5-Hydroxytryptamine Induces Mast Cell Adhesion and Migration

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5-Hydroxytryptamine Induces Mast Cell Adhesion and Migration

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The neurotransmitter serotonin (5-hydroxytryptamine (5-HT)) is implicated in enhancing inflammatory reactions of skin, lung, and gastrointestinal tract. To determine whether 5-HT acts, in part, through mast cells (MC), we first established that mouse bone marrow-derived MC (mBMMC) and human CD34 derived MC (huMC) expressed mRNA for multiple 5-HT receptors. We next determined the effect of 5-HT on mouse and human MC degranulation, adhesion, and chemotaxis. We found no evidence that 5-HT degranulates MC or modulates IgE-dependent activation. 5-HT did induce huMC and huMC adherence to fibronectin; and immature and mature mBMMC and huMC migration. Chemotaxis was accompanied by actin polymerization. Using receptor antagonists and pertussis toxin, we identified 5-HT1A as the principal receptor mediating the effects of 5-HT on MC. mBMMC from the 5-HT1A receptor knockout mouse (5-HT1AR) did not respond to 5-HT. 5-HT did induce accumulation of MC in the dermis of 5-HT1A+/+ mice, but not in 5-HT1A−/− mice. These studies are the first to demonstrate an effect of 5-HT on MC. Furthermore, both mouse and human MC respond to 5-HT through the 5-HT1A receptor. Our data are consistent with the conclusion that 5-HT promotes inflammation by increasing MC at the site of tissue injury. The Journal of Immunology, 2006, 177: 6422–6432.

Materials and Methods

Animals used

Female wild-type C57BL/6 mice were purchased from The Jackson Laboratory; wild-type BALB/c mice were from Taconic Farms; 5-HT1A receptor knockout mice (that the point mutation results in a nonfunctional protein) (5-HT1AR−/−) and wild-type C57BL/6 littermate controls (5-HT1AR+/+) were maintained at the Department of Pharmacology, Weill Medical College of Cornell University (22). Mice were kept and sacrificed in accordance with National Institutes of Health and Weill Medical College of Cornell University animal care and use guidelines. The studies have been reviewed and approved by an appropriate institutional review committee.

mBMMC culture

Cells, obtained from mouse femur, were cultured in RPMI 1640 medium supplemented with 10% FCS, glutamine (4 mM), sodium pyruvate (1 mM), penicillin (100 U/ml), streptomycin (100 µg/ml), nonessential amino acids (1 mM), HEPES (25 mM), 2-mercaptoethanol (50 mM), and mouse rIL-3 (30 ng/ml; PeproTech) as described (23). Cell cultures were maintained at 37°C in 5% humidified CO2 up to 6 wk. Immature cultures were used to study selective migration of immature mBMMC (24, 25); mature MC were obtained at 5–6 wk.

huMC culture

huMC were cultured as described (26–28). In brief, peripheral blood CD34 progenitor cells were collected from healthy donors after informed consent and were processed by affinity column apheresis. CD34 cells...
FIGURE 1. mBMMC expression of 5-HT receptors: effect of 5-HT on IgE-mediated degranulation. A, mBMMC at 5 wk were analyzed for 5-HT receptor expression. Mouse brain total RNA was used as a positive control. Results are representative of three separate experiments using separate MC cultures initiated from different mice.

B, mBMMCs were sensitized overnight with IgE anti-DNP and activated by DNP-HSA. 5-HT at concentrations noted was added to the wells simultaneously with Ag. H9252 release was determined after 30 min. “Spont” equals spontaneous release; “0,” H9252 release when cells were activated with IgE/DNP-HSA alone. Results are representative of three experiments, each initiated from different mice.

C, mBMMC (10^6 cells/condition) were activated through FcγRI or were treated with 5-HT and analyzed by Western blot for protein phosphorylation at 30 and 60 min. Line 1 represents levels of protein phosphorylation in unstimulated control cells. Syk was used for protein normalization.

D, Ca^2+ flux was measured in mBMMC exposed over 300 s to stimuli indicated.

