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ACCELERATED COMMUNICATION

5-Carboxamido-tryptamine, CP-122,288 and Dihydroergotamine but not Sumatriptan, CP-93,129, and Serotonin-5-*O*-carboxymethyl-glycyl-tyrosinamide Block Dural Plasma Protein Extravasation in Knockout Mice that Lack 5-Hydroxytryptamine$_{1B}$ Receptors

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SUMMARY

We studied the dural plasma protein extravasation response after unilateral electrical stimulation of the trigeminal ganglion in mice lacking serotonin 5-HT$_{1B}$ (5-HT$_{1D\beta}$) receptors by modifying a technique previously described in rats or guinea pigs. We investigated the inhibitory effects of six 5-HT$_1$ receptor agonists in this model: 3-(1,2,5,6-tetrahydropyrid-4-yl)pyrrolo[3,2-*b*]pyrid-5-one (CP-93,129), sumatriptan, serotonin-5-*O*-carboxymethyl-glycyl-tyrosinamide (GTI), 5-methylaminosulfonylmethyl-3-(*N*-methylpyrrolidin-2*R*-ylmethyl)-1*H*-indole (CP-122,288), 5-carboxamido-tryptamine (5-CT), and dihydroergotamine. The plasma extravasation response did not differ between wild-type and mutant after vehicle injection. The potency of sumatriptan, CP-122,288, CP-93,129, and 5-CT in wild-type mice was similar to that previously reported for rats. CP-122,288 (1 nmol kg), 5-CT (1 nmol/kg), and dihydroergotamine (72 nmol/kg) inhibited plasma protein extravasation within dura mater after electrical trigeminal ganglion stimulation in both wild-type and knockout mice, which suggests that these agonists act predominantly via receptors other than 5-HT$_{1B}$. Unlike in wild-type mice, CP-93,129 (1.4 μmol/kg), a specific 5-HT$_{1B}$ receptor agonist, had no effect in knockout mice. The same held true for sumatriptan (0.7 μmol/kg) and GTI (0.6 μmol/kg). These results suggest that CP-93,129, sumatriptan, and GTI exert their effects via 5-HT$_{1B}$ (5-HT$_{1D\beta}$) receptors in mice.

Neurogenic inflammation within the meninges has been proposed as an important event in the pathogenesis of migraine headaches (1). Abortive migraine drugs such as sumatriptan and ergot derivatives block neurogenic inflammation within dura mater, a response that was proposed to be mediated by 5-HT$_{1B}$ (rats) or 5-HT$_{1D}$ (guinea pigs) receptors located prejunctionally on trigeminovascular fibers (2). However, the potency of 5-CT and two conformationally restricted sumatriptan derivatives (CP-122,288 and 5-methylaminosulfonylmethyl-3-(pyrrolidin-2*R*-ylmethyl)-1*H*-indole) in this model is several orders of magnitude higher than that predicted from their affinities at 5-HT$_{1D}$ receptors (3, 4). In addition, the 5-HT$_1$ receptor antagonist metergoline does not block the effects of 5-CT and only partially reverses those of sumatriptan and CP-122,288. It has been suggested that more than a single receptor subtype or mechanism may be involved (4).

Molecular cloning techniques have revealed the existence

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ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; 5-CT, 5-carboxamido-tryptamine; GTI, serotonin-5-*O*-carboxymethyl-glycyl-tyrosinamide; DHE, dihydroergotamine; CP-122,288, 5-methylaminosulfonylmethyl-3-(*N*-methylpyrrolidin-2*R*-ylmethyl)-1*H*-indole; CP-93,129, 3-(1,2,5,6-tetrahydropyrid-4-yl)pyrrolo[3,2-*b*]pyrid-5-one; BSA, bovine serum albumin.

of two subtypes of 5-HT_{1D} receptors, 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ (5). In humans and guinea pigs, the pharmacological profiles of these receptors are almost identical, although the 5-HT_{2A} receptor antagonists ketanserin and ritanserin have been shown to preferentially block the former subtype (6). Rats and mice also possess two subtypes of 5-HT_{1D} receptors, but a single mutation in their 5-$HT_{1D\beta}$ gene confers a very distinct pharmacology to the encoded receptors, which is most commonly called 5-HT_{1B} (5, 7). In all species investigated, the 5-$HT_{1D\beta}$ (5-HT_{1B}) receptor subtype seems to predominate in the brain parenchyma (8, 9), where they fulfill the same function [i.e., presynaptic inhibition of transmitter release (10)]. 5-$HT_{1D\beta}$ receptors are also present in vascular tissues (11).

