebral circulation.[15] Moreover, blocking CGRP or its receptor with monoclonal antibodies, which do not cross the blood–brain barrier, has revealed that migraine attacks can be prevented through peripheral blockade of CGRP.[16,44]

For almost 30 years, the triptans, 5-HT$_{1B/1D}$ receptor agonists, of which some also display affinity for the 5-HT$_{1F}$ receptor,[41] have been the specific treatment of choice for terminating migraine attacks. It has previously been shown that during a migraine attack, triptans normalize the elevated CGRP plasma levels by inhibiting further release from trigeminal afferents and, consequently, decrease nociceptive transmission from the periphery to the central nervous system.[14] However, due to their peripheral vasoconstrictor potential, including coronary vasoconstriction, they are contraindicated in migraine patients with cardiovascular diseases.[35] This problem led to the search for antimigraine agents lacking vasoconstrictor activity, which culminated in the development of lasmiditan.

Lasmiditan is a novel high-affinity, lipophilic, highly selective 5-HT$_{1F}$ receptor agonist ("ditan," **Table 1**) that is currently under development for the acute treatment of migraine.[37] Phase III trials revealed clinical efficacy in migraine patients, and preclinical data showed that it is devoid of vasoconstrictor properties in several blood vessels including human isolated coronary arteries.[40] However, few studies have investigated its exact (antimigraine) mechanism(s) of action. Lasmiditan may possess central as well as peripheral antinociceptive effects because it reduced c-Fos expression in the trigeminal nucleus caudalis and inhibited the dural plasma protein extravasation, both induced by electrical stimulation of the trigeminal ganglion.[37] However, the latter effect has not been demonstrated to occur in migraine patients, and several plasma protein extravasation inhibitors failed to be effective in clinical trials.[10] Therefore, based on the neurobiology

Table 1

Summary of functional (pEC$_{50}$ values of cAMP assays) and binding (pIC$_{50}$ values of radioligand binding assays) data of sumatriptan and lasmiditan at 5-HT receptors.

Compound	Species	Receptor							
		5-HT$_{1B}$		5-HT$_{1D}$		5-HT$_{1F}$		5-HT$_7$	
		pEC$_{50}$	pIC$_{50}$	pEC$_{50}$*	pIC$_{50}$	pEC$_{50}$	pIC$_{50}$	pEC$_{50}$	pIC$_{50}$
Sumatriptan succinate	Rat	6.44	7.26†	7.57	7.7	ND	7.2	ND	6.3†
	Human	7.32	7.81	8.30	8.31	8.03	7.13	5.22	6.10
Lasmiditan hemisuccinate	Rat	ND	<5	ND	<5	ND	8.48	ND	ND
	Human	<5	5.98†	6.64	5.87†	8.43	8.66†	<5	<5.52†

Data for sumatriptan[40] and lasmiditan[37] human recombinant 5-HT receptors. Data for lasmiditan rat 5-HT$_1$ receptors.[27] Data for sumatriptan rat 5-HT$_{1B}$,[4,13,19] 5-HT$_{1D}$,[43] 5-HT$_{1F}$,[33] and 5-HT$_7$ receptors.[45]

* pEC$_{50}$ values correspond to [^{35}S] GTPγS assay.

† pK$_i$; ND: no data were available.

of migraine headache, we set out to analyze the effects of lasmiditan on peripheral components as well as central projections of the trigeminovascular CGRPergic system, and compared our results with the responses to sumatriptan, as a positive control. For this purpose, we investigated the ex vivo CGRP release in the peripheral (dura mater and trigeminal ganglion) and central (trigeminal nucleus caudalis) trigeminal components of mice, as well as the dural vasodilation induced by endogenous (capsaicin and electrical stimulation) and exogenous CGRP in rats in vivo. Our results show that selective 5-HT$_{1F}$ receptor activation by lasmiditan inhibits CGRP release and, consequently, may attenuate nociceptive transmission in the trigeminovascular system.

2. Material and methods

2.1. Experimental animals

Experiments were performed in 36 male C57BL/6J (22-24 g; 13-14 weeks of age) mice and 54 male Sprague-Dawley (300-350 g; 8-10 weeks of age) rats, purchased from Charles River. Animals were housed under a 12-hour dark–light cycle in a special room at constant temperature (22 ± 2°C) and humidity (50%), with free access to food and water. Only male animals were used to avoid the well-known interaction of CGRP with cycling estrogen,[29] previously reported in the cranial-window model.[22] All experimental protocols of the study were approved by the Erasmus University Medical Center's institutional animal ethics committee (permission protocol numbers EMC 2891/3393), in accordance with the European directive 2010/63/EU and ARRIVE (Animal Research: Reporting In Vivo Experiments) reporting guidelines for the care and use of laboratory animals. All animals were randomly assigned into the different experimental protocols. For the CGRP assays (see next section), the analyst was not blinded to the compounds but to the research hypothesis. For the in vivo experiments, values were calculated using the dose–response autoanalyse selection feature of LabChart.