Table I. Primer sequences for 5-HT receptors

<table>
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<tr>
<th>Primer 1 (sense 5'- to 3')</th>
<th>Primer 2 (antisense 5'- to 3')</th>
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<td>β-Actin^b</td>
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</tbody>
</table>

^a Primers from Ref. 18.
^b Primer from Ref. 56.
^c Primer from Ref. 30.
were cultured in StemPro-34 SFM (Invitrogen Life Technologies) in the presence of rIL-3 (first week only), rIL-6, and recombinant human stem cell factor (rhSCF) (PeproTech). All cultures were maintained up to 10 wk at 37°C and 5% CO₂. One-half of the cytokine-supplemented medium was replaced weekly.

**Cell activation and degradation**

mBMMC were sensitized with mouse monoclonal anti-DNP IgE (100 ng/ml; Sigma-Aldrich) and activated with DNP-human serum albumin (HSA) conjugate (100 ng/ml; Sigma-Aldrich). huMC were sensitized with biotinylated IgE (100 ng/ml) overnight and activated with streptavidin (100 ng/ml; Sigma-Aldrich). Degranulation was measured by granule-associated β-hexosaminidase (β-hex) release as described (29). 5-HT was prepared before each experiment.

**RT-PCR**

A total of 3–4 × 10⁶ cells was collected by centrifugation and processed with TRIzol (Invitrogen Life Technologies). The RNA layer was collected and transferred to an RNaseasy column for further purification according to the manufacturer’s instructions (Invitrogen Life Technologies). Five- to 7-μg samples were also treated with 1 U of DNase to eliminate residual DNA contamination (30). One microgram of each sample was used in the RT-PCR (SuperScript III One-Step RT-PCR System; Invitrogen Life Technologies) with specific primers designed (Bio-Synthesis) for murine and human receptors (Table I). β-Actin was used as sample loading control. cDNA synthesis and PCR amplification were performed with a DNA Engine PTC-200 cycler (Biorad Laboratories) programmed with the following cycles: cDNA synthesis: 30 min at 55°C; denaturation: 2 min at 94°C; PCR amplification (30 cycles): 15 s at 94°C (denature), 30 s at 60°C (anneal); 2 min at 68°C (extend); final extension at 5 min at 68°C. Total mouse brain RNA (BD Biosciences) was used as a positive control for 5-HT receptor expression. For conformational analysis of the mutated nonfunctional insert in MC cultured from 5-HT₁₆⁻ R⁻/⁻ and 5-HT₁₆⁺ R⁺/⁺ mouse bone marrow, specific primers were used (Table I).

**Cytokine measurement**

mBMMC (1 × 10⁷/100 μl per well) were cultured for 24 h in 5-HT (10⁻³–10⁻⁶ M) with or without prior activation through FceRI. Supernatants were collected following centrifugation at 300 × g for 5 min. TNF-α (sensitivity, <0.18 pg/ml; R&D Systems) and IL-6 (sensitivity, <10 pg/ml; R&D Systems) were measured following R&D protocols.

**Flow cytometry**

MC were identified by flow cytometry as double-positive cells for FcεRI and Kit. Propidium iodide staining was used to exclude the dead cell population. FcεRI/IgE, Kit (CD117), and adhesion molecule surface expression. FcεRI aggregation, addition of 5-HT or both (33). Proteins were separated on 4–12% NuPage Tris-Bis gels using NuPage MES-SDS running buffer (Invitrogen Life Technologies), transferred onto nitrocellulose membranes, and probed with the following Abs: anti-Fos polyclonal Ab (pAb) and anti-Jun pAb (Santa Cruz Biotechnology); anti-Syk mAb (4D10) from (sensitization); values of spontaneous adhesion were subtracted from all data sets. Effect of 5-HT alone, or in the presence of 1 ng/ml (B), or 100 ng/ml (D) SCF. Values of p were calculated by two-way ANOVA; dose-dependent differences were assessed by Bonferroni’s posthoc test or Student’s t test. *, p < 0.05; **, p < 0.01, 5-HT vs spontaneous; #, <0.05, #, <0.001, SCF vs spontaneous; ##, <0.05, 5-HT/SCF vs SCF alone. Data are represented as mean ± SEM (n = 3–5).

