Histamine-driven responses are sustained via a bioactive metabolite

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Histamine-driven responses are sustained via a bioactive metabolite

Tania E. Velez, B.S.¹, Adam J. Byrne, Ph.D.², Joshua B. Wechsler, M.D.¹, Rebecca A. Krier-Burris, M.S.¹, Kathryn E. Hulse, Ph.D.¹*⁸, Paul J. Bryce, Ph.D.¹.³⁵

¹Northwestern University Feinberg School of Medicine, Allergy and Immunology Division,
²Imperial College London, National Heart and Lung Institute
³Current address: Immunology & Inflammation Therapeutic Area, Sanofi USA.
⁵These authors contributed equally to this work

*Corresponding Author

240 E. Huron St, McGaw Room M315
Chicago, IL 60611
Phone: 312-503-2854
Fax: 312-503-0078
Email: k-hulse@northwestern.edu

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Abbreviations:

BMMCs: Bone marrow derived-mast cells
IAA: Imidazole Acetic Acid
Capsule Summary

A histamine metabolite, imidazole acetic acid (IAA), recapitulates key histamine-driven biology, including recruitment of eosinophils, induction of itch, and induction of anaphylaxis. IAA may perpetuate anaphylactic and other allergic responses after the initial release and metabolism of histamine.

Declaration of Interests: The authors declare that they have no relevant conflicts of interest.
To the Editor,

Anaphylaxis is a life-threatening allergic response driven by IgE-mediated activation of mast cells and basophils and the release of granule-stored mediators. A key mediator of this biology is histamine, which functions via four receptors: histamine receptor 1-4 (H1R-H4R). Histamine elicits anaphylactic symptoms, such as hypotension, cutaneous flushing, headache, and airway obstruction, through H1R and/or H2R signaling(1). While histamine is rapidly released within minutes of antigen exposure, it becomes undetectable within minutes of its release due to degradation into metabolites, which can be detected in urine for several hours after symptom onset. Despite this, antihistamines that target H1R or H2R are effective treatments well after immediate hyperreactivity(2), suggesting that histamine-associated biology persists during later phases of anaphylaxis.

Early work showed that a metabolite of histamine, imidazole acetic acid (IAA), could induce eosinophil migration in mice(3). IAA is relatively stable compared to histamine, and accumulates in tissues during anaphylaxis, before being excreted into the urine(4). Therefore, we aimed to determine whether IAA had any functions in allergic responses in vivo.

