ACS Pharmacology & Translational Science

pubs.acs.org/ptsci Article

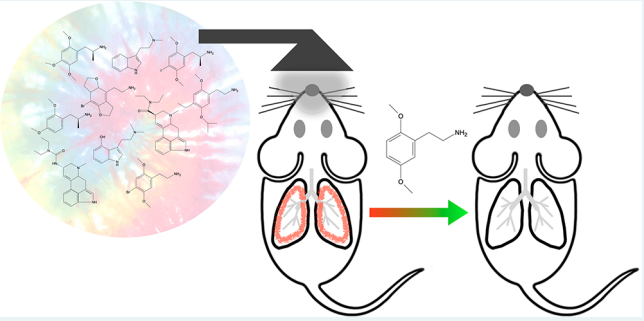
Structure–Activity Relationship Analysis of Psychedelics in a Rat Model of Asthma Reveals the Anti-Inflammatory Pharmacophore

Thomas W. Flanagan, Gerald B. Billac, Alexus N. Landry, Melaine N. Sebastian, Stephania A. Cormier, and Charles D. Nichols*

Cite This: *ACS Pharmacol. Transl. Sci.* 2021, 4, 488–502 Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Psychedelic drugs can exert potent anti-inflammatory effects. However, anti-inflammatory effects do not appear to correlate with behavioral activity, suggesting different underlying mechanisms. We hypothesized that the distinct structural features of psychedelics underlie functionally selective mechanisms at the target 5-HT_{2A} receptor to elicit maximal anti-inflammatory effects. In order to test this hypothesis, we developed a new rat-based screening platform for allergic asthma. Next, we investigated 21 agonists at the 5-HT_{2A} receptor from the three primary chemotypes (phenylalkylamine, ergoline, and tryptamine) for their ability to prevent airways hyperresponsiveness as a measure of pulmonary inflammation. Furthermore, we assessed each drug for *in vitro* activation of the canonical signaling pathway, calcium mobilization, from the 5-HT_{2A} receptor. We find that the drug 2,5-dimethoxyphenethylamine (2C-H) represents the pharmacophore for anti-inflammatory activity and identify structural modifications that are either permissive or detrimental to anti-inflammatory activity. Additionally, there is no correlation between the ability of a particular psychedelic to activate intracellular calcium mobilization and to prevent the symptoms of asthma or with behavioral potencies. Our results support the notions that specific structural features mediate functional selectivity underlying anti-inflammatory activity and that relevant receptor activated pathways necessary for anti-inflammatory activity are different from canonical signaling pathways. Our results inform on the nature of interactions between ligands at the 5-HT_{2A} receptor as they relate to anti-inflammatory activity and are crucial for the development of new 5-HT_{2A} receptor agonists for anti-inflammatory therapeutics in the clinic that may be devoid of behavioral activity.



KEYWORDS: *psychedelic, asthma, 5-HT_{2A} receptor, inflammation, anti-inflammatory, chemical structure*

Downloaded via UNIV OF LIVERPOOL on June 8, 2021 at 09:15:00 (UTC).
See https://pubs.acs.org/sharingguidelines for options on how to legitimately share published articles.

Serotonin (5-hydroxytryp tamine, 5-HT) is a small molecule and hormone that mediates many aspects of physiology and behavior. Its effects are mediated through action at seven families of 5-HT receptors.[1] With the exception of the ligand-gated 5-HT_3 receptors, all are of the seven-transmembrane-spanning G-protein-coupled receptor (GPCR) superfamily. Importantly, 5-HT has been shown to influence numerous components of the immune system,[2] including proinflammatory cytokine production and activation of specific immune constituents, with serotonin itself largely considered a proinflammatory mediator.[3,4] Accordingly, several 5-HT receptors are expressed in many immune-related tissues, with interactions at specific 5-HT receptors modulating aspects of the immune response and inflammation.[5]

Of all the mammalian serotonin receptors, the 5-HT_{2A} subtype is the most widely expressed and is found in nearly every tissue and cell type (e.g., muscle, endothelial, endocrine, and central nervous system (CNS)).[1] In the CNS, 5-HT_{2A} receptors are principally distributed in serotonin-rich terminal areas and are linked to complex behaviors associated with cognition and memory.[6] In immune-related tissues and cells, including the spleen, thymus, and circulating lymphocytes,[7] activity at 5-HT_{2A} receptors can profoundly impact immune responses, where the effects of serotonin are predominantly proinflammatory.[2]

We have previously discovered that activation of 5-HT_{2A} receptors with psychedelics[8] produces potent anti-inflammatory effects *in vitro*[9] and in whole animal studies,[10] including disease models for asthma[11,12] as well as cardiovascular and metabolic disease.[13] In those studies, we primarily used the 5-HT_2 receptor selective agonist (*R*)-2,5-dimethoxy-4-iodoamphetamine [(*R*)-DOI]. Whereas (*R*)-DOI affords potent and efficacious anti-inflammatory activity, we found other psychedelics, including

Special Issue: Psychedelics

Received: June 4, 2020
Published: August 13, 2020

© 2020 American Chemical Society

https://dx.doi.org/10.1021/acsptsci.0c00063
ACS Pharmacol. Transl. Sci. 2021, 4, 488–502