Both 5-HT_{1D} receptor subtypes are part of the larger family of 5-HT_1 receptors that comprises 5-HT_{1A}, 5-HT_{1E}, and 5-HT_{1F} receptors (12). The latter subtype is of particular interest, as it also displays a high affinity for sumatriptan and CP-122,288 (13). In addition, 5-CT has been shown to bind with a high affinity to 5-HT_5 and 5-HT_7 receptor recognition sites (14).

In the absence of selective antagonists, we used knockout mice lacking 5-HT_{1B} receptors to investigate further which receptor subtypes mediate the inhibitory effects of 5-HT_1 agonists in the trigeminovascular system.

Materials and Methods

5-HT_{1B} knockout mice were generated as described previously (15) at Columbia University (New York, NY). Wild-type SV-129 mice were obtained from Charles River Laboratories (Wilmington, MA). All animals weighed 23–30 g. Electrical trigeminal stimulation was performed on mice anesthetized with pentobarbitone (100 mg/kg intraperitoneal) and placed in a stereotaxic frame (DKI900, David Kopf Instruments, Tujunga, CA) with the incisor bar set at 0 mm. Symmetrical burr holes were drilled 1.5 mm lateral and 2.0 mm posterior from bregma. ^{125}I-BSA (200 μCi/kg)was injected in the femoral vein. After 5 min, electrodes were lowered 5.5 mm from dura mater. The right ganglion was stimulated (5 min, 0.6 mA, 5 Hz, 5-msec duration) (Pulsemaster A300 and Stimulus Isolator A365, World Precision Instruments, San Carlos, CA). At 10 min before stimulation and 5 min before ^{125}I-BSA administration, animals were injected intravenously with CP-93,129, sumatriptan, GTI, CP 122,288, 5-CT, and DHE. Immediately after stimulation, the animals were perfused via the left cardiac ventricle with 10 ml of saline for 2 min to flush out the tracer from the lumen of blood vessels. The skull was then opened, the brain was removed, and the cranial cavity was rinsed with saline. The dura mater was dissected bilaterally and quickly rinsed with saline, and radioactivity was determined on each side with a γ-counter (Micromedic 4/600, Micromedic Systems, Huntsville, AL) as previously described (1).

In preliminary experiments, capsaicin and substance P were also administered in to establish the model in the mouse. The left femoral vein was exposed in pentobarbitone-anesthetized mice. Five minutes later, ^{125}I-BSA (200 μCi/kg) was injected as a bolus. After an additional 5 min, capsaicin (4 μmol/kg) or substance P (40 nmol/kg) was infused over 3 min in the femoral vein. Ten minutes later, animals were perfused with saline, and the dura mater was dissected on both sides as described above.

^{125}I-BSA (New England Nuclear, Boston, MA) was diluted in saline. Sumatriptan (Glaxo Ware, Hertfordshire, UK), DHE, 5-CT (Research Biochemicals, Natick, MA), and GTI (Immunotech, Marseille, France) were dissolved in 0.9% saline or distilled water. CP-93,129 and CP-122,288 (Pfizer Inc., Groton, CT) were dissolved in dimethysulfoxide/saline (1:9) and diluted 10 times with saline for injection.

Values are given as mean ± standard error. ^{125}I-BSA extravasation is expressed as a ratio [cpm/μg of wet weight (stimulated side)/cpm/μg of wet weight (unstimulated side)]. In experiments with capsaicin or substance P, the ratio is expressed as percent cpm/mg (wet weight) in substance P- or capsaicin-treated animals compared with vehicle-treated animals. Paired t test was used only for statistical comparison between stimulated and unstimulated sides. To compare the extravasation ratios after the different pretreatments, analysis of variance followed by Dunnett's multiple-range test was performed in wild-type mice (values were obtained after vehicle administration were pooled). For the mutant mice, each treatment was compared with its own vehicle values with the use of t test. Values of $p < 0.05$ were considered significant.

Results

Unilateral electrical stimulation of the trigeminal ganglion increased leakage of iodinated albumin within dura mater of vehicle-treated animals from 650 ± 64 to 1090 ± 108 cpm/mg wet weight (knockout mice, $p < 0.001$, 19 experiments) and from 720 ± 50 to 1200 ± 96 cpm/mg wet weight (wild-type mice, $p < 0.001$, 23 experiments). The ratios between the stimulated and unstimulated sides were 1.70 ± 0.05 and 1.70 ± 0.04 in knockout and wild-type mice, respectively.

Intravenously administered capsaicin and substance P also caused the leakage of tracer in dura mater: 171 ± 20% (capsaicin; six experiments) and 168 ± 15% (substance P; eight experiments) of extravasation compared with vehicle-treated animals.

