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Histamine-driven responses are sustained via a bioactive metabolite

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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 4 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] Although 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 with 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 (500 μL; 6.0 mM, 0.6 mM, or 0.06 mM) of wild-type (WT) mice and H2R knock-out (KO) mice. Twenty-four hours after injection, peritoneal lavages were collected, and eosinophils were quantified from cytospins using Kwik-Diff (Thermo Fisher, Waltham, Mass) staining. IAA was sufficient to induce the recruitment of eosinophils in an H2R-dependent manner, similar to histamine (Fig 1, *A*). To investigate histamine-induced pruritus, which is primarily mediated through H1R,[5] IAA or histamine was administered by intradermal injection (100 μL, 0.01 mol) into WT and H1R KO mice in the back of the neck (shaved). This injection

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site is accessible only 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 Mac-Book Pro. Itch was measured by counting the number of bouts of scratching in the 30-minute period immediately after injection. A bout of scratching was defined as 3 or more individual rapid scratch movements with the hind paws to the area around the injection site (ie, the back of the neck). IAA induced itch in an H1R-dependent manner, similar to histamine (Fig 1, *B*). 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 (100 μL; 0.1 mol) into WT and H1R/H2R double KO (DKO) mice. Rectal temperatures were recorded every 10 minutes to monitor the response. Both histamine and IAA induced anaphylaxis in an H1R/H2R-dependent fashion (Fig 1, *C*). Taken together, these data establish that IAA induces physiological responses similarly to histamine, and via the same receptors. These data also suggested that IAAwas 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 (Fig 2, *A*). To test whether IAA could directly induce histamine release, IAA or PBS was administered by retro-orbital injection as above, and blood was collected before injection and at 1, 3, 10, and 30 minutes after injection. Serum histamine levels were quantified by ELISA (Abcam, Cambridge, Mass). Injection of IAA resulted in a significant increase in histamine levels compared with injection of PBS after 1 minute, and histamine levels diminished thereafter (Fig 2, *B*), indicating that IAA could directly induce histamine release.

To determine whether histamine was necessary for the *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 (Fig 2, *C*), establishing that the effects of IAA required endogenous histamine. Because 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^{sh}) were reconstituted with bone marrow–derived mast cells (BMMCs) from WT or HDC KO animals and were challenged with IAA (see this article's Methods section in the Online Repository at www.jacionline.org). In the absence of mast cells, IAA did not induce anaphylaxis (Fig 2, *D*), indicating that mast cells were necessary for this response. Furthermore, W^{sh} animals reconstituted with WT BMMCs had a significantly larger temperature change after IAA injection than did W^{sh} mice reconstituted with HDC KO BMMCs (Fig 2, *D*). 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

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anaphylaxis.[9] We found that nischarin mRNA was expressed by both human and murine mast cells (see this article's Methods section) (Fig 2, *E* and *F*). To determine whether nischarin was necessary for the effects of IAA, we pretreated mice with efaroxan, a reported nischarin inhibitor, at 10 mg/kg 30 minutes before challenging mice with IAA. IAA did not induce anaphylaxis in mice pretreated with efaroxan (Fig 2, *G*), 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_1) 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.

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REFERENCES

1. Reber LL, Hernandez JD, Galli SJ. The pathophysiology of anaphylaxis. J Allergy Clin Immunol 2017;140:335–48. [PubMed: 28780941]
2. Boyce JA, Assa'ad A, Burks AW, Jones SM, Sampson HA, Wood RA, et al. Guidelines for the diagnosis and management of food allergy in the United States: summary of the NIAID-sponsored Expert Panel Report. J Allergy Clin Immunol 2010;126:1105–18. [PubMed: 21134568]
3. Turnbull LW, Kay AB. Eosinophils and mediators of anaphylaxis—histamine and imidazole acetic-acid as chemotactic agents for human eosinophil leukocytes. Immunology 1976;31:797–802. [PubMed: 992712]
4. Johnson HL. Nonmast-cell histamine kinetics, IV: interrelationships between synthesis, binding and catabolism of 3 H-histamine in vivo. J Pharmacol Exp Ther 1971;177:579–90. [PubMed: 5568812]
5. Rossbach K, Nassenstein C, Gschwandtner M, Schnell D, Sander K, Seifert R, et al. Histamine H-1, H-3 and H-4 receptors are involved in pruritus. Neuroscience 2011;190:89–102. [PubMed: 21689731]
6. Kuraishi Y, Nagasawa T, Hayashi K, Satoh M. Scratching behavior induced by pruritogenic but not algesiogenic agents in mice. Eur J Pharmacol 1995;275:229–33. [PubMed: 7539379]
7. Wechsler JB, Schroeder HA, Byrne AJ, Chien KB, Bryce PJ. Anaphylactic responses to histamine in mice utilize both histamine receptors 1 and 2. Allergy 2013;68:1338–40. [PubMed: 24112077]
8. Besnard J, Ruda GF, Setola V, Abecassis K, Rodriguiz RM, Huang XP, et al. Automated design of ligands to polypharmacological profiles. Nature 2012;492:215–20. [PubMed: 23235874]
9. Head GA, Mayorov DN. Imidazoline receptors, novel agents and therapeutic potential. Cardiovasc Hematol Agents Med Chem 2006;4:17–32. [PubMed: 16529547]