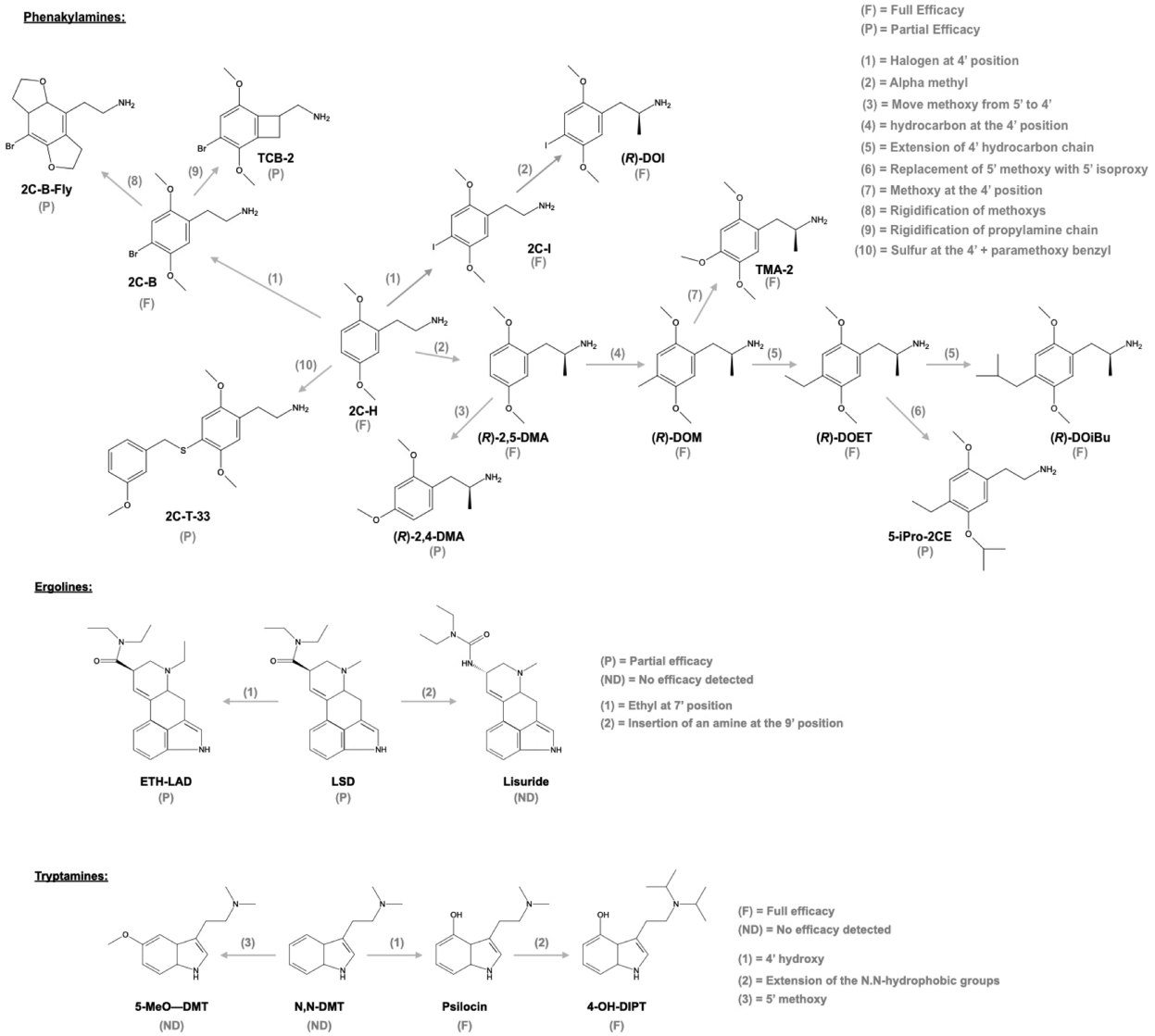


Figure 1. Chemical structures. The structures of the drugs used for SAR analysis are shown. Particular structural relationships relevant to data interpretation are indicated as gray arrows and marked with a number referring to the nature of the relationship between the molecules connected with a particular arrow as defined in the text to the right of the structures. Efficacy of a particular drug to prevent AHR at the 0.5 mg/kg (inhaled) dose is also indicated under the name of the drug with an F (fully efficacious), P (partially efficacious), or ND (no efficacy detected). For the phenylalkylamines, the 2C-H pharmacophore anti-inflammatory molecule is at the center. For ergolines, LSD is presented in the center as the prototypic ergoline. For tryptamines, *N,N*-DMT is shown at the center as the prototypic, least-modified tryptamine.

those closely related in structure and pharmacological properties to (*R*)-DOI, are significantly less effective. These findings led us to hypothesize that certain structural features of 5-HT_{2A} receptor agonists underlie the ability of some agonists to be more potent than others at the receptor with respect to anti-inflammatory activity through functionally selective mechanisms. Furthermore, regardless of general affinity for the receptor and the potency/efficacy to activate canonical effector pathways, some 5-HT_{2A} receptor agonists are able to independently recruit anti-inflammatory effector pathways.

To test this hypothesis, we examined the ability of several agonists of differing chemical structures to prevent airways hyperresponsiveness (AHR) in a rodent model of allergic asthma, rather than a cell-based system. First, testing in an *in vivo* model of asthma provides greater translational relevance to results. Second, airway response in rodent models of asthma takes into account not only pulmonary inflammation but also other aspects of disease processes involving immune cell function. Demonstrating suppression of AHR in a rodent model of asthma indicates pleiotropic therapeutic effects are occurring that together prevent inflammation in the lung and enhance our ability to detect anti-inflammatory effects regardless of its nature or source.