We investigated whether IAA was sufficient to induce similar biological responses driven by histamine such as eosinophil recruitment, pruritus, and anaphylaxis. Histamine induces eosinophil recruitment via H2R, and so we injected IAA or histamine into the peritoneum (500µL; 6.0mM, 0.6mM, or 0.06mM) of WT mice and H2R knock-out (KO) mice. 24hrs following injection peritoneal lavages were collected, and eosinophils were quantified from cytospins using Kwik-Diff™ (Thermofisher) staining. IAA was sufficient to induce the recruitment of eosinophils in an H2R-dependent manner, similar to histamine (Figure 1A). In order to
investigate histamine-induced pruritus, which is primarily mediated through H1R(5), IAA or
histamine were administered by intradermal injection (100µL, 0.01M) into WT and H1R KO mice
in the back of the neck (shaved). This injection site is only accessible by the animal's hind paws,
and therefore scratching behavior can be separately identified from grooming, which is
performed by the forelimbs(6). Mice were placed in transparent chambers and filmed with
iMovie™ software using a MacBook Pro. Itch was measured by counting the number of bouts of
scratching in the 30-min period immediately following injection. A bout of scratching was defined
as three or more individual rapid scratch movements with the hind paws to the area around the
injection site (i.e. the back of the neck). IAA induced itch in an H1R-dependent manner, similar
to histamine (Figure 1B). H1R and H2R are both necessary to induce anaphylaxis to histamine
in mice,(7) thus we assessed whether IAA could also induce anaphylaxis by administering
histamine or IAA by retro-orbital injection (100µL; 0.1M) into WT and H1R/H2R double KO
(DKO) mice. Rectal temperatures were recorded every 10 mins to monitor the response. Both
histamine and IAA induced anaphylaxis in an H1R/H2R-dependent fashion (Figure 1C). Taken
together, these data establish that IAA induces physiological responses similarly to histamine,
and via the same receptors. These data also suggested that IAA was either inducing histamine
release, or binding to the histamine receptors to mediate these responses. In collaboration with
the NIMH Psychoactive Drug Screening Program(8), we initially determined that IAA did not
demonstrate any competitive binding to a panel of receptors and ion channels, including all the
histamine receptors (Figure 2A). To test whether IAA could directly induce histamine release,
IAA or PBS was administered by retro-orbital injection as above, and blood was collected prior
to injection and at 1 min, 3 min, 10min, and 30 min after injection. Serum histamine levels were
quantified by ELISA (Abcam). Injection of IAA resulted in a significant increase in histamine
levels compared to PBS after 1 min, and histamine levels diminished thereafter (Figure 2B),
indicating that IAA could directly induce histamine release.
In order to determine whether histamine was necessary for the \textit{in vivo} effects of IAA, histidine decarboxylase KO mice (HDC KO), which are unable to synthesize histamine, or WT animals were injected with IAA or histamine to induce anaphylaxis. IAA induced anaphylaxis in WT animals but not in HDC KO animals (Figure 2C), establishing that the effects of IAA require endogenous histamine. Since mast cells are the primary source of histamine, we wanted to determine whether mast cell-derived histamine was necessary for IAA-driven anaphylaxis. Mast cell deficient mice (W$^s$) were reconstituted with bone marrow-derived mast cells (BMMC) from WT or HDC KO animals and were challenged with IAA (see supplementary methods). In the absence of mast cells, IAA did not induce anaphylaxis (Figure 2D), indicating that mast cells were necessary for this response. Further, W$^s$ animals reconstituted with WT BMMCs had a significantly larger temperature change after IAA injection than W$^s$ mice reconstituted with HDC KO BMMCs (Figure 2D). These data indicate that mast cell-derived histamine plays an important role in the induction of anaphylaxis after IAA injection.

Finally, we wanted to examine which receptors on mast cells were responsible for the IAA-induced release of histamine. Of the imidazole receptors known to facilitate the neurological functions of IAA, I$_1$ and I$_3$ (9), I$_1$, also known as nischarin, is the most well described. Interestingly, nischarin has been shown to promote arachidonic acid release and S1P-mediated calcium flux, both of which are critical pathways for mast cell activation during anaphylaxis(9). We found that nischarin mRNA was expressed by both human and murine mast cells (see supplementary methods) (Figure 2E, F). To determine whether nischarin was necessary for the effects of IAA, we pre-treated mice with efaxoxan, a reported nischarin inhibitor, at 10mgs/kg 30 mins before challenging mice with IAA. IAA did not induce anaphylaxis in mice pretreated with...
efaroxan (Figure 2G), suggesting that IAA may function via nischarin to induce histamine release from mast cells.

In conclusion, this work has characterized an unrecognized mechanism that may play a key role in perpetuating anaphylactic responses. We have found that the histamine metabolite IAA recapitulates several key functions of histamine, including recruitment of eosinophils, induction of itch, and induction of anaphylaxis; these responses to IAA are dependent on H1R and H2R, as well as on mast-cell derived histamine. Finally, we have demonstrated that IAA may function to induce histamine release from mast cells by binding to nischarin (I) on mast cells. Because IAA is more stable than histamine, this may provide a mechanism to perpetuate anaphylactic, or other allergic, responses long after the initial release of histamine.