2.2. Ex vivo: calcitonin gene-related peptide release in trigeminal nucleus caudalis, trigeminal ganglion, and dura mater

This technique has been previously reported[8]; in brief, mice were anesthetized using intraperitoneal (i.p.) sodium pentobarbital (80 mg/kg) and decapitated at the atlanto-occipital joint. The scalp was retracted from the cranium. First, the trigeminal caudal nuclei, which run 9 to 13 mm caudally from bregma, were isolated from the brainstem. The skull was divided into halves by a clear cut along the sagittal suture, and the cerebral hemispheres were carefully removed while the cranial dura was left attached to the skull. Second, the trigeminal ganglia were harvested by dissection 1 mm proximal and distal to the point where the mandibular nerve branches off. Third, the remaining brain tissues were extracted from the hemisected skulls without damaging the dura of the middle cranial fossa. Each trigeminal nucleus caudalis, trigeminal ganglion, and hemisected skull with the dura mater were immersed and washed in carbogenated synthetic interstitial fluid, containing (mM): NaCl (108), KCl (3.48), MgSO$_4$ (3.5), NaHCO$_3$ (26), NaH$_2$PO$_4$ (11.7), CaCl$_2$ (1.5), sodium gluconate (9.6), glucose (5.55), and sucrose (7.6) for 30 minutes at 37°C. The isolated tissues were randomized and placed in a 24-well plate containing 500-μL synthetic interstitial fluid. The 24-well plate was fixed in a water bath that formed a closed humid chamber of 37°C. Basal CGRP values were measured in each tissue; then, CGRP release was induced by superfusion with 60-mM KCl. Vehicle (saline), sumatriptan, or lasmiditan (30 μM each, concentration based on our earlier experiments with sumatriptan)[8] was applied 10 minutes before the challenge with 60-mM KCl. For every sample, including the baseline measurement, the solution was collected after 10-minute incubation and mixed with aprotinin (500 KIU/mL). For the assessment of CGRP content, samples were stored at −20°C until processed with a commercial CGRP RIA kit according to the manual (Phoenix Pharmaceuticals, Burlingame, CA). Calcitonin gene-related peptide (pg/mL) absorbance values were calculated through an interpolation method using an equation derived from the standard curve. The assay has a detection level of ~0.1 pg/mL. Blank wells without CGRP were used as a control to exclude possible false-positive measurements, as previously described.[21]

2.3. In vivo: intravital microscopy and dural artery vasodilation

Rats were anaesthetized throughout the experiments using sodium pentobarbital (60 mg/kg, i.p. followed by 18 mg/kg intravenously [i.v.] per hour). The adequacy of anesthesia was judged by a negative tail flick test, mean arterial pressure levels, and the absence of ocular reflexes, among others. Tracheal intubation was performed, and a capnograph was used to monitor pCO$_2$ levels. As previously described,[50] the femoral vein and artery were cannulated for i.v. administration of drugs and for continuous monitoring of mean arterial pressure, respectively. During the experiment, the core temperature of each animal was maintained between 36.5°C and 37.5°C using a homeothermic blanket system for rodents (Harvard Instruments, Edenbridge, United Kingdom). Subsequently, the rat was placed in a stereotaxic frame, and the parietal bone was drilled thin until the dural middle meningeal artery was clearly visible. Because the rat skull

is thin, care was taken to drill with constant application of ice-cold saline. The drilled area was covered with saline to prevent drying of the skull and to facilitate visualization of the artery. The dural artery diameter was captured with an intravital microscope (Leica MZ 16; Leica Microsystem Ltd, Heerbrugg, Switzerland), using a cyan filter on a cold light source. A zoom lens (80-400x magnification) and camera (DCx V3.52, Thorlabs LTD, Ely, United Kingdom) were used to capture the image of the dural artery, which was displayed and measured on a computer using a dedicated software package (IDA-Intravital Dimension Analyser; http://www.beneryx.co.uk) integrated with a ADC/DAC board (DI-158, DATAQ instruments, 's-Hertogenbosch, The Netherlands). Dural artery diameter was calculated from the area under the curve of the intensity measured and expressed in arbitrary units. For periarterial electrical stimulation, a bipolar stimulating electrode (NE 200X, Clark Electromedical, Edenbridge, Kent, United Kingdom) was placed on the surface of the bone approximately 200 μM from the artery. The surface of the cranial window was stimulated at 5 Hz, 1 ms for 10 seconds (Stimulator model S88, Grass Instruments, West Warwick, RI) with a current intensity of 100 μA (monitored on an oscilloscope, model 54601A; Hewlett Packard, Palo Alto, CA) and increasing with 50-μA steps until a maximal level of vasodilation was achieved, usually at 200 μA.[23] Data of dural artery diameter and mean arterial blood pressure were recorded using a LabChart data acquisition system (AD Instruments Ltd, Oxford, United Kingdom). Dural artery vasodilator responses produced by periarterial electrical stimulation (100-200 μA), capsaicin (10 μg/kg, i.v.), or exogenous α-CGRP (1 μg/kg, i.v.) were elicited before and after consecutive i.v. bolus injections of vehicle (saline), sumatriptan, or lasmiditan (0.3, 1, 3, and 10 mg/kg each). These doses were based on clinically relevant doses previously calculated[40] as well as, for sumatriptan, previous studies performed in the same model.[50] In brief, for sumatriptan, assuming a 25 to 100 mg oral dose in a 70-kg adult with a 15% bioavailability would be equivalent to i.v. doses of about 0.05 to 0.21 mg/kg. For lasmiditan, a 50 to 200 mg oral dose in a 70-kg adult with a 40% bioavailability would be equivalent to i.v. doses of about 0.28 to 1.14 mg/kg. Each dose of the aforementioned compounds was administered 5 minutes before a subsequent treatment with electrical stimulation, capsaicin, or CGRP. Dural artery diameter and mean arterial pressure values, were both restored to preinjection levels by the time the next vasodilation was induced, and 30 minutes was allowed to elapse after each of the vasodilator treatments for the recovery of baseline diameter.