We desired to test several compounds from the three main chemotypes of psychedelics: phenylalkylamines, ergolines, and tryptamines. Unfortunately, mice have a very active form of monoamine oxidase[14] that rapidly deaminates tryptamines like psilocybin and its active metabolite psilocin to an inactive form in a matter of minutes.[15] Ergolines like LSD are also rapidly metabolized in mice, likely through P450 enzymes. These short half-lives are predicted to not be compatible with the experimental design of drug treatment prior to OVA exposure (~20 min) and the length of the OVA exposure (30 min), which require drug to be present at therapeutic levels for nearly an

https://dx.doi.org/10.1021/acsptsci.0c00063
ACS Pharmacol. Transl. Sci. 2021, 4, 488−502

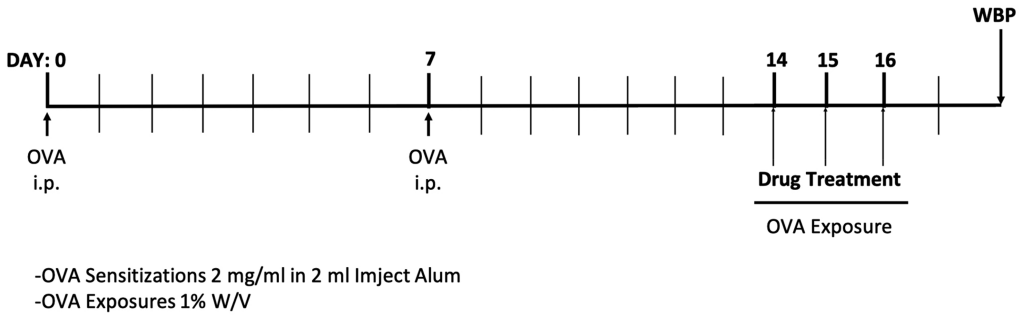


Figure 2. Sensitization and challenge protocols for rat model of allergic asthma. Adult male Brown Norway rats received two intraperitoneal sensitizations with 2 mg of chicken egg ovalbumin emulsified in 2 mL of Imject Alum on days 0 and 7. From day 14−16, rats were challenged with either 1% ovalbumin or sterile saline aerosol for 30 min. Thirty minutes prior to OVA challenge, each rat was exposed to indicated drugs in a total volume of 4.5 mL using an inExpose nose-only inhalation system for 15 min. On day 18, AHR was measured using whole body plethysmography.

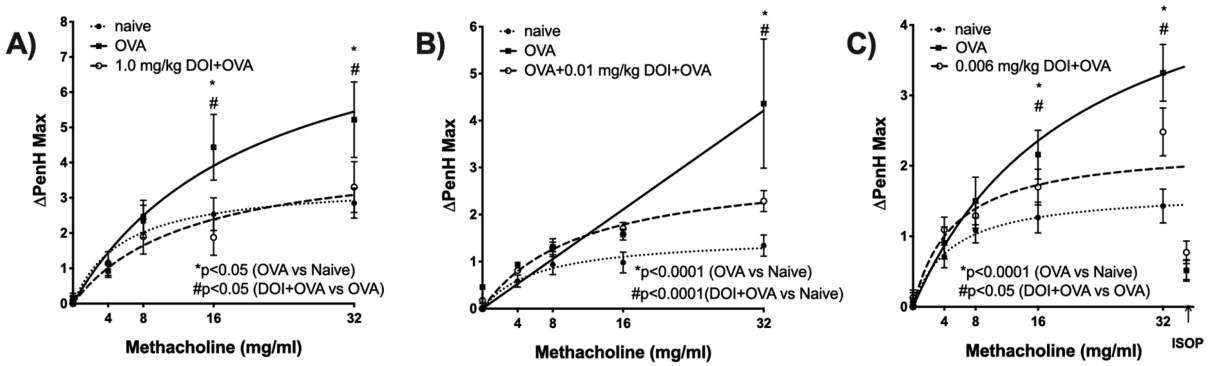


Figure 3. (*R*)-DOI robustly prevents the development of AHR in an acute rodent allergic airways disease model. Results from whole body plethysmography on awake, freely moving rats with (A) 1.0 mg/kg (*R*)-DOI, (B) 0.01 mg/kg (*R*)-DOI, $n = 5-6$ animals/treatment group, and (C) 0.006 mg/kg (*R*)-DOI with 30 mg/mL isoproterenol exposure after 32 mg/mL methacholine challenge. *, $p < 0.0001$ OVA vs naïve; #, $p < 0.0001$ OVA vs (*R*)-DOI+OVA; #, $p < 0.05$ 0.006 mg/kg DOI+OVA vs OVA. Error bars represent ± SE; 2-way ANOVA with Bonferonni posthoc test.