Tania E. Velez, B.S. Northwestern University
Adam J. Byrne, Ph.D. Imperial College London
Joshua B. Wechsler, M.D. Northwestern University
Rebecca A. Krier-Burris, M.S. Northwestern University
Kathryn E. Hulse, Ph.D. Northwestern University
Paul J. Bryce, Ph.D. Sanofi USA
References


**Figure Legends**

**Figure 1.**

IAA recapitulates the effects of histamine *in vivo*. Effects of histamine and IAA on 
A. eosinophil migration, B. induction of pruritus, and C. induction of anaphylaxis. Data are representative of the mean ± SEM from 3-9 mice per concentration and condition.

**Figure 2.**

IAA mediates pathology through nischarin in a histamine-dependent manner. A. Ligand binding screen of histamine and IAA. B. Plasma histamine after IAA injection. *p<0.05 by student’s t-test. Data representative of mean ± SEM for 3-4 animals per condition. C. Induction of anaphylaxis by IAA in WT and HDC KO mice. D. Anaphylaxis after IAA injection in W<sup>sh</sup>, and W<sup>sh</sup> mice reconstituted with WT or HDC KO BMMC. E. Murine nischarin gene expression in PC-12 neuronal cells, RBL-2H3 mast cells, MC/9 mast cells, and BMMC. Values above bars represent average delta Ct for nischarin versus β-actin. Relative copies of Nisch represents number of nischarin mRNA copies per 10<sup>4</sup> copies of β-actin. n=3 individual wells for each. F. Human nischarin gene expression from cultured iPSC-neuron cells, ROSA<sup>KIT WT</sup> mast cells, and cultured skin mast cells. Values above bars represent average delta Ct versus GAPDH. Relative copies of NISCH represents number of nischarin mRNA copies per 10<sup>4</sup> copies of GAPDH, n=1-3 individual wells for each. G. Effect of Efaroxan administered 30 minutes prior to IAA challenge. *p<0.05 by student’s t-test. Data representative of mean ± SEM from 3-13 mice per condition.
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¹Northwestern University Feinberg School of Medicine, Allergy and Immunology Division,
²Imperial College London, National Heart and Lung Institute
³Current address: Immunology & Inflammation Therapeutic Area, Sanofi USA.
Supplementary Methods

Bone Marrow Derived Mast Cells and Wsh Reconstitutions

Bone marrow cells from femurs and tibias were isolated and cultured in RPMI 1640 supplemented with 2mM L-glutamine, 1 mM sodium pyruvate (Sigma), 0.1 mM nonessential amino acids (Sigma), 10% FBS (Atlanta Biologicals), 25 mM HEPES (Sigma), 100 U/ml penicillin, 100 mg/ml streptomycin (Corning) and 0.05 mM 2-Mercaptoethanol (Sigma). Cells were culture for 4-6 weeks in 30 ng/ml recombinant mouse (m)IL-3 (Miltenyi Biotec. BMMC) were retro-orbitally injected into Wsh mice (4-6 weeks old) were injected via retro-orbital route day 0 and with 10^6 at week 8. 12 weeks after the first injection, mice were challenged with 130 mg/kg of IAA.

Nicharin qPCR

Murine RNA was isolated from PC-12 (ATCC® CRL-1721™) neuronal cells, RBL-2H3 (ATCC® CRL-2256™) mast cells, MC/9 (ATCC® CRL-8306™) mast cells, and BMMC using RNeasy RNA isolation kit (QIAGEN). Human RNA was isolated from cultured iPSC-neuron cells (provided by Dr. John Kessler), ROSA^{KIT^{WT}} mast cells (provided by the Dr. Bruce Bochner), and cultured skin mast cells (provided by Dr. Bruce Bochner). Ambion RNAqueous micro kit was used to isolate RNA from iPSC-neuron cells, and RNAeasy RNA isolating kit (QIAGEN) was used to isolate RNA from ROSA mast cells and isolated skin mast cells. Real-time PCR was conducted on ABI 7500 using TaqMan probes ([murine] Nisch, 4331182; [murine] Actb, 4331182; [human] NISCH, 4351372; [human] GAPDH, 4331182) (Applied Biosystems).