**Actin polymerization (F-actin)**

Cells were washed and resuspended in HEPES buffer with 1% BSA at 1 × 10⁶ cells/ml. Cells were kept at 37°C in a water bath and stimulated with 5-HT or the 5-HT₁₆ receptor agonist R-(+)-8-(OH)-DPAT (Sigma-Aldrich) as indicated (see Fig. 6 legend). For inhibition experiments, 5-HT and the 5-HT₁₆ receptor antagonist WAY100635 (Sigma-Aldrich) were both added to cells. Cells were fixed and permeabilized with 4% paraformaldehyde, 0.5% saponin, and 1.7 μg/ml FITC-phalloidin conjugate (Invitrogen Life Technologies) for 10 min on ice, washed, and analyzed by flow cytometry (32). For immunostaining, 5 × 10⁴ cells were grown overnight on glass chamber slides, and then washed and stimulated with 5-HT, R-(+)-8-OH-DPAT or 5-HT with WAY100635. Staining for F-actin was performed as above. Cells were visualized and photographed using an Axioshot microscope (Zeiss) at ×63 (lens objective).

**Signaling studies**

Protein phosphorylation, in sensitized MC, was performed following FceRI aggregation, addition of 5-HT or both (33). Proteins were separated on 4–12% NuPage Tris-Bis gels using NuPage MES-SDS running buffer (Invitrogen Life Technologies), transferred onto nitrocellulose membranes, and probed with the following Abs: anti-Fos polyclonal Ab (pAb) and anti-Jun pAb (Santa Cruz Biotechnology); anti-Syk mAb (4D10) from

*FIGURE 2.* Effects of 5-HT on mBMMC adhesion to fibronectin in the presence or absence of SCF. mBMMC adhesion was analyzed using 96-well fibronectin precoated plates. Cells (5 × 10⁴/well) were treated with various concentrations of 5-HT alone or in combination with SCF. Spontaneous adhesion was subtracted from all data sets. Effect of 5-HT alone (A) or in the presence of 1 ng/ml (B), 10 ng/ml (C), or 100 ng/ml (D) SCF. Values of p were calculated by two-way ANOVA; dose-dependent differences were assessed by Bonferroni’s posthoc test or Student’s t test. *, p < 0.05; **, p < 0.01, 5-HT vs spontaneous; #, <0.05, #, <0.001, SCF vs spontaneous; ##, <0.05, 5-HT/SCF vs SCF alone. Data are represented as mean ± SEM (n = 3–5).
UBI; anti-phospho-AKT (Ser(P)-473) pAb, anti-phospho-ERK (Thr(P)-202 and Tyr(P)-204), anti-phospho-Jun (Ser(P)-73) pAb, and anti-phospho-NF-H9260 B (Ser(P)-536) pAb (Cell Signaling); and anti-phospho-PLC-H9253 (Tyr(P)-783) pAb (Biosource). The membranes were then incubated with the appropriate secondary Ab conjugated to HRP (1:3000) (Amersham Biosciences). The immunoreactive bands were visualized using a Renaissance chemiluminescence kit (NEN) according to the manufacturer’s protocol. Calcium flux was measured as described (34) using fura 2-AM as a probe.

**Adhesion assay**

The adhesion assay was performed as described (35) with a slight modification. Serocluster 96-well flat-bottom microtiter culture plates (Costar) were coated overnight with 5 μg/ml human fibronectin (Sigma-Aldrich) at 4°C, and then blocked with 4% BSA over 1 h in 37°C and washed. Cells at 1 x 10^5/ml were incubated in RPMI 1640 and then incubated with 2 μg/ml calcein-AM (Invitrogen Life Technologies) for 30 min at 37°C and resuspended in HEPES/0.01% BSA, followed by washing and incubation in RPMI 1640 for another 30 min. Labeled cells were plated at a final concentration of 5 x 10^4 cells/well and stimulated with the 5-HT1A receptor agonist R-(+)-8-OH-DPAT, 5-HT, or 5-HT with or without pertussis toxin (Sigma-Aldrich) or antagonists WAY100635, the nonselective 5-HT1 antagonist ketanserin, the 5-HT1B/1D/1A antagonist GR 127935, the 5-HT2B/2C antagonist SB 200646 (Sigma-Aldrich), or the 5-HT7 antagonist sumatriptan (GlaxoSmithKline) at a concentration selected from the published reports (18, 36) and where pilot experiments had demonstrated a maximal effect. After incubation at 37°C for 60 min (for mBMMC) or 90 min (for huMC), nonadherent cells were carefully removed. Fluorescence was read on a TECAN GENios plate reader (ReTiSoft) at an excitation wavelength of 485 nm and an emission of 530 nm. The percentage of adherent cells was calculated as follows (35): fluorescence of adherent cells/fluorescence of total cells x 100 = % adherent cells.