hour. For that reason, we performed testing in an allergic asthma model using rats, which do not rapidly metabolize tryptamines and ergolines and where drug half-lives are compatible with the experimental design time considerations. Whereas the vast majority of rodent-based allergic asthma models utilize mice[16,17] and several studies have used rats,[18,19] a thorough literature search revealed few adult-rat-based models of allergic asthma that used or were suitable for drug screening.[20−22] Therefore, we developed protocols to generate an adult-rat-based experimental system for use as a screening platform. Validation experiments were performed by comparing results of (*R*)-DOI in this system with our previous results in mice.[11] We chose to use the Brown Norway rat because it has a robust Th2-mediated immune response compared to other strains[23,24] and has been used in asthma studies by others.[25−27] After developing and validating our experimental system, we tested several compounds from the three main chemotypes of psychedelics for their ability to prevent the development of AHR as a proxy for anti-inflammatory activity. We focused on phenylalkylamines because they have greater selectivity for 5-HT_2 receptors than tryptamines and ergolines, which can have affinity and efficacy at other serotonin receptors. In addition to *in vivo* testing, we examined the ability of each drug to activate canonical canonical signaling pathways in HEK293 cells heterologously expressing human 5-HT_{2A} receptors. We then determined whether *in vivo* anti-asthma activity correlated with activation of *in vitro* canonical signaling pathways. We further explored the therapeutic efficacy of our prototype molecule, (*R*)-DOI, by performing dose−response experiments and testing efficacy after different routes of administration.

RESULTS AND DISCUSSION

(*R*)-DOI Prevents the Development of Symptoms Associated with Asthma in Rats. In order to test several of our compounds (see Figure 1 for complete library) without the confounding rapid metabolic issues present in mice,[14] we used rats. Unfortunately, little work has been done by others with adult rats as a model for allergic asthma. Therefore, we needed to develop and validate an appropriate rat model. On the basis of our prior work with a BALB/c mouse model,[11] and certain protocols in the literature for rats,[25,26] we developed a treatment paradigm as described in the "Methods" section (Figure 2) that produces robust symptoms of allergic asthma in the Brown Norway rat.[28,29] The validation of our model involved demonstrating similar physiological responses to OVA treatment with respect to the mouse model we previously used, where treatment with low doses of (*R*)-DOI prevents the development of asthma-related symptoms. As anticipated, rats challenged with OVA develop pronounced AHR in response to methacholine (MeCh), similar to what was observed in our mouse model (Figure 3A). Pretreatment with very low doses of nose-only (*R*)-DOI (0.01 mg/kg) also completely prevents increases in enhanced pause (PenH) (Figure 3B).

Lung Remains Responsive to Other Asthma Treatment Modalities after Exposure to (*R*)-DOI. To determine whether the lung remains responsive to a conventional treatment for asthma following (*R*)-DOI administration, we

https://dx.doi.org/10.1021/acsptsci.0c00063
ACS Pharmacol. Transl. Sci. 2021, 4, 488−502