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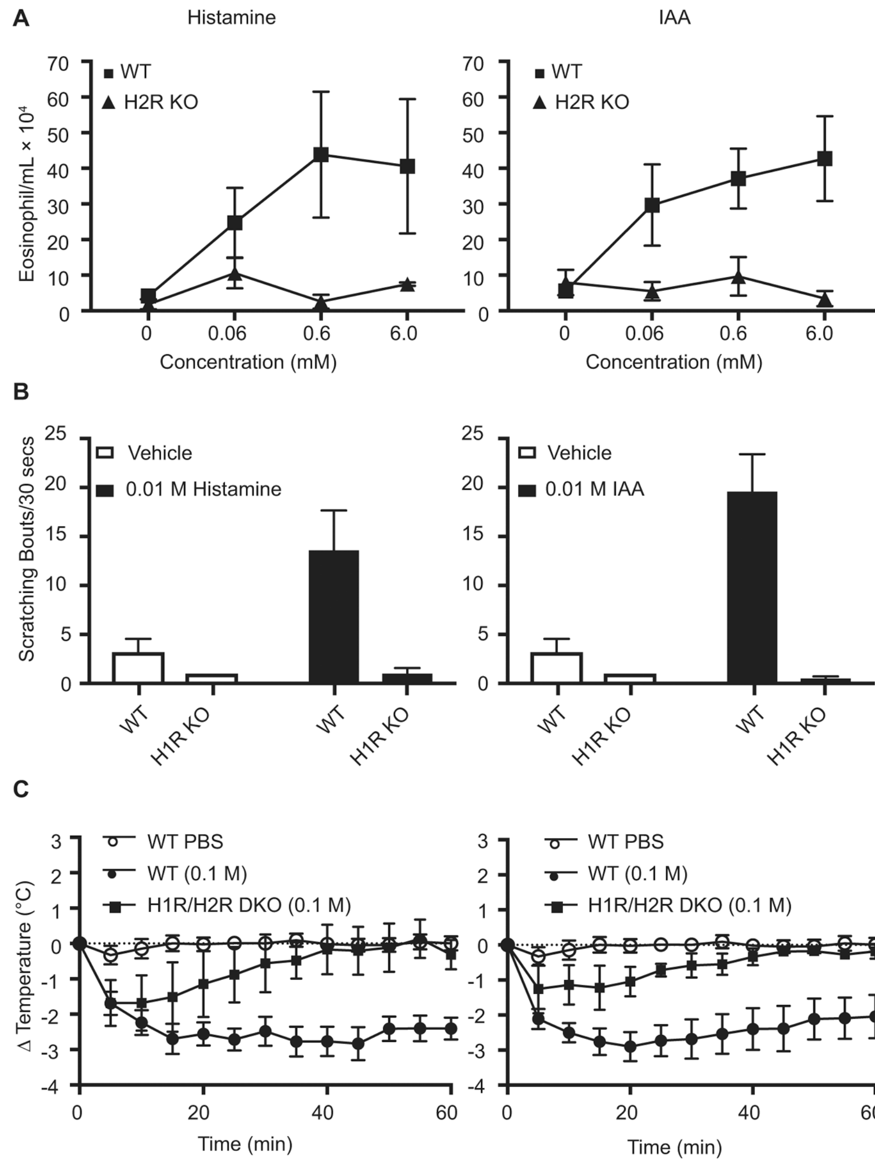

FIG 1.
IAA recapitulates the effects of histamine *in vivo*. Effects of histamine and IAA on eosinophil migration (A), induction of pruritus (B), and induction of anaphylaxis (C). Data are representative of the mean ± SEM from 3 to 9 mice per concentration and condition.