Dose-response curves for these agonists are shown in Fig. 1. CP-122,288 and 5-CT yielded shallow dose-response curves (Hill coefficient = 0.43 ± 0.14 and 0.56 ± 0.15, respectively), whereas Hill coefficients for sumatriptan and CP-93,129 were not significantly different from 1. The pEC_{50} values (−log mol/kg ± standard error) for sumatriptan, CP-93,129, CP-122,288, and 5-CT in wild type mice were 6.3 ± 0.2, 6.8 ± 0.03, 11.6 ± 0.3, and 11.9 ± 0.02, respectively.

The effects of pretreatment of wild-type and knockout mice with CP-93,129 (1.4 μmol/kg), sumatriptan (0.7 μmol/kg),

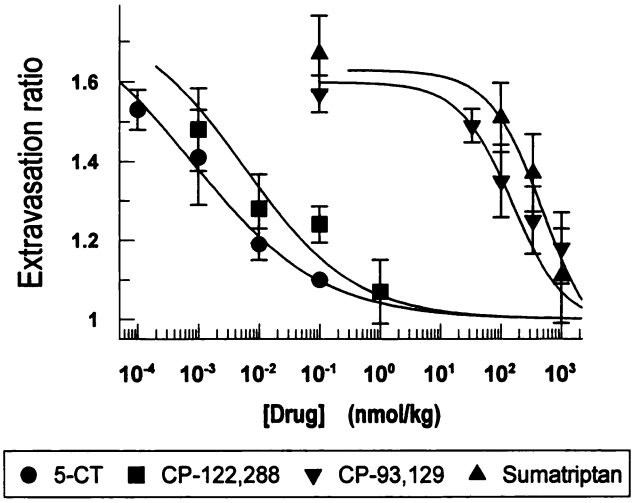

Fig. 1. Dose-response curves of sumatriptan, CP-93,129, CP-122,288, and 5-CT on the leakage of ^{125}I-BSA in the dura mater (stimulated side divided by nonstimulated side; see text for experimental details) of wild-type mice after electrical stimulation of the trigeminal ganglion. CP-122,288 and 5-CT display shallow dose-response curves and are 3 orders of magnitude more potent than sumatriptan and CP-93,129, which suggests that they act at multiple sites.

CP-122,288, 5-CT (1 nmol/kg), GTI (0.6 μmol/kg), and DHE (72 nmol/kg) are shown in Table 1. The drugs did not affect the absolute amount of extravasated ^{125}I-BSA on the unstimulated side (not shown).

Discussion

The characterization of the 5-HT receptor subtype that mediates the inhibition of plasma protein extravasation by sumatriptan, 5-CT, DHE, CP-93,129, and CP-122,288 has been hampered by the lack of a selective antagonist (2–4). In a previous study, metergoline partially inhibited the effect of sumatriptan and CP-122,288 (3) but was ineffective against 5-CT. At high concentrations (1 mg/kg), metergoline showed significant agonist activity in the rat (3). In the absence of a suitable antagonist, we used mutant mice with a deletion of the 5-HT$_{1B}$ receptor gene; this study shows that the profile of the receptor(s) that mediate the inhibition of plasma protein extravasation in the mouse is very similar to that previously characterized in rats (3). It is also similar to that described in the guinea pig (4),[2] with the exception that the selective 5-HT$_{1B}$ agonist CP-93,129 is inactive in this species (16). More importantly, the present study shows that sumatriptan, CP-93,129, and GTI exert their effect prominently via 5-HT$_{1B}$ receptors in the mouse. Finally, the fact that 5-CT, CP-122,288, and DHE retain their activity in knockout mice indicates that they act on a site other than 5-HT$_{1B}$ receptors.

In addition to 5-HT$_{1B}$ (5-HT$_{1D\beta}$) receptors, CP-122,288 has a high affinity for 5-HT$_{1D\alpha}$ and 5-HT$_{1F}$ receptors (13). However, an action at this site can be ruled out, as sumatriptan also displays a high affinity for these sites and is inactive in knockout mice. 5-CT selectivity is poor, as it shows a high affinity for 5-HT$_{1A}$, 5-HT$_{5A}$, and 5-HT$_7$ receptors (14). An action at 5-HT$_{1A}$ receptors is unlikely, as the selective 5-HT$_{1A}$ agonist 8-hydroxy-dipropylaminotetralin acts weakly in the extravasation model (3). The affinity of CP-122,288 at rat and guinea pig 5-HT$_7$ receptors is low (17), which rules out a possible role of these receptors in the inhibition of plasma extravasation by this agonist. Finally, methiothepin is an antagonist at 5-HT$_{1A}$, 5-HT$_{5A}$, and 5-HT$_7$ receptors, and it does not affect the inhibition of extravasation by 5-CT (3).