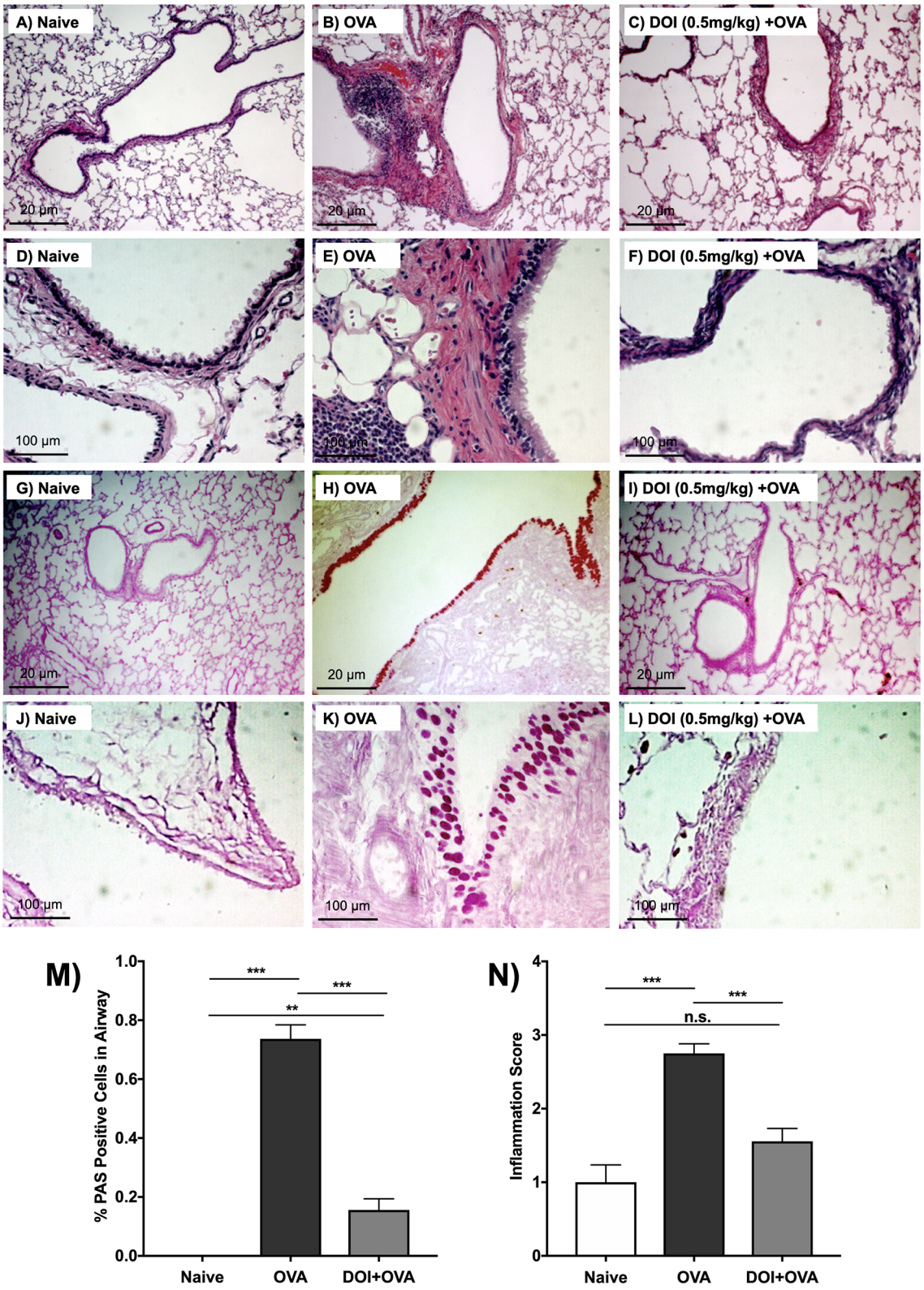


Figure 4. Inhaled (*R*)-DOI prevents pulmonary inflammation and mucus overproduction. Nasally administered (*R*)-DOI (0.5 mg/kg) inhibits OVA-induced lung inflammation and mucus hyperproduction. Representative sections of airways (4 μm) stained with the hematoxylin and eosin (H&E, A−F) and periodic acid−Schiff (PAS, G−L) techniques are shown in this figure to highlight inflammation (dark purple color) and mucus (bright pink color). Saline-treated animals have normal airway morphology and no mucus or inflammation (A, D, G, J). OVA-alone-treated animals have thickened airways with a significant amount of peribronchial inflammation (B, E) and mucus-stained goblet cells (H, K). Animals pretreated with (*R*)-DOI (0.5 mg/kg) exhibit normal airway morphology, with little to no detectable mucus or inflammation (C, F, I, L). (M) Inflammatory index scored to peribronchial and perivascular inflammation degree. (N) Fraction of airways cells containing mucus, as determined by PAS staining. Airways were scored by 5 unbiased observers for 3 animals/treatment (n = 3). ***, $p < 0.0001$; **, $p < 0.01$; n.s. = no-significance. Error bars represent ± SE; ANOVA with Bonferonni post hoc test.

https://dx.doi.org/10.1021/acsptsci.0c00063
ACS Pharmacol. Transl. Sci. 2021, 4, 488−502

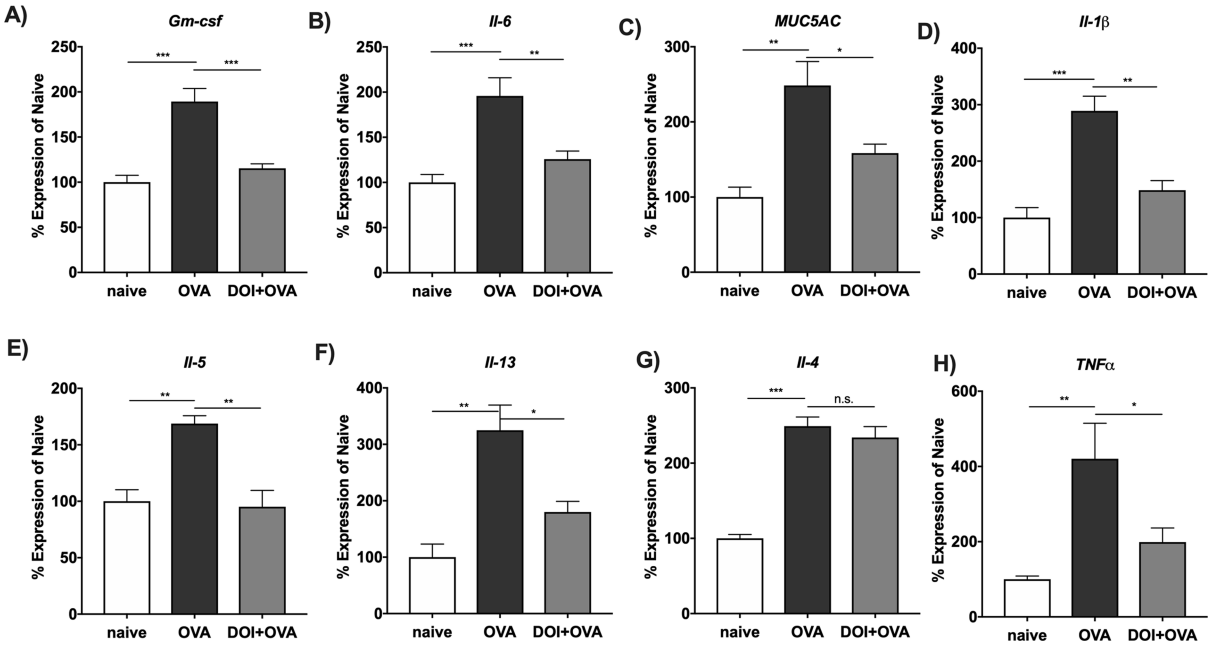


Figure 5. Inhaled (*R*)-DOI selectively inhibits proinflammatory gene expression in the whole lung after allergen challenge. Quantitative RT-PCR measurement of mRNA expression levels of several inflammatory markers is shown. OVA produces a significant increase in the mRNA levels of all genes shown. (*R*)-DOI prevents OVA-induced increases in all but *IL-4*. ***, $P < 0.0001$; **, $P < 0.01$; *, $P < 0.05$; n.s. = no significance. $n = 5$ animals for the Naïve group, $n = 4-6$ for the OVA group, and $n = 5-6$ animals for the (*R*)-DOI+OVA treatment groups. Error bars represent ± SE; ANOVA with Tukey post hoc test.

