

Letter to the Editor

Bitter taste receptor (TAS2R) agonists inhibit IgE-dependent mast cell activation

To the Editor:

Previously, bitter taste receptors (TAS2Rs) were considered to be exclusively located on the tongue, where their activation

enables our perception of bitter taste. However, several studies have now also linked TAS2Rs with the respiratory system. TAS2Rs have been found to be expressed in human airway smooth muscle (ASM) and agonists caused relaxation of isolated ASM and dilation of airways.^{1,2} Moreover, Orsmark et al³ recently found increased expression of TAS2Rs in leukocytes from children with severe asthma and inhibition of certain

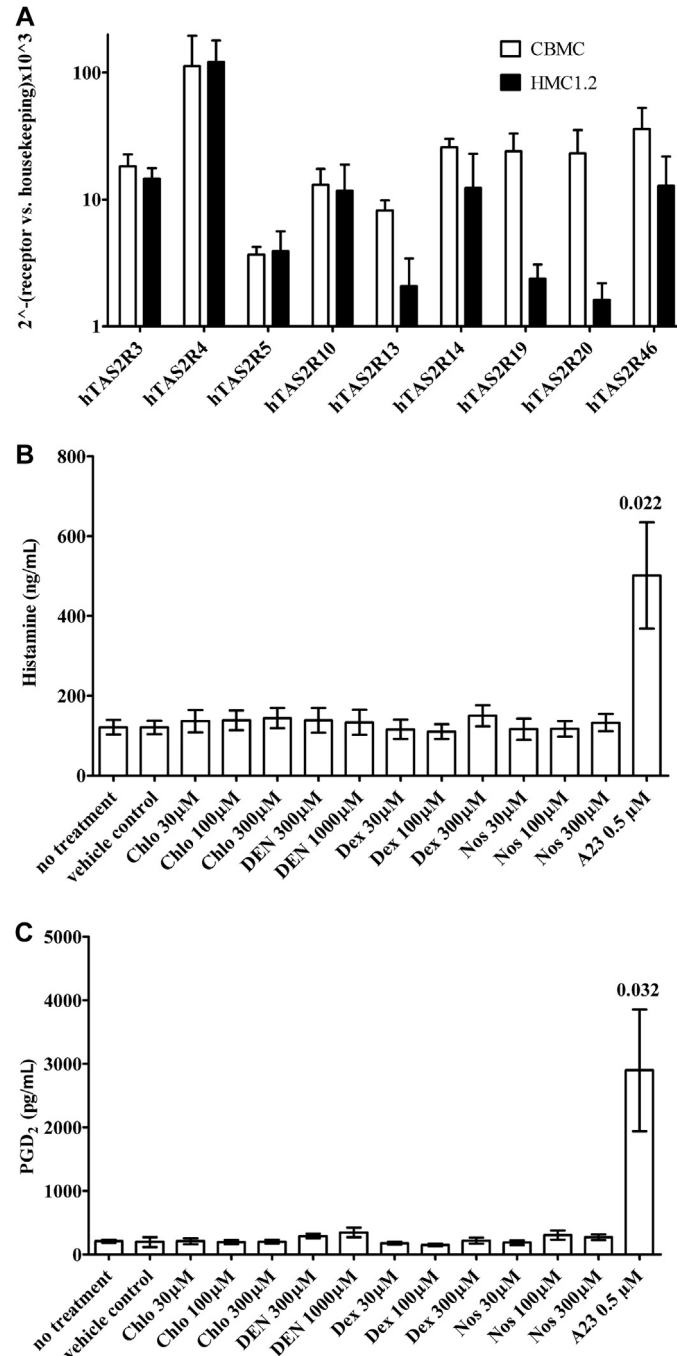


FIG 1. A, TAS2R mRNA expression of CBMCs and the human mast cell line HMC-1.2, measured with real-time PCR (N = 3). TaqMan primers for human TAS2Rs and GAPDH, ACTB, and HPRT-1 were used. Gene expression was calculated according to the $2^{-\Delta CT}$ formula, and expressed as follows: $2^{-(\text{receptor cyclic threshold [CT]} - \text{average CT of all housekeeping genes})} \times 10^3$. **B and C,** TAS2R agonists (60-minute incubation) do not induce release of histamine or PGD₂ in CBMCs.