The fact that neither metergoline nor methiothepin is able to block the response to 5-CT is puzzling, as these antagonists have been shown to block most of the known 5-HT receptor subtypes, except 5-HT$_3$ and 5-HT$_4$ (14). This fact, taken together with the unusually high potency of 5-CT, might suggest that this drug acts on a site that is not a known 5-HT receptor. The existence of a large receptor reserve could also account for the lack of effect of the antagonists that have been tested to date. The shallow dose-response curves of 5-CT and CP-122,288 contrast with those obtained with CP-93,129 and sumatriptan; these patterns are identical to those observed in the guinea pig (4)[3] and suggest that 5-CT and CP-122,288 act on multiple targets.

There are no obvious structural characteristics to explain why CP-122,288, 5-CT, and DHE, and not sumatriptan and GTI, are active in knockout mice. All compounds contain an indole moiety. CP-122,288 is a conformationally restricted sumatriptan derivative, in which the aminoethyl side chain has been constrained within a stereogenic pyrrolidine ring (18). DHE also contains its aminoethyl side chain in a conformationally restricted fashion. However, its structural constraint seems to be different from that in CP-122,288. Although both DHE and CP-122,288 are tertiary amines, 5-CT is a primary amine with no conformational constraint in its aminoethyl side chain. The structural characteristics of these three compounds seem to be disparate. However, comparisons of 5-benzyloxytryptamine with 5-CT and comparison of sumatriptan with CP-122,288 might shed some light on the structural requirements for potent inhibition of neurogenic inflammation. Although 5-benzyloxytryptamine has not been tested in the knockout mice, it has been shown in the rat to be more than 1000 times less potent than 5-CT (3). 5-CT and 5-benzyloxytryptamine differ only in their functional group at C5 of the indole template. The carboxamide in 5-CT can function within a receptor as both a hydrogen bond donor and an acceptor, whereas the benzyloxy group in 5-benzyloxytryptamine can only function as a hydrogen bond acceptor.

Examination of 5-CT, DHE, and CP-122,288 reveals that all three compounds possess strong hydrogen bond donors proximate to the C5 position of their indole rings, which indicates that feature as an important structure-activity relationship requirement for potent inhibition of neurogenic inflammation. Sumatriptan and CP-122,288 differ only in

[2] X.-J. Yu and M. A. Moskowitz ,unpublished observations.

[3] X.-J. Yu and M. A. Moskowitz, unpublished observations.

TABLE 1

Effects of pretreatment with various 5-HT$_1$ receptor agonists on the extravasation ratio ± standard error of ^{125}I-BSA in the dura mater of wild-type and 5-HT$_{1B}$ knockout mice after electrical stimulation of the trigeminal ganglion

Pretreatment	Wild-type mice (n = 3–4)	5-HT$_{1B}$ knockout mice	
		Corresponding vehicle (n)	Treatment (n)
Vehicle	1.70 ± 0.04[a]		
CP-93,129 (1.4 μmol/kg)	1.12 ± 0.08[b]	1.71 ± 0.09 (7)	1.65 ± 0.09 (7)[c]
Sumatriptan (0.7 μmol/kg)	1.09 ± 0.03[b]	1.67 ± 0.06 (7)	1.54 ± 0.06 (7)[c]
GTI (0.6 μmol/kg)	1.15 ± 0.06[b]	1.76 ± 0.16 (3)	1.63 ± 0.12 (4)[c]
5-CT (1 nmol/kg)	1.11 ± 0.03[b]	1.74 ± 0.10 (5)	1.00 ± 0.08 (6)[d]
CP-122,288 (1 nmol/kg)	1.12 ± 0.11[b]	1.64 ± 0.06 (5)	1.19 ± 0.07 (7)[d]
Dihydroergotamine (72 nmol/kg)	1.23 ± 0.18[b]	1.76 ± 0.16 (3)	1.31 ± 0.11 (4)[d]

[a] n = 19.
[b] $p < 0.05$ compared with the vehicle-treated group (analysis of variance followed by Dunnett's).
[c] no significant difference as compared with the vehicle-treated knockout group.
[d] $p < 0.05$ compared with the corresponding vehicle-treated group (t test).