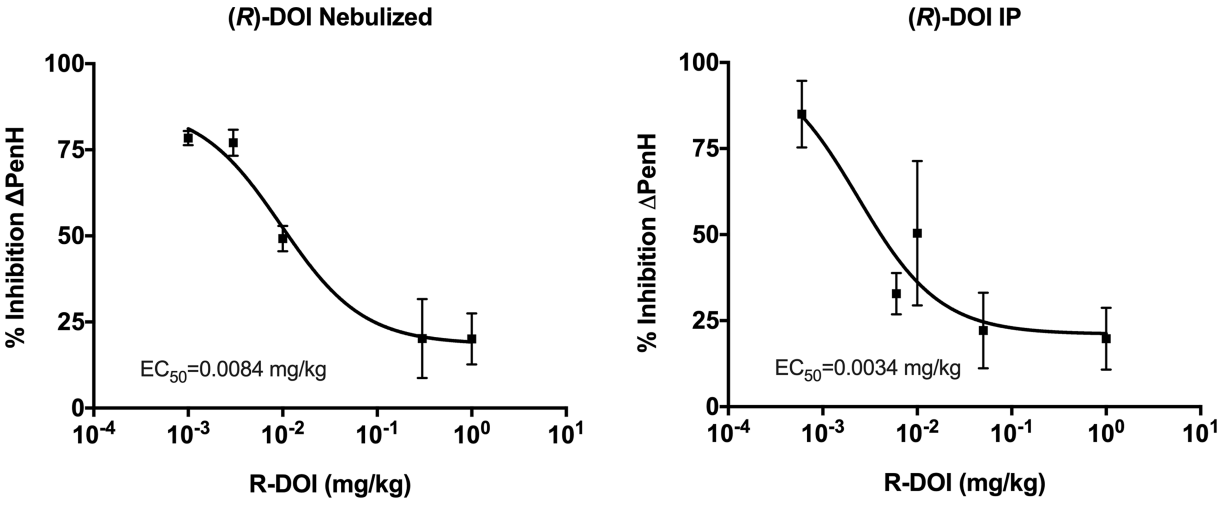


Figure 6. Dose response. Dose−response curves were determined for the effects of (*R*)-DOI on maximal PenH at concentrations ranging from 0.001 mg/kg to 1.0 mg/kg administered either (A) nebulized nose-only or (B) via intraperitoneal injection (i.p.). Peak enhanced pause values represent baseline-normalized values. $n = 5-6$ animals/treatment group; error bars represent ± SEM.

assessed the ability of a β2-receptor agonist, isoproterenol, to lower PenH following administration of an approximate EC_{50} dose of (*R*)-DOI and 32 mg/mL methacholine. As expected, isoproterenol lowers PenH values to nearly those of naïve levels that were equivalent between experimental treatment groups (Figure 3C). These results suggest that 5-HT_{2A}-receptor-mediated therapy for asthma in the clinic may be compatible with traditional types of rescue therapy like bronchodilators.

(*R*)-DOI Treatment Prevents OVA Exposure-Mediated Pulmonary Inflammation and Mucus Overproduction. Histological analysis of the lungs reveals significant OVA-induced pulmonary inflammation (Figure 4B,E,M) and mucus overproduction (Figure 4H,K,N) in the OVA-alone treated animals. Pretreatment with (*R*)-DOI (0.5 mg/kg; nose-only) prevents OVA-induced pulmonary inflammation (Figure 4C,F,M) and mucus over production (Figure 4I,L,N).

(*R*)-DOI Prevents OVA-Induced Proinflammatory Related Gene Expression in the Lung. We validated our rat model at the gene expression level by assessing the pulmonary expression of certain genes associated with the pathology of asthma by qRT-PCR analysis. These included examining mRNA levels of *IL-1β*, *IL-4*, *IL-5*, *IL-6*, *IL-13*, *Muc5ac*, *TNFα*, and *Gm-csf* from whole lung homogenates. Similar to our earlier results with the acute OVA mouse model, (*R*)-DOI pretreatment prevented the expression of OVA-induced increases in the mRNAs for *IL-5*, *IL-6*, *IL-13*, *Gm-csf*, and *Muc5ac* in the lung

https://dx.doi.org/10.1021/acsptsci.0c00063
ACS Pharmacol. Transl. Sci. 2021, 4, 488−502