**Direct fluorescence of negative controls (each chemical compound without cells) was assessed in parallel.** Baseline (spontaneous) adhesion of cells to fibronectin was calculated for each separate experiment and subtracted from all data sets (average spontaneous mBMMC adhesion was 12–14%, and for huMC it was 8–10%).

**In vitro chemotaxis**

Chemotaxis was analyzed using a 96-well microchemotaxis system with 8-μm pore size membranes (Neuroprobes) using direct fluorescence as described (37). Thus, cells were resuspended at 1 x 10^6/ml in HEPES plus 0.1% BSA, labeled with calcein-AM, and allowed to settle on the chemotaxis membranes for 10 min. Agents to be tested were diluted from stock solutions in HEPES/0.1% BSA and placed in the lower wells. Inhibitors, when tested, were added to the upper and lower wells at 10^−5 M before plate assembly. mBMMC were allowed to migrate for 60 min and huMC for 90 min, filters were removed, and migrated cells were counted based on direct fluorescence. A titration curve of fluorescence per cell was used for calculation of migrated cell number (linear regression in all experiments) (37). Human or murine rSCF (Sigma-Aldrich) at 10 ng/ml was used as a positive control (38). Chemokinesis was assessed by comparison of the migration of cells in the presence of the activation factor in both upper and lower wells. Baseline chemotaxis was subtracted (−4000 for mBMMC and 2000 for huMC).

**Immature MC chemotaxis**

Two-week-old immature cultured mBMMC as described (24) were studied for chemotaxis. Cells were plated without fluorescent staining on the chemotaxis filters and allowed to settle for 10 min. The filter was assembled with the lower plate filled with chemotactic agent(s) and placed at 37°C for 60 or 90 min. The lower well content (30 ml of PBS/0.1% BSA with transmigrated cells) was collected and stained with FITC-antiGr-1 (RB6-8C5) (BD Biosciences) and PE-CD117 (7B8) (BD Biosciences) for 30 min.
on ice. Propidium iodide (BD Biosciences) was added 5 min before analysis. Total cell number was determined for each sample. Migrated immature MC were defined as cells positive for CD117 and negative for Gr-1 (granulocyte marker).

Migration of MC in vivo

Wild-type mice, between 2 and 2.5 mo of age, were used for skin injections (National Institutes of Health protocol ASP LAD 2). Bilateral flanks were
shaved and washed with alcohol. 5-HT creatinine sulfate complex (Sigma-Aldrich) was diluted in sterile PBS to a final concentration of 10 μM. Fifty microliters of 5-HT or vehicle (PBS alone) was injected (left flank and right flank). Two schedules were used as follows: 1 day with three injections 5 h apart; 2 days with three injections 4 h apart on each day. Mice were sacrificed 1 h after the last injection. Skin biopsies (10 × 10 mm) were obtained at the 5-HT injection site and from the contralateral control site, which had received only PBS. Skin specimens were transferred to Carnoy’s fixative (60% ethanol, 30% chloroform, and 10% acetic acid) for 3 h at 4°C. The presence of tissue MC was assessed in 3-micrometer sections. MC were counted by light microscopy per injection site (three slides per specimen from three different animals per each condition) using a 10 × 10 μm grid at ×10 magnification, and average MC number per square millimeter was determined. Sections were number-coded so that the observer was blinded to the identity of individual specimens. Tissue digital photography of representative slides (one per group of three animals, each injected in contralateral sites with vehicle or 5-HT) was performed on Axiophot Ziax microscope at ×10 (lens objective).

Statistical analysis

One-way or two-way mixed factorial ANOVA followed by the Bonferroni’s post hoc test or Dunnet’s multiple comparison test were used to determine statistical significance of response variations. Paired Student’s t test ANOVA was used to assess dose-dependent effect on in vivo model. Differences were accepted to be statistically significant at p < 0.05 (* or #), p < 0.01 (** or ##), and p < 0.001 (*** or ###).