2.4. Statistical analysis

All data are expressed as mean ± SEM. Calcitonin gene-related peptide release in the different trigeminal components of the mice was expressed as relative stimulated CGRP release, which was calculated by the ratio of KCl-induced CGRP release and basal CGRP values.[12] Statistical differences of the basal CGRP values, the relative stimulated CGRP release in the presence of vehicle, and the % inhibition of CGRP release between groups were calculated using a one-tailed Mann–Whitney test for unpaired observations, as previously described.[8] For comparing the relative stimulated CGRP release in the presence of vehicle to its corresponding treatment (sumatriptan or lasmiditan), a one-tailed Wilcoxon matched-pairs test for nonparametric analysis of paired was used. The peak changes in dural artery diameter were expressed as percent change from baseline. Changes in mean arterial pressure were expressed in absolute values (mm Hg). The differences between the variables within one group of animals

were compared by using a one-way repeated-measures analysis of variance followed by Dunnett test. Statistical significance was accepted at $P < 0.05$.

2.5. Compounds

The compounds used in this study (obtained from the sources indicated) were: rat α-CGRP (NeoMPS S.A., Strasbourg, France); sumatriptan succinate and capsaicin (Sigma Chemical, Co, Steinheim, Germany); and lasmiditan hydrochloride (provided by Eli Lilly & Co, Indianapolis, IN). Calcitonin gene-related peptide, sumatriptan, and lasmiditan were dissolved in physiological saline. Capsaicin (1 mg/mL) was dissolved in a mixture of Tween-80, ethanol 70%, and water (1:1:8). The doses mentioned in the text refer to the free base of substances in all cases.

3. Results

3.1. Ex vivo: basal calcitonin gene-related peptide levels and relative stimulated calcitonin gene-related peptide release after KCl stimulation

A total of 108 tissues were analyzed. Tissues with basal CGRP measurement errors (CGRP below detection limit, n = 2) or that did not generate CGRP release in response to 60 mM KCl (n = 14) were excluded. In addition, in one experiment, the positive control (inhibition of CGRP by sumatriptan) failed (instead we measured an increase in CGRP release of >15 times); this measurement was a statistically significant outlier ($P = 0.016$, Dixon outlier test) and was therefore excluded. Because data were paired between the left side and right side, in these cases, both the left and right sides were excluded.

There were no significant differences between the basal CGRP levels from the left and right side components of the trigeminovascular system in each experimental group (data not shown). Moreover, basal CGRP levels (in absolute values; pg/mL) were not different between the sumatriptan (S) and lasmiditan (L) groups in the dura mater (S: 10.7 ± 1.8 vs L: 9.7 ± 3.0; n = 10 and 9 respectively; $P = 0.302$), trigeminal ganglion (S: 14.7 ± 3.3 vs L: 18.4 ± 10.2; n = 12 each; $P = 0.130$), and trigeminal nucleus caudalis (S: 25.9 ± 13.2 vs L: 48.5 ± 21.9; n = 10 and 9 respectively; $P = 0.219$). Moreover, the basal CGRP release values were not modified by the incubation per se of vehicle, sumatriptan, or lasmiditan in all the components of the trigeminovascular system studied (data not shown). The relative stimulated CGRP release (ie, the fold increase compared to baseline) induced by KCl in the presence of vehicle (control) was comparable between both groups in the dura mater (S: 6.0 ± 1.4 vs L: 6.4 ± 1.2; n = 10 and 9, respectively; $P = 0.275$), trigeminal ganglion (S: 5.4 ± 1.5 vs L: 7.4 ± 2.6; n = 12 each; $P = 0.267$), and trigeminal nucleus caudalis (S: 9.2 ± 2.8 vs L: 10.9 ± 3.0; n = 10 and 9 respectively; $P = 0.330$).