their presentation of their aminoethyl side chains to a receptor. The conformational restriction in CP-122,288 apparently places the aminoethyl side chain in the low energy conformation recognized by the receptor that mediates inhibition of neurogenic inflammation. The flexibility of the *N,N*-dimethylaminoethyl group in sumatriptan might preclude it from easily adopting that recognized conformation. These structure-activity relationship interpretations suggest that the primary amine analogue of sumatriptan [i.e., 5-aminomethylsulfonylmethyl-3-(2-aminoethyl)indole] should have significantly increased activity in our model compared with sumatriptan, whereas the tertiary amine analogue of 5-CT [i.e., 5-carboxamido-3-(2-*N,N*-dimethylaminoethyl)indole] should be significantly less potent than 5-CT itself. Efforts are under way to examine these hypotheses.

It is not clear whether 5-CT, CP-122,288, and DHE act on the same unknown site. Although 5-CT and CP-122,288 are not known to possess significant affinity for non-5-HT receptors, DHE is known to be a potent agent (i.e., affinity value <100 nM) at dopamine D_2 and α_1- and α_2-adrenergic binding sites in addition to 5-HT_{1A}, 5-HT_{1D}, 5-HT_{2C}, 5-HT_{2A} (19), and 5-HT_7 receptors (20). DHE also displays moderate affinity (i.e., affinity values = 100-1000 nM) for β-adrenergic and dopamine D_1 sites and is completely inactive at 5-HT_3, muscarinic, and benzodiazepine receptors (18). α_2-Adrenergic receptor agonists inhibit plasma extravasation (21); however, DHE is known to be an α_2-adrenergic antagonist. The effect of dopamine and β-adrenergic receptor stimulation has never been investigated in the plasma extravasation model. β-Adrenergic binding sites have been found in the trigeminal ganglion,[4] but their functional significance is unknown.

The likely sites of actions for sumatriptan are 5-HT_{1D} (α or β, see below) receptors located prejunctionally on trigeminovascular fibers, where they inhibit neuropeptide release (22). This hypothesis is based on the fact that sumatriptan blocks extravasation provoked by electrical stimulation but has no effect when the extravasation is caused by direct substance P administration. Similar evidence has been provided for DHE (22), 5-CT,[5] and CP-122,288 (4), which indicates that their site of action is also likely to be prejunctional.

As mentioned above, we had to use 5-HT_{1B} knockout mice because selective antagonists are lacking and the knockout technology is not available in the guinea pig. Species differences are known to exist, however, in 5-HT_{1D} receptor pharmacology (5, 10). Rats and mice possess a 5-$HT_{1D\beta}$ receptor (called 5-HT_{1B} in these species) in which a single amino acid substitution accounts for a pharmacological profile markedly different from that of guinea pig and human receptors (7). In human (8), as in rat (9) and mouse (15), brain, the 5-$HT_{1D\beta}$ (or 5-HT_{1B}) receptor seems to predominate. mRNA for 5-HT_{1B} receptors has been found in rat trigeminal ganglion (23), whereas human and guinea pig trigeminal ganglia seem to contain a higher proportion of 5-$HT_{1D\alpha}$ receptor mRNA (24, 25). Caution should thus be exerted when extrapolating rodent data to humans. Preliminary findings show that the selective 5-HT_{1D} antagonist GR127,935 (26) inhibits the effect of sumatriptan but not that of CP-122,288 and 5-CT in the guinea pig extravasation paradigm (27), which indicates that an additional site exists in species other than mice. Interestingly, a recent report has shown that 5-CT, but not sumatriptan, inhibits 5-HT release via non-5-HT_{1B} receptor sites in several brain regions of 5-HT_{1B} knockout mice (28); this finding represents a departure from the previously accepted belief that serotonergic autoreceptors belong to the 5-HT_{1B} subtype, at least in rats and mice (10). Further studies are needed to establish whether this additional site corresponds to the site that mediates the effect of 5-CT on dural plasma extravasation.

Sumatriptan represents a major advance in the acute treatment of migraine and vascular headache, as it displays a improved selectivity over previously used ergot alkaloids (19). Its action at 5-$HT_{1D\beta}$ receptors might also cause the vascular side effects associated with sumatriptan administration. Preliminary studies have shown that CP-122,288 is not more potent than sumatriptan in constricting cerebral blood vessels despite its 1000-fold increased potency in the extravasation model (29). Targeting drugs at the site(s) mediating these enhanced effects may yield drugs with an improved safety profile. Further experiments and selective agents will be important to determine the identity and characteristics of this site.

[4] C. Waeber and M. A. Moskowitz, unpublished observations.
[5] W. S. Lee and M. A. Moskowitz, unpublished observations.

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