Results

mBMMC express 5-HT receptors

As the first step in the analysis of whether 5-HT affects mBMMC function, expression of mRNA for 5-HT receptors was determined (Fig. 1A). mBMMC were found to express mRNA for the 5-HT1A, 5-HT1B, 5-HT1D, 5-HT2A, 5-HT2B, 5-HT6, and 5-HT7 receptors as well as for serotonin transporter (SERT) (39). No mRNA was detected for 5-HT2C and 5-HT4 receptors in mBMMC. Thus, mBMMC expressed mRNA for receptors for 5-HT that have been reported to mediate the effect of 5-HT on other cells of hemopoietic origin (16–21).

5-HT does not affect MC degranulation and cytokine release

Because MC are known to degranulate in inflammation in the dermis where 5-HT is also found to be increased (6, 40), we initially determined whether 5-HT directly induces MC degranulation or alters IgE-mediated degranulation. As Fig. 1B shows, 5-HT neither induced mBMMC degranulation, nor did it alter IgE-mediated degranulation. In data not shown, production of either TNF-α or IL-6 was similarly not altered after culture of mBMMC in 5-HT

FIGURE 5. Comparison of adhesion and chemotaxis to 5-HT in mBMMC cultured from 5-HT1A−/− and 5-HT1A+R+/+ mice. A, Adhesion of mBMMC cultured from 5-HT1A−/− and 5-HT1A+R+/+ mice in the presence of 5-HT. Data are presented as a mean ± SEM of six experiments performed in triplicate. Two-way ANOVA was applied to these data to compare differences in adhesion of 5-HT1A−/− and 5-HT1A+R+/+ mBMMC (p < 0.001) followed by Bonferroni’s post hoc test to compare values within each concentration. B, Comparison of 5-HT1A−/− and 5-HT1A+R+/+ mBMMC migration to SCF (○, 5-HT1A−R−/−; △, 5-HT1A−/−) or 5-HT alone (●, 5-HT1A−R−/−; ○, 5-HT1A−/−) or 5-HT1A+R+/+ in the presence of WAY100635 (□). Two-way ANOVA was used to compare effect of 5-HT on 5-HT1A−R−/−, 5-HT1A−/−, and 5-HT1A+R+/+ mBMMC exposed to inhibitor (p < 0.01). Bonferroni’s multiple comparison test compared variables within each concentration. *, p < 0.05; **, p < 0.01; ***, p < 0.001.

FIGURE 6. 5-HT induced actin polymerization in mBMMC. A, mBMMC were stimulated with 10−8 M 5-HT or R-(+)-8-OH-DPAT for 10 and 60 s on multichamber glass slides, after which cells were stained with PE-conjugated phalloidin toxin bound to polymerized F-actin. Note the x-axis is not a linear scale. B–D, Fluorescent confocal microscopy of an mBMMC before 5-HT exposure (B), or 10 s after exposure to 5-HT (C), or 60 s after exposure of 5-HT (D). E, mBMMC 60 s after exposure of 5-HT and WAY100635.
5-HT induces adhesion of MC to fibronectin

We next examined whether 5-HT enhances mBMMC adhesion to fibronectin, a component of tissue matrix. As Fig. 2A shows, 5-HT induced mBMMC adhesion to fibronectin in a concentration-dependent manner. Maximal adhesion was observed at 10^{-7}–10^{-6} M 5-HT. Adhesion induced by 5-HT was comparable to that induced by SCF alone at 1 ng/ml (Fig. 2B, first column), a known adhesion promotion factor for MC. When SCF at 1 ng/ml with increasing concentrations of 5-HT were simultaneously added, there was an additive effect on MC adhesion. SCF at 10 or 100 ng/ml resulted in a higher level of adhesion than at 1 ng/ml, and 5-HT had less effect in combination (Fig. 2, C and D), presumably because at these concentrations of SCF, adhesion is near maximal. These data show that 5-HT alone induces MC adhesion to fibronectin, and this effect is additive at lower concentrations of SCF.

Pertussis toxin blocks 5-HT-induced adhesion of MC to fibronectin

5-HT receptors are members of the G-protein-coupled receptor family, Goi subunit, which is pertussis toxin sensitive (15), binds only to 5-HT receptors 5-HT1A, 5-HT1B, and 5-HT1D. As can be seen in Fig. 3A, pertussis toxin preincubation abolished 5-HT-induced adhesion of mBMMC to fibronectin, suggesting that 5-HT-induced adhesion is through the 5-HT1A, 5-HT1B, or 5-HT1D.