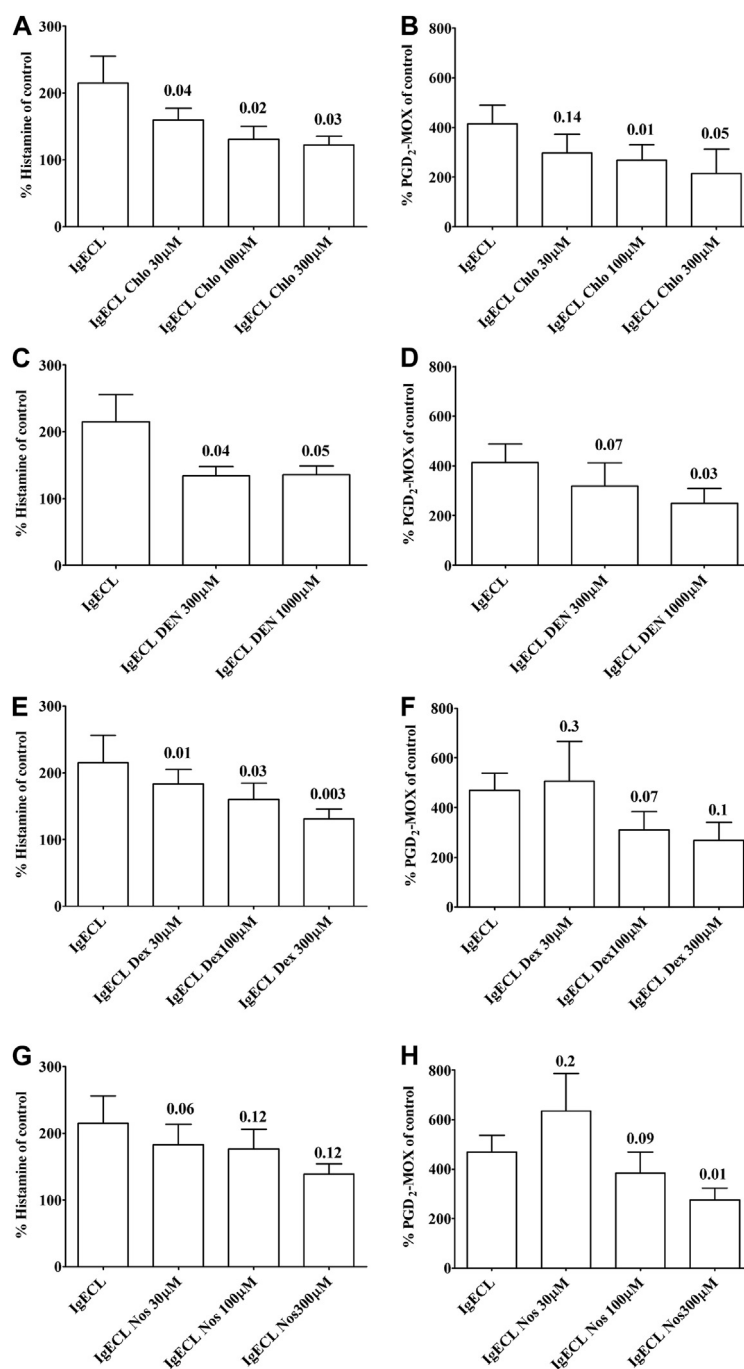


FIG 2. TAS2R agonists inhibit histamine and/or PGD₂ release after IgECL of CBMCs (N = 6-8). Cells were incubated with 1 μg/mL IgE (Calbiochem) overnight and agonists were added 30 minutes before activation with 2 μg/mL α-IgE (Sigma). **A** and **B**, The effect of chloroquine on release. **C** and **D**, The effect of denatonium on release. **E** and **F**, The effect of dextromethorphan on release. **G** and **H**, The effect of noscaphine on release.

functional responses by the TAS2R agonists chloroquine and denatonium. These findings suggest that TAS2Rs may be potential targets for the development of a new class of more efficacious agonists for bronchodilation and treatment of asthma.

Mast cells are associated with several aspects of asthma. Mast cell accumulation and redistribution have been described and correlated with lung function impairment. Moreover, it is currently accepted that mast cell infiltration of ASM in asthma contributes to airway hyperresponsiveness in asthma.⁴ In this context, it is

possible that in addition to bronchodilatory effects on ASM, TAS2Rs may affect signaling in mast cells. There are a few early reports on the effect of chloroquine on the release of mediators from rat mast cells, in which chloroquine in a dose-dependent manner inhibits histamine release from cells activated with, for example, compound 48/80 or calcium ionophore A23187.^{5,6} The aim of this study was to test the hypothesis that functionally important TAS2Rs are expressed on mast cells and further our understanding of the effects of TAS2R agonists on mast cells.