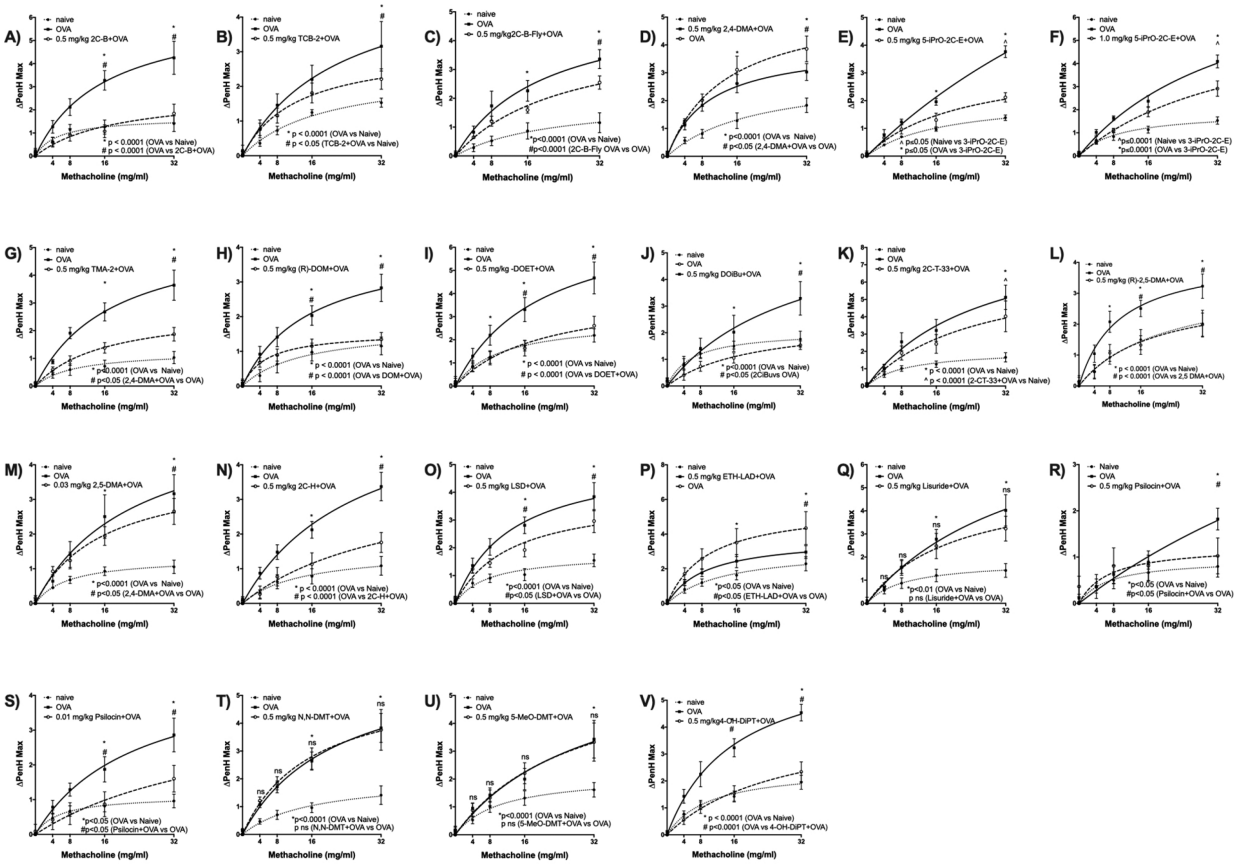


Figure 7. PenH analysis of psychedelics. The individual curves for determination of airways hyperresponsivity to increasing doses of methacholine to determine PenH and the ability of drug pretreatment to prevent OVA-induced increases are shown. Phenalkylamines (A-N); Ergolines (O-Q); Tryptamine (R-V). $n = 5-6$ animals/treatment group; *, $p < 0.0001$ OVA vs naïve; #, $p < 0.0001$ OVA vs drug+OVA; #, $p < 0.05$; ns = no significance. Error bars represent ± SEM; 2-way ANOVA with Bonferonni post hoc test.

(Figure 5A–F). Also similar to our previous results from the acute OVA mouse model, (*R*)-DOI does not affect the expression of mRNAs for other cytokines such as *IL-4* (Figure 5G), indicating that as in the mouse (*R*)-DOI is not acting as a general immunosuppressant and is only affecting a subset of proinflammatory/asthma-related genes.

Determination of Potency and Efficacy by Different Routes of Administration for (*R*)-DOI. To determine the potency of (*R*)-DOI to prevent the symptoms of allergic asthma, we performed dose–response experiments. We also compared different routes of administration to determine EC_{50} values for both nebulized and intraperitoneal drug administration. The use of whole-body plethysmography (WBP) as a proxy to generate dose–response curves and test the effects of biological alterations to the bronchoconstriction response has been described elsewhere.[30,31] Dose–response curves were generated for the effects of different concentrations of (*R*)-DOI (0.0001–1.0 mg/kg) plotted against the highest dose of methacholine administered (32 mg/mL). We determined the EC_{50} dose of (*R*)-DOI to be 0.008 mg/kg when delivered via nose-only (Figure 6A) and 0.003 mg/kg when delivered via intraperitoneal (i.p.) injection (Figure 6B). These doses are roughly 30-fold less than the minimal threshold dose for behavioral effects in rats when administered by i.p. injection (0.1 mg/kg)[32−35] and are consistent with our earlier studies in mice demonstrating therapeutic efficacy at levels far below those necessary to produce behaviors. The nearly equivalent potency of noninhaled drug may at first seem surprising, but this may be due to the lung serving as a depot for drugs of this class. In rats, [^{131}I]-labeled DOI shows the highest tissue concentrations in the lung after systemic injection.[36] Rapid localization to the lung is also observed in full body scans in humans for the closely related compound 2,5-dimethoxy-4-bromoamphetamine (DOB) after systemic administration of radiolabeled DOB.[37] Furthermore, in rats given oral and subcutaneous doses of DOB, the highest concentration of DOB is found in the lungs.[38] Therefore, we predict that i.p.-injected (*R*)-DOI is rapidly accumulating in the lung to provide effects equivalent to those of the nebulized drug. Together, our data indicate that the potential anti-inflammatory therapeutic levels for diseases like asthma in the clinic with drugs like (*R*)-DOI would be sub-behavioral and potentially orally available.