A 5-HT1A receptor antagonist inhibits 5-HT induced adhesion of MC to fibronectin

We next examined the effect of WAY100635 (Fig. 3B), GR127935 (C), ketanserin (D), SB200646 (E), and sumatriptan (F) on 5-HT-induced mBMMC adhesion. We found that WAY100635 at 10 μM completely blocked and GR127935 at 1 μM significantly inhibited 5-HT induced adhesion (see Fig. 3). Other antagonists were less effective in blocking 5-HT induced adhesion. These data are consistent with the conclusion that 5-HT, acting through the 5-HT1A receptor, mediates increased adhesion to fibronectin.

5-HT1A-R^{-/-}-derived mBMMC exhibit defective 5-HT-mediated adhesion

To confirm that 5-HT, acting via the 5-HT1A receptor, is mediating MC adhesion to fibronectin, we next examined mBMMC derived from 5-HT1A receptor knockout mice (5-HT1AR^{-/-}). We first confirmed the presence of the 5-HT1A R mutation in mBMMC by RT-PCR with specific primers. As expected, total MC mRNA derived from 5-HT1A-R^{-/-} MC samples were positive with R^{-/-} primers but not with R^{+/+} specific primers, whereas MC mRNA derived from 5-HT1A-R^{+/+} mice were positive only for the R^{+/+} specific primers (Fig. 4A). Then, we compared the cellular phenotype of 5-HT1A-R^{-/-} mBMMC with 5-HT1A-R^{+/+} mBMMC. No significant differences in expression of Kit and FcεRI were found between cultures (Fig. 4B).

Similarly, there was no significant difference in β-hex release in response to FcεRI aggregation or to 5-HT stimulation between mBMMC from 5-HT1A-R^{+/+} and 5-HT1A-R^{-/-} mice (Fig. 4C). 5-HT1A-R^{+/+} mBMMC had similar levels of cell surface markers
involved in adhesion to fibronectin, including CD11a, CD49e, CD51, and CD54 (Fig. 4D). Thus, we found no difference in 5-HT1A-R<sup>−/−</sup> and 5-HT1A-R<sup>+/+</sup> MC in terms of Kit and FcεRI expression, degranulation, or expression of adhesion molecules. When 5-HT1A-R<sup>−/−</sup> mBMMC were exposed to 5-HT, no 5-HT-induced adhesion was observed, whereas 5-HT1A-R<sup>+/+</sup> mBMMC did adhere to fibronectin (Fig. 5A). Taken together, these observations establish that the 5-HT1A receptor is the principal receptor responsible for mediating the effect of 5-HT on mBMMC adhesion.

mBMMC chemotaxis to 5-HT is mediated through 5-HT<sub>1A</sub> receptor

We then examined whether mBMMC would migrate to 5-HT. As can be seen in Fig. 5B, 5-HT1A-R<sup>+/+</sup> mBMMC exhibited dose-dependent chemotaxis to 5-HT. When 5-HT1A-R<sup>−/−</sup> MC were exposed to 5-HT in the presence of WAY100635, chemotaxis was abolished. 5-HT1A-R<sup>−/−</sup> MC demonstrated defective chemotaxis in response to 5-HT. These findings show that mBMMC migrate to 5-HT and that the action of 5-HT on mBMMC migration is mediated by the 5-HT1<sub>A</sub> receptor.

5-HT promotes actin polymerization (F-actin) in mBMMC

We next examined actin polymerization, which accompanies signals that induce cell migration. Flow cytometry was used in the analysis of actin polymerization as assessed by monitoring fluorescence of bound phalloidin toxin in mBMMC following exposure to 5-HT (Fig. 6A). Baseline fluorescence (G<sub>mean</sub> = 47.76) was tripled when mBMMC were exposed to 5-HT (G<sub>mean</sub> = 150) or to the 5-HT1<sub>A</sub> receptor selective agonist R-(-)-8-OH-DPAT (G<sub>mean</sub> = 175) at 1 μM. Polymerization was observed within 5 s. Actin polymerization was also detected in mBMMC grown on glass chamber slides. Leading edges could be observed as early as 10 s, followed by cytoskeletal reorganization and cell shape changes, including cell elongation and membrane ruffling (Fig. 6, B–E). Similar observations were made in other cell types exposed to chemotactic stimuli consistent with our data that 5-HT acting through 5-HT1<sub>A</sub> receptor signals mBMMC movement (32).