3.2. Ex vivo: relative stimulated calcitonin gene-related peptide release in the presence of sumatriptan and lasmiditan

The effects of pretreatment with sumatriptan or lasmiditan (30 μM) on CGRP release in the trigeminovascular components are shown in **Figure 1**. In the presence of sumatriptan, relative stimulated CGRP release was significantly attenuated in the dura mater (6.0 ± 1.4 vs 3.0 ± 0.5; n = 10; $P = 0.032$), trigeminal ganglion (5.4 ± 1.5 vs 2.2 ± 0.6; n = 12; $P = 0.013$), and trigeminal nucleus caudalis (9.2 ± 2.8 vs 2.8 ± 0.7; n = 10; $P = 0.032$).

Moreover, lasmiditan also significantly attenuated the relative stimulated CGRP release in the dura mater (6.4 ± 1.2 vs 2.6 ± 0.5;
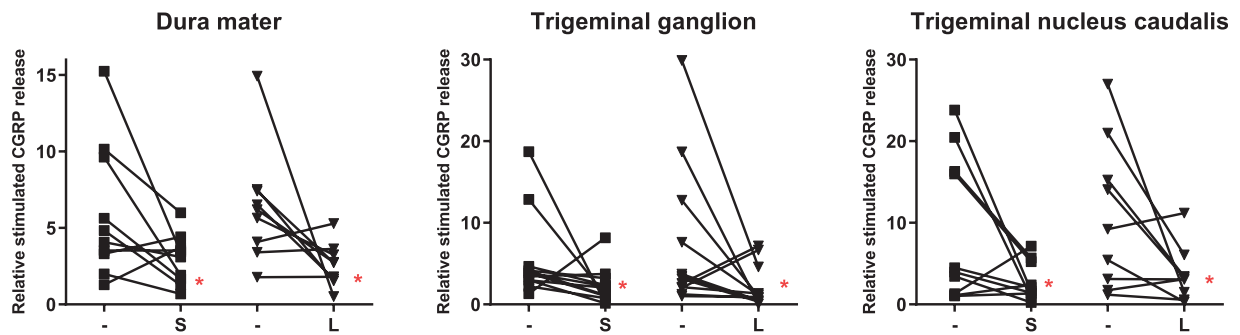
Figure 1. Relative stimulated CGRP release after KCl in the absence (−) or presence of sumatriptan (S) and lasmiditan (L) in the dura mater (n = 9-10), trigeminal ganglion (n = 12), and trigeminal nucleus caudalis (n = 9-10). *P < 0.05 vs vehicle response. CGRP, calcitonin gene-related peptide.

n = 9; P = 0.027), trigeminal ganglion (7.4 ± 2.6 vs 2.1 ± 0.7; n = 12 P = 0.032), and trigeminal nucleus caudalis (10.9 ± 3.0 vs 3.6 ± 1.1; n = 9; P = 0.037). Furthermore, both compounds were equieffective in inhibiting CGRP release in the dura mater (S: 50% vs L: 59%; n = 9-10; P = 0.257), trigeminal ganglion (S: 59% vs L: 71%; n = 12; P = 0.244), and trigeminal nucleus caudalis (S: 70% vs L: 67%; n = 9-10; P = 0.275).

3.3. In vivo: effects of ES, capsaicin, and calcitonin gene-related peptide on mean arterial pressure and dural diameter

At the beginning of the experiments, the average mean arterial pressure from all animals was 99 ± 2 mm Hg. Because there were no significant differences on the hemodynamic and dural artery changes in the control interventions (ES, capsaicin, and CGRP) between groups, data were pooled for further analysis. In none of the experiments, periarterial ES (100-200 μA) affected the mean arterial pressure. By contrast, there was a significant decrease in mean arterial pressure as compared to baseline after the i.v. administration of 10 μg/kg capsaicin (27 ± 5 mm Hg, n = 19) or 1 μg/kg CGRP (29 ± 4 mm Hg, n = 14), which was not different between capsaicin and CGRP (P = 0.744). At the end of the experiments, the value of mean arterial pressure (97 ± 2 mm Hg) was not significantly different from the initial baseline value (P = 0.140; n = 53).

3.4. In vivo: dural artery dilation induced by electrical stimulation, capsaicin, and calcitonin gene-related peptide

Periarterial ES (100-200 μA) increased dural artery diameter by 62 ± 3% (n = 20), whereas i.v. administration of 10 μg/kg capsaicin or 1 μg/kg CGRP increased the diameter by 59 ± 3% (n = 20) and 66 ± 3% (n = 14), respectively. In accordance with our previous controls, repeated treatment (up to 5 times) with electrical stimulation (P = 0.349; n = 4), capsaicin (P = 0.878; n = 4), or CGRP (P = 0.912; n = 4) produced reproducible increases in dural artery diameter.