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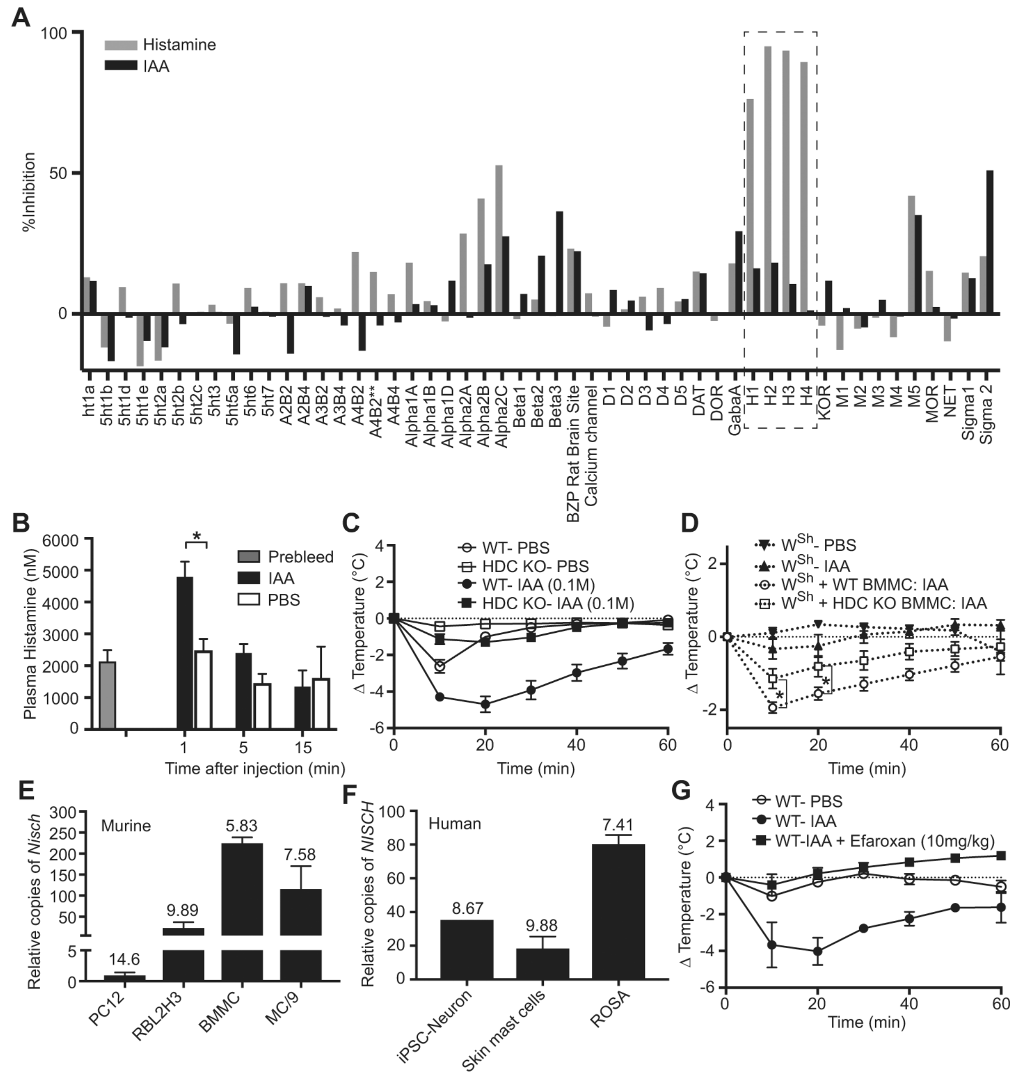

FIG 2.

IAA mediates pathology through nischarin in a histamine-dependent manner. A, Ligand binding screen of histamine and IAA. B, Plasma histamine after IAA injection. $*P < .05$ by Student t test. Data representative of mean ± SEM for 3 to 4 animals per condition. C, Induction of anaphylaxis by IAA in WT and HDC KO mice. D, Anaphylaxis after IAA injection in W^{sh} mice, and W^{sh} mice reconstituted with WT or HDC KO BMMCs. E, Murine nischarin gene expression in PC-12 neuronal cells, RBL-2H3 mast cells, MC/9 mast cells, and BMMCs. Values above bars represent average delta Ct for nischarin versus β-actin. Relative copies of Nisch represent the number of nischarin mRNA copies per 10^4 copies of β-actin. n = 3 individual wells for each. F, Human nischarin gene expression from cultured iPSC-neuron cells, $ROSA^{KIT\ WT}$ mast cells, and cultured skin mast cells. Values above bars represent average delta Ct versus GAPDH. Relative copies of NISCH represent the number of nischarin mRNA copies per 10^4 copies of GAPDH (n = 1–3 individual wells for each). G, Effect of efaroxan administered 30 minutes before IAA