Immature MC migrate to 5-HT

It has recently been reported that immature mBMMC migrate to leukotriene B<sub>4</sub> (LTB<sub>4</sub>) but not to chemokines, indicating that LTB<sub>4</sub> is important in regulating tissue MC numbers (24). To determine whether 5-HT could similarly attract immature MC, we examined immature MC for their response to 5-HT. We monitored movement of immature MC across a membrane by determining the accumulation of immature Kit<sup>+</sup> mBMMC in the lower chamber. Gr-<sup>1</sup> cells that thus meet the definition of granulocytes (remaining in 2-wk MC cultures) were excluded (Fig. 7A). The numbers of migrated Kit<sup>+</sup>Gr-<sup>1</sup> cells were monitored, and we observed a significant dose-dependent increase (Fig. 7B). Maximal migration of immature MC was observed using 5-HT in concentrations between 1 × 10<sup>−7</sup> and 1 × 10<sup>−6</sup> M. Migration of immature mBMMC induced by 10 ng/ml SCF was significantly less than 5-HT-induced migration, consistent with the report that SCF does not play a major role in chemotaxis of immature mBMMC (24). Thus, as is reported for LTB<sub>4</sub>, 5-HT is a potent chemoattractant for immature mBMMC.

Effect of 5-HT on human MC adhesion and chemotaxis

We next investigated whether, similar to mBMMC, huMC are also affected by 5-HT. We first determined that cultured 8-wk-old huMC express mRNA for the 5-HT<sub>1A</sub>, 5-HT<sub>1B</sub>, 5-HT<sub>1E</sub>, 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub>, 5-HT<sub>2C</sub>, 5-HT<sub>3</sub>, 5-HT<sub>4</sub>, and 5-HT<sub>7</sub> receptors (Fig. 8A). As with mBMMC, huMC released β-hex following FcεRI aggregation, but 5-HT neither altered FcεRI-mediated degranulation nor induced degranulation (Fig. 8B). However, huMC showed increased adhesion to fibronectin when exposed to 5-HT (Fig. 8C).

FIGURE 8. HuMC adhesion and migration induced by 5-HT. A, huMC express message for multiple 5-HT receptors. Data presented are representative of three separate experiments, each from cultured huMC from a separate donor. B, huMC degranulate to IgE-mediated signals, but not to 5-HT alone. 5-HT does not alter IgE-mediated degranulation. “0,” β-hex release when cells were activated by FcεRI aggregation only. Representative of two independent experiments. C, 5-HT induced huMC adhesion. SCF-induced adhesion was used as a positive control (on average 40%); n = 2; mean ± SEM. One-way ANOVA (p < 0.001) with Bonferroni’s posthoc test was used to analyze the data. *** p < 0.01; **** p < 0.001. D, huMC migration to 5-HT was compared with migration induced by SCF. One-way ANOVA (p < 0.01) was followed by Bonferroni’s ANOVA. *, p < 0.05.
and migrated to 5-HT (D). 5-HT-induced migration was abolished in the presence of WAY100635 consistent with the conclusion that it is 5-HT₁A dependent (data not shown).

5-HT-dependent MC accumulation in vivo

Because 5-HT induces MC migration and adhesion in vitro, we asked whether 5-HT could lead to MC accumulation in vivo. To examine this possibility, 5-HT was injected into mouse skin, and MC number was determined. Injection of 5-HT into the dermis of wild-type mice led to a significant increase in the accumulation of MC at the site of 5-HT injections after 48 h (Fig. 9A) and 7 h (B). However, this effect was not observed in the 5-HT₁AR⁻/⁻ mouse (Fig. 9B), indicating that 5-HT affects MC migration in vivo via a 5-HT₁A receptor-dependent mechanism.
Discussion

The role of 5-HT in MC function has not yet been investigated, and here we demonstrate that both huMC and mBMMC adhesion and migration respond to 5-HT. We also demonstrate that these biological functions are mediated by the 5-HT1A receptor. The preservation of responses to 5-HT from rodent to human supports the notion that these pathways are evolutionarily consequential. Not all functions of mouse MC are conserved in human MC. For instance, whereas mBMMC will proliferate in the presence of IL-3 or SCF, huMC only show significant proliferation to SCF (41, 42). Note we found no evidence that 5-HT either could induce MC degranulation or cytokine production, nor alter the consequences of IgE-mediated degranulation.