3.5. In vivo: effects of sumatriptan and lasmiditan per se on mean arterial pressure and dural diameter

Because there were no significant differences on the hemodynamic and dural artery effects among sumatriptan (or lasmiditan) doses in the experimental interventions, data were pooled for further analysis. As shown in **Figure 2** (upper panels), sumatriptan injection produced a significant (P < 0.0001, n = 21) short-lasting and dose-dependent vasodepressor response starting at clinically relevant doses. After each injection, mean arterial pressure

returned to baseline values before the next systemic vasodilation was elicited. By contrast, lasmiditan was devoid of significant (P = 0.274, n = 20) effects on mean arterial blood pressure at all doses tested. When considering dural artery diameter (**Fig. 2**; lower panels), all doses of sumatriptan significantly (P < 0.0001, n = 21) and dose-dependently increased the artery diameter, whereas lasmiditan only increased it significantly (P = 0.006, n = 20) after injecting the 2 highest doses.

3.6. In vivo: effects of sumatriptan and lasmiditan on dural artery dilation induced by electrical stimulation, capsaicin, and calcitonin gene-related peptide

As shown in **Figure 3**, as compared to their corresponding control, the pretreatment with high doses (3 and 10 mg/kg) of sumatriptan significantly inhibited the vasodilation induced by electrical stimulation (control increase in dural artery diameter: 60 ± 3% vs 3 mg/kg: 30 ± 3%; 10 mg/kg: 21 ± 2%; P < 0.0001; n = 8) and capsaicin (control: 65 ± 6% vs 3 mg/kg: 44 ± 3%; 10 mg/kg: 34 ± 3%; P < 0.0001; n = 8). By contrast, i.v. dural artery diameter responses to exogenous (i.v.) CGRP were not affected by any dose of sumatriptan (P = 0.210; n = 5).

In contrast to sumatriptan, already lower doses of lasmiditan induced a significant dose-dependent attenuation of the vasodilator responses to electrical stimulation (control increase in dural artery diameter: 61 ± 2% vs 0.3 mg/kg: 41 ± 4%; 1 mg/kg: 39 ± 3%; 3 mg/kg: 34 ± 3%; 10 mg/kg: 21 ± 2%; P < 0.0001; n = 8) and capsaicin (control: 56 ± 5% vs 0.3 mg/kg: 38 ± 7%; 1 mg/kg: 31 ± 5%; 3 mg/kg: 30 ± 5%; 10 mg/kg: 23 ± 4%; P < 0.0001; n = 8) as compared to their corresponding control. Similar as with sumatriptan, the exogenous CGRP responses were not affected by any dose of lasmiditan (P = 0.911; n = 5).

4. Discussion

In this study, we investigated the potential antimigraine site of action of the selective 5-HT$_{1F}$ receptor agonist lasmiditan in relation to the trigeminovascular CGRPergic system, through inhibition of chemically induced (KCl and capsaicin, through voltage-gated calcium and TRPV1 channel activation, respectively) and electrically induced CGRP release from trigeminal sensory fibers.

We compared our results with data obtained with sumatriptan because this was the first triptan developed, and this class of drugs is the current gold standard for the specific acute treatment of migraine attacks. In accordance with our previous work,[8] pretreatment with 30-μM sumatriptan attenuated KCl-relative stimulated CGRP release in the peripheral and central components of the trigeminovascular system of mice ex vivo (**Fig. 1**). This
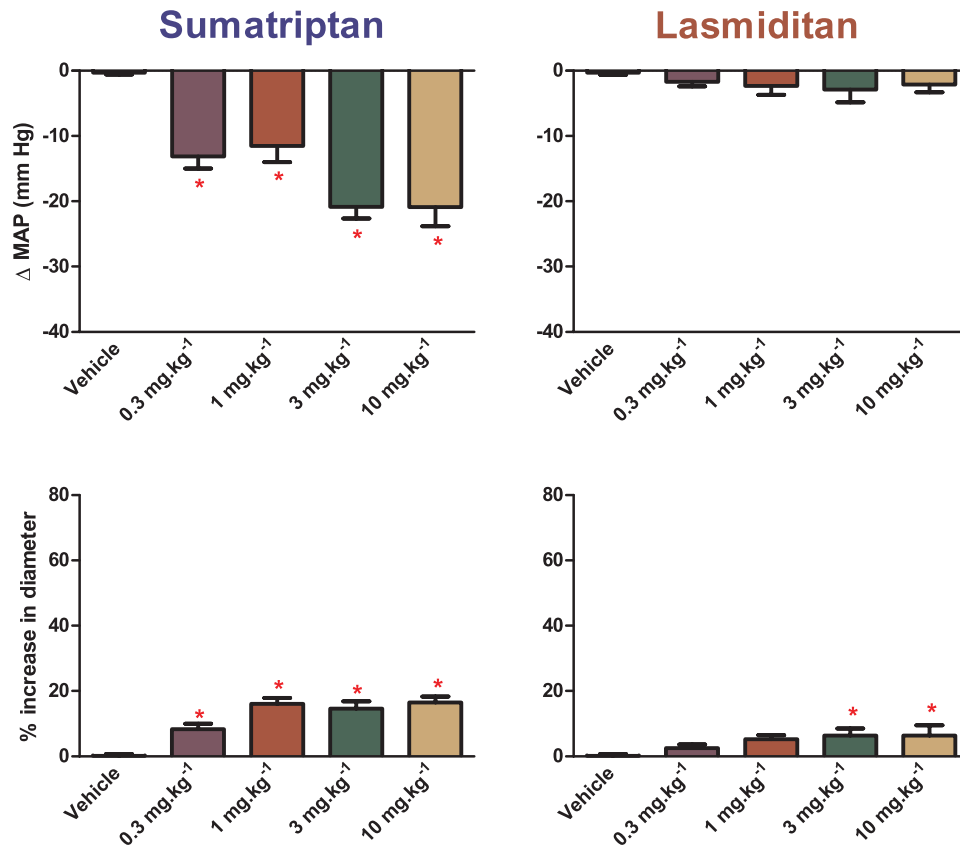
Sumatriptan   Lasmiditan