We investigated the expression of 9 TAS2Rs on human mast cells and the effect of 4 TAS2R agonists⁷—chloroquine, denatonium, dextromethorphan, and noscapine—on human mast cell-mediated release of histamine and prostaglandin D₂ (PGD₂) after activation via the Fc ϵ receptor I (ie, IgE-receptor crosslinkage [IgERCL]). We found that cord blood-derived mast cells (CBMCs) and the mast cell line HMC1.2 expressed several TAS2Rs (Fig 1, A). CBMCs and HMC1.2 expressed all TAS2Rs tested (4>46>14>19>20>3>10>13>5 and 4>3>46>14>10>5>19>13>20, respectively), but we were not able to consistently detect TAS2R expression in the mast cell line LAD-2 (data not shown).

The expressed TAS2Rs on CBMCs are known to bind chloroquine (TAS2R3 and 10), denatonium (TAS2R4, 10 and 46), dextromethorphan (TAS2R10), and noscapine (TAS2R14).⁷ We therefore investigated the possible effects of these compounds on mast cell mediator release by incubation of CBMCs (N = 6-8) with varying concentrations (ranging from 30 to 1000 μ M) of these agonists for 60 minutes before supernatants were collected and analyzed for histamine and PGD₂ release (Fig 1, B and C) (RefLab [Copenhagen, Denmark] and Cayman Chemical immunoassay [Cayman Chemical, Ann Arbor, Mich], respectively). We found that the TAS2R agonists chloroquine, denatonium, dextromethorphan, and noscapine by themselves did not induce mast cell release of histamine or PGD₂, compared with the spontaneous release of untreated cells, as analyzed using Student *t* test (significant *P* value $\leq$.05). The positive control, calcium ionophore A23187, induced release of histamine and PGD₂.

We next investigated the effects of the agonists (chloroquine, denatonium, dextromethorphan, and noscapine) on CBMCs subjected to IgERCL. Mast cells were incubated with 1 μ g/mL IgE (Calbiochem, Merck Millipore, Darmstadt, Germany) overnight and activated using 2 μ g/mL α -IgE (Sigma, St Louis, Mo). For inhibition studies, the TAS2R agonists were added 30 minutes before activation. After 30 minutes of activation, the supernatants were collected for the measurement of histamine and PGD₂. Chloroquine significantly inhibited the IgERCL-induced release of histamine at all concentrations tested (30, 100, and 300 μ M) and the release of PGD₂ at 100 and 300 μ M (Fig 2, A and B, Student *t* test with significant *P* value $\leq$.05). Similarly, denatonium significantly inhibited IgERCL-induced release of histamine at all concentrations tested (300 and 1000 μ M) and the release of PGD₂ at 1000 μ M (Fig 2, C and D). For dextromethorphan, there was an inhibitory effect on histamine but not PGD₂ release (Fig 2, E and F) whereas the TAS2R agonist noscapine did not have an effect on histamine release and there was only an inhibitory effect on PGD₂ release at the highest concentration of 300 μ M (Fig 2, G and H).

To summarize our results, TAS2Rs were expressed in human mast cells and agonists known to bind to these particular TAS2Rs mediated significant inhibition of the release of histamine and PGD₂ from IgE-receptor-activated primary human mast cells. The findings suggest that TAS2R may mediate anti-inflammatory responses in addition to previously documented bronchodilatory properties.^{1,2} This is in line with previous indications of inhibitory effects of some TAS2R agonists on leukocyte function.³ The 4 different TAS2R agonists used in our study displayed varying capacities for inhibition, and the

mechanisms behind these patterns require more detailed functional studies. Nevertheless, our findings reinforce the possibility that bitter compounds, alone or in combination with other therapies, may have a future in asthma treatment. Interestingly, a previous study found beneficial therapeutic effects of treatment with chloroquine in asthma,⁸ supporting that it is timely to revisit the actions of chloroquine and related compounds in asthma from a new point of view. It may be more than a coincidence that both chromoglicate and nedocromil have affinities for TAS2Rs⁷ and that their mode of action to date remains unclear.

Maria Ekoff, PhD^{a,b}

Jeong-Hee Choi, MD, PhD^{a,b,c,*}

Anna James, PhD^{a,b}

Barbro Dahlén, MD, PhD^{b,d}

Gunnar Nilsson, PhD^{b,c}

Sven-Erik Dahlén, MD, PhD^{a,b}

From ^athe Institute for Environmental Medicine, Experimental Asthma and Allergy Research, ^bthe Centre for Allergy Research, ^cthe Department of Medicine at Karolinska University Hospital Solna, Clinical Immunology and Allergy Research, and ^dthe Department of Medicine at Karolinska University Hospital Huddinge, Karolinska Institutet, Stockholm, Sweden. E-mail: Sven-Erik.Dahlen@ki.se.

^{*}Dr Choi is now with the Department of Pulmonology and Allergy, Hallym University College of Medicine, Chuncheon, Korea.

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