MC of rodent origin (43) and human origin (44) both synthesize and concentrate 5-HT. Indeed, the uptake of [3H]5-HT in rodent MC in culture with its subsequent release following IgE-mediated degranulation is commonly used in studies of MC function (45–47). The possibility thus exists of an autocrine loop where 5-HT, released following MC activation, enhances the subsequent events by increasing MC at the site of inflammation.

5-HT is reported to influence behavior of inflammatory cells, inducing up-regulation of phagocytosis by macrophages (16), protecting NK cells from oxidative stress (17), promoting mitogen-activated T and B cell survival (21, 48), inducing migration of eosinophils (19), and modulating cytokine release from dendritic cells (18). The principal receptor involved in this process, when determined, has been identified as the 5-HT1A receptor (17, 21). Several lines of evidence support the notion that MC respond to 5-HT through the 5-HT1A receptor including the pertussis toxin-dependent inhibition of adhesion, the ability to disrupt adhesion and chemotaxis with the 5-HT1A receptor antagonist WAY100635, and the lack of response of MC derived from 5-HT1A−/− mice. 5-HT2A is predominantly expressed in human MC; however, the evidence is compelling that 5-HT2A is not the predominant 5-HT receptor that allows the human MC to respond to 5-HT in adhesion and chemotaxis. This is because in human MC pertussis toxin significantly abrogates the response to 5-HT, and pertussis toxin is known to inhibit 5-HT1A (Gq-coupled), but not 5-HT2A (Gq, Gi-coupled) responses. This observation, coupled with the inhibition of 5-HT1A with specific antagonist allows us to feel comfortable with the conclusion that the 5-HT1A is the predominant receptor involved in human MC adhesion and chemotaxis to 5-HT.

The possible in vivo relevance of 5-HT-induced MC adhesion and migration is illustrated by the increase of MC at sites of 5-HT injection in mouse dermis. MC accumulation did not occur in 5-HT1AR−/− mice, demonstrating that the 5-HT1A receptor is also involved in responses in vivo. However, we cannot exclude the possibility that the effect of 5-HT in vivo is working indirect on MC. For instance, 5-HT could act on another cell in mouse dermis, which in turn could release a chemotactic factor attracting MC.

The origin of MC that accumulate at the sites of 5-HT injection is not known. It is possible that MC near the site of injection are attracted to the injection site over a few hours. MC progenitors additionally might be recruited from the blood if the level of 5-HT remains persistently elevated at the tissue site. Although not confirmed in vivo, in vitro experiments strongly suggest that 5-HT recruits MC progenitors, much like LTB4 (24), and may be implicated in influencing MC numbers in tissues. Note there are reports of SCF down-regulation of Kit in mature MC over several hours (49). However, the ability SCF to down-regulate Kit may not be meaningful in interpreting our experiments that take place over 60 min and involve use of progenitor MC. However, in the final analysis, Kit+ progenitor cells did migrate, although one could argue that the method of analysis might underestimate the number of cells migrating, and the conclusion remains valid.

The possibility that tissue 5-HT levels, which increase in inflammation of the dermis, might play a role in MC recruitment (5–8) is an intriguing possibility, and may be relevant in inflammation of other tissues. For instance, 5-HT is known to be present in pulmonary tissues where the source may be pulmonary neuroendocrine cells (50, 51) or platelets (52), and thus could facilitate MC accumulation in inflammatory conditions of the lung. Similarly, 5-HT is known to be synthesized by enterochromaffin cells and nerve ganglia in the gastrointestinal system (53–55), and might influence accumulation of MC at these sites. In summary, we, for the first time, have shown that 5-HT has the ability to influence the biology of the MC. Data additionally clearly document that the effects of 5-HT are principally through the HT1A receptor. These observations also support the conclusion that pharmaceutical agents that more selectively block the action of 5-HT1A receptor could be explored as adjunct therapies in the treatment of allergic inflammation.

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Disclosures

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References


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