Figure 2. Effects per se of increasing doses of sumatriptan or lasmiditan on mean arterial pressure (MAP) and dural artery diameter. n = 20 to 21. *$P < 0.05$ vs vehicle response.

inhibition has previously been shown to be mediated through activation of 5-HT$_{1D}$ and 5-HT$_{1F}$ receptors in rats, whereas a lower concentration (3 μM) of sumatriptan was ineffective.[3] Moreover, 30-μM lasmiditan was equieffective in attenuating CGRP release in the dura mater, trigeminal ganglion, as well as trigeminal nucleus caudalis; however, because theoretically multiple 5-HT$_1$ (ie, 5-HT$_{1A}$ and 5-HT$_{1F}$) inhibitory-G$_i$ protein receptor subtypes expressed in the mice trigeminovascular system[7] could be activated at the nonselective concentration used,[40] we performed more in-depth experiments with a range of clinically relevant doses in the in vivo rat closed cranial window, an experimental neurovascular model of migraine.[50]

In accordance with the ex vivo experiments, high doses (3 and 10 mg/kg) of sumatriptan were required to inhibit the neurogenic dural vasodilation in response to i.v. capsaicin and periarterial electrical stimulation. These high-dose inhibitory effects have been previously shown with sumatriptan, and also with riza-triptan, whereas low doses of both compounds (1 mg/kg) were ineffective in this model.[50,51] Based on data available on receptor affinity profiles and pharmacological antagonism,[5,41] the inhibition of CGRP release by triptans seems to be mediated through activation of 5-HT$_{1D}$ and possibly also 5-HT$_{1F}$ receptors; unfortunately, highly selective antagonists for the 5-HT$_{1F}$ receptors are not yet commercially available to further determine the receptor(s) involved. Remarkably, lasmiditan inhibited the neurogenic dural vasodilation in response to i.v. capsaicin and periarterial electrical stimulation starting at lower, clinically relevant doses; this effect is most likely mediated by selective activation of the 5-HT$_{1F}$ receptor. By contrast, like sumatriptan, lasmiditan was not capable of attenuating the nonneurogenic

dural vasodilation in response to exogenous CGRP. Thus, we demonstrate that lasmiditan prejunctionally inhibits the release of CGRP from trigeminal sensory fibers innervating the dural vasculature.

The activation of either 5-HT$_{1B}$, 5-HT$_{1D}$, or 5-HT$_{1F}$ receptors on trigeminal fibers results in a direct inhibition of adenylyl cyclase and a subsequent decrease in the cAMP-signaling pathway,[1] which in turn alters the phosphorylation of TRPV1 channels[6] and decreases the release of CGRP, as evidenced by the inhibition of CGRP- and capsaicin-induced dural vasodilation by sumatriptan and lasmiditan. In addition, activation of 5-HT$_1$ receptors also inhibits neurotransmitter release occurring after stimulation with KCl or electrical stimulation.[8,18,20] However, if both compounds act through the same second messenger cascade, why does agonism of the 5-HT$_{1F}$ receptor seem to be more effective?

Although the mRNA expression of the 5-HT$_{1D}$ and 5-HT$_{1F}$ receptors has been detected in the peripheral and central components of the rat trigeminovascular system,[3] and both receptors have been found to be colocalized with CGRP,[2,34] a small study (n = 3) found that the 5-HT$_{1D}$ receptor is more abundantly expressed in the trigeminal ganglion, whereas the highest concentration of 5-HT$_{1F}$ receptors was found in the trigeminal nucleus caudalis, and this expression pattern could result in diverse inhibition profiles.[3] However, more in-depth studies have found that the 5-HT$_{1B}$, 5-HT$_{1D}$, and 5-HT$_{1F}$ receptors are equally expressed in the trigeminal ganglion.[9,39] Therefore, the difference between sumatriptan and lasmiditan inhibition results does not seem to be explained by the expression/density profile of the 5-HT$_{1B/1D/1F}$ receptors, and may involve (although this study does not prove it) drug-dependent
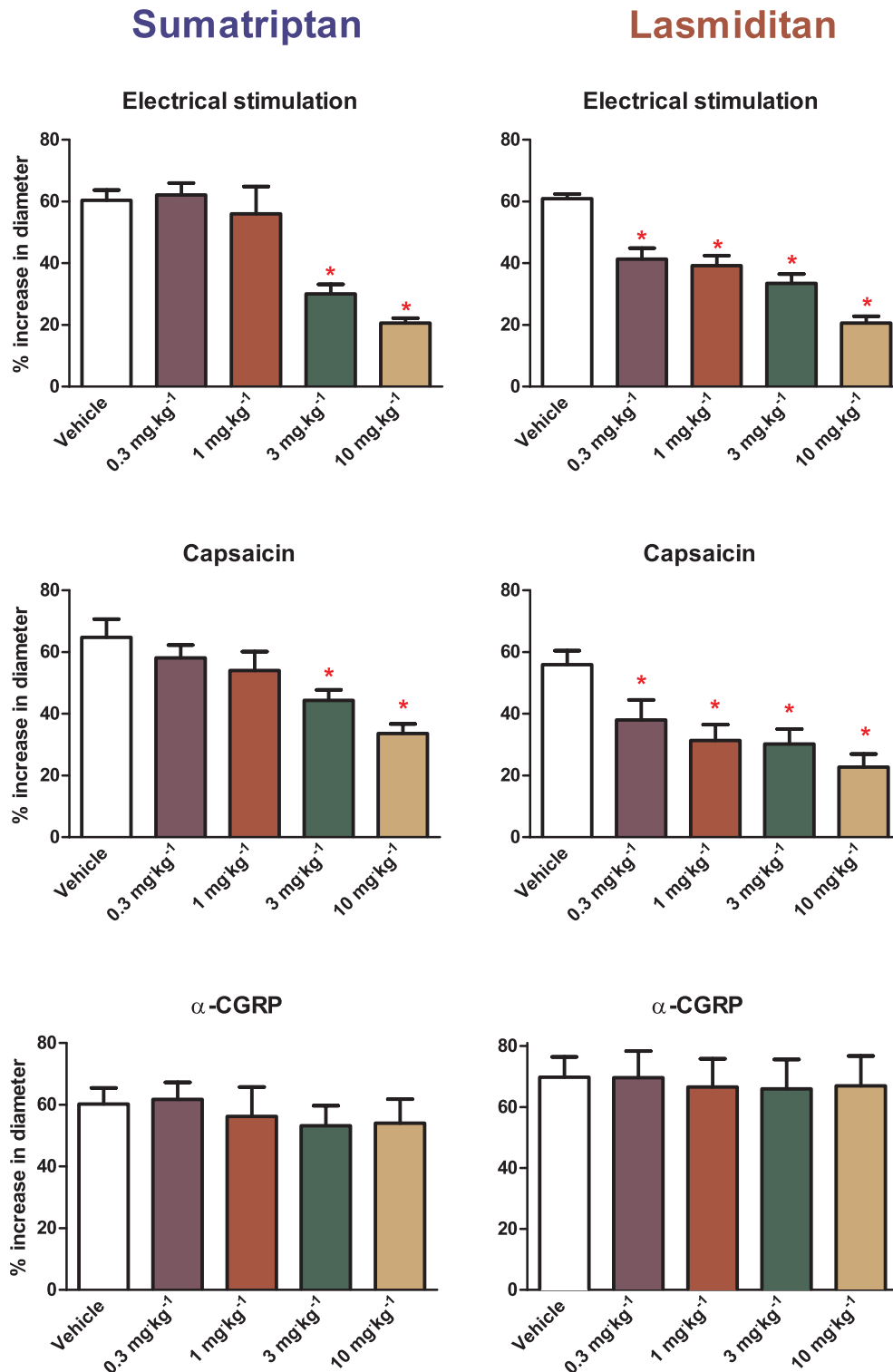
Figure 3. Effects of increasing doses of sumatriptan or lasmiditan on the vasodilation of the dural artery induced by electrical stimulation (upper panels, n = 8 each), capsaicin (middle panels, n = 8 each), and CGRP (lower panels, n = 5 each). *$P < 0.05$ vs vehicle response. CGRP, calcitonin gene-related peptide.

factors such as the affinity and intrinsic activity of these compounds for each of these receptors at the specific components of the trigeminovascular system.

It is noteworthy that species differences have been described for the 5-HT$_1$ receptor subtypes mediating the inhibition of trigeminal CGRP release by 5-HT$_{1B}$ and 5-HT$_{1D}$ receptor agonists (including sumatriptan). This inhibition has previously

been attributed to activation of 5-HT$_{1B}$ receptors in rats and mice, and 5-HT$_{1D}$ receptors in guinea pigs, cats, and humans.[49] Moreover, the rat and mouse 5-HT$_{1B}$ receptor has been considered to represent the counterpart to the 5-HT$_{1D}$ receptor in other species.[24] Thus, sumatriptan's receptor affinity for the 5-HT$_1$ receptors in rats (5-HT$_{1D}$ >5-HT$_{1B}$) correlates with the high doses required to inhibit CGRP release through 5-HT$_{1B}$ receptors

and, possibly also, 5-HT$_{1F}$ receptors. In addition, as described above, the CGRPergic system is influenced by cycling estrogen, which we have previously described in both the human[26] and rodent[22] trigeminovascular system, whereas a link between testosterone and CGRP release remains to be demonstrated. Thus, in future studies, it would be interesting to investigate the effects of lasmiditan on trigeminovascular CGRP release in female animals with different hormonal status, but also in male animals that are intact or orchiectomized.

It should be kept in mind that our rodent model is suitable for studying inhibition of CGRP release, but not hemodynamic changes in response to 5-HT$_1$ receptor stimulation. In support of this is the fact that lower doses of i.v. sumatriptan immediately activated systemic vascular 5-HT receptors before reaching the 5-HT$_{1D}$ (and later 5-HT$_{1B}$) receptors in the trigeminal sensory fibers. Consequently, the injection of already clinically relevant doses of sumatriptan resulted in a significant drop in mean arterial pressure and dural artery vasodilation (rather than vasoconstriction; **Fig. 2**), which have been shown to be mediated through activation of 5-HT$_7$ receptors in rodents.[46] However, as previously pointed out, rodents are not suitable for studying hemodynamic changes associated with 5-HT$_1$ receptor activation because receptor expression and vascular responses are not similar to nonrodent species, including humans. Hence, we have recently addressed the vasoconstrictor effects of sumatriptan in appropriate and translational (human isolated arteries and canine in vivo) models.[40] Similar to our previous findings, i.v. infusions of sumatriptan, through 5-HT$_{1B}$ receptor activation,[32] increased systemic and pulmonary arterial pressure in humans,[36] which represents a major limitation for triptans use in cardiovascular compromised patients. By contrast, lasmiditan was devoid of hemodynamic effects at all doses tested. Thus, as demonstrated by the above-mentioned studies, clinically relevant doses of lasmiditan are not associated with changes in mean arterial pressure or dural artery diameter. These results suggest that the antimigraine efficacy of lasmiditan is unrelated to direct vasoconstrictive mechanisms, although the inhibition of CGRP release may indirectly affect dural artery diameter.

The 5-HT$_{1F}$ receptor is also widely expressed throughout the central nervous system, and because lasmiditan is a lipophilic drug that crosses the blood–brain barrier (in contrast with sumatriptan), it has been associated with mild central side effects. The most common side effects described in clinical trials are dizziness, paresthesia, somnolence, fatigue, nausea, and lethargy.[17,28] Given that lasmiditan inhibits the peripheral components of the trigeminovascular system, it is tempting to speculate what would happen to the side-effect profile or antimigraine efficacy of a selective 5-HT$_{1F}$ receptor agonist that does not enter the brain. Whether inhibiting the trigeminovascular system centrally as well as peripherally is more effective than just peripherally or centrally, or whether a mixed mechanism is associated with increased side effects remains to be determined. Unfortunately, there are no hydrophilic (selective) 5-HT$_{1F}$ receptor agonists available yet.

Additional (antimigraine) mechanisms of action described with previous 5-HT$_{1F}$ receptor agonists include modulation of glutamate release from trigeminal sensory fibers and mitochondrial biogenesis.[38,42] Clearly, future experiments, beyond the scope of our study, are needed to determine whether lasmiditan can inhibit glutamatergic neurons in the central nervous system and/or enhance mitochondrial biogenesis. Moreover, it would be interesting to evaluate whether lasmiditan is able to attenuate cortical spreading depression, a key pathogenic event in migraine with aura,[47] and whether these mechanisms are also associated with its clinical antimigraine efficacy.

In conclusion, our results indicate that lasmiditan prejunctionally inhibits CGRP release in peripheral and central trigeminal nerve terminals. This effect may, at least partly, explain the clinical efficacy of lasmiditan. Because activation of 5-HT$_{1F}$ receptors is not associated with vasoconstriction, lasmiditan may represent a cardiovascular safety advantage over the vasoactive triptans.

Conflict of interest statement

Acknowledgments

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