Structure–Activity Relationship Analysis. We tested the efficacy of a panel of several known ligands with different structural features in our rat allergic asthma OVA model to determine how structural modifications alter responses. All compounds were initially tested at the same screening dose of 0.5 mg/kg delivered via nose-only route and dissolved in sterile saline. Our rationale for this dose choice was that it should provide for complete normalization of PenH and inflammation based on our dose–response study with (*R*)-DOI and be high enough so as to be a fully efficacious dose for drugs that may have significantly lower potency than (*R*)-DOI. Therefore, in this study we are comparing only efficacy (E_{MAX}) and not potency (EC_{50}) of the compounds tested to reduce the magnitude of OVA-induced AHR in response to MeCh

https://dx.doi.org/10.1021/acsptsci.0c00063
ACS Pharmacol. Transl. Sci. 2021, 4, 488−502

challenge. Our results were grouped into three categories based on their ability to rescue/normalize MeCh-induced PenH: fully efficacious, partially efficacious, and no significant effect. For a few drugs, like psilocin, we performed additional testing at different doses to address potency issues. The rationale for examining only efficacy at a high dose of test compound as the primary comparator was that given our throughput of one test compound/dose per week, it simply was not feasible to generate full dose−response curves for 20+ different drugs, which would have required nearly three years of continual weekly testing. Figure 7 shows the PenH curves for phenylalkylamines (Figure 7A−N), ergolines (Figure 7O−Q), and tryptamines (Figure 7R−V) screened.

Phenylalkylamines. Our primary focus in this study was on the phenylalkylamine chemotype. Phenylalkylamines are not only more selective for 5-HT_2 receptors but also better suited for drug development because they are generally more chemically stable than ergolines and tryptamines. Knowledge of the structure−activity relationships (SAR) of this class of drug with respect to anti-inflammatory activity will enable potential development of next generation 5-HT_{2A} receptor agonist therapeutics with less behavioral liability. Starting with (*R*)-DOI as the prototype anti-inflammatory agonist, neither removal of the α-carbon from the side chain or replacing the 4-iodo with a bromine had a measurable effect on activity, as 2C-B had full efficacy (Figure 7A).

Modification of the side chain by rigidifying it (TCB-2) resulted in reduced efficacy (Figure 7B). It should be noted that each of these drugs that demonstrate reduced efficacy in our system are known to be highly potent and efficacious drugs for canonical signaling and inducing mouse head twitch behaviors, with nearly the same pharmacological properties as (*R*)-DOI. Therefore, we propose that a 2-carbon side chain ending in a primary amine is necessary and sufficient, as well as able to tolerate the addition of an alpha-methyl, but any other more extreme types of modification are not well tolerated for full anti-inflammatory efficacy.

The 2- and 5-methoxy substituents for phenethylamines are hypothesized to serve as hydrogen bond acceptors to orient the compound in the correct orientation for proper receptor interaction.[39] Therefore, both moieties must be taken into account when considering any modification to the basic DO*x* or 2C structure. 2C-B-Fly, which tethers the 2- and 5-oxygens into a dihydrofuran structure, has affinity for the 5-HT_{2A} receptor and agonist activity at canonical pathways nearly equal to (*R*)-DOI. However, 2C-B-Fly has significantly reduced efficacy compared to (*R*)-DOI for reducing AHR (Figure 7C). Loss of the 5-methoxy (2,4-DMA) is also detrimental to efficacy (Figure 7D), as is substitution of the 5-methoxy with an isopropoxy moiety (5-iPro-2C-E) (Figure 7E). Together, these data indicate that conformational restraint of the methoxys at the 2- and 5- position is detrimental, and at least a methoxy at the 5-position is necessary for full anti-inflammatory efficacy.

The nature of the 4-position substituent of phenethylamine psychedelics has been previously linked to 5-HT_2 receptor selectivity as well as agonist properties at 5-HT_2 receptors.[40] Analysis of the 4-position demonstrated that the identity of the moiety at this position was rather flexible. Fully efficacious substitutions at the 4-position included the halogens iodine and bromine (*R*)-DOI (Figure 3), 2C-B (Figure 7A), methoxy (TMA-2) (Figure 7G), short-chain hydrocarbons (*R*)-DOM (Figure 7H), (*R*)-DOET) (Figure 7I), and a branched hydrocarbon (DOiBu) (Figure 7J). An exception was 2C-T-33 (Figure 7K), which has a large hydrophobic 2-methoxythiophenyl at the 4′-position that demonstrated reduced efficacy. That may be due to the overall bulk at the 4-position or to the thiol. Further testing of additional 2C-T compounds is necessary to answer this issue.

Although all of these 4-substituted compounds, with the exception of 2C-T-33, demonstrated full efficacy at 0.5 mg/kg inhaled, potencies may differ. To examine this possibility, we tested (*R*)-2,5-DMA (Figure 7L) at 0.03 mg/kg, a fully efficacious dose for (*R*)-DOI, and found that (*R*)-2,5-DMA was only partially efficacious at this lower dose (Figure 7M). Conversely, for drugs that only demonstrated partial efficacy at 0.5/mg/kg inhaled, their efficacy may be due to not providing sufficient levels to achieve full efficacy. To test for this possibility, we doubled the dose of 5-iPrO-2C-E to 1.0 mg/kg (Figure 7F). No increase in efficacy was found between the 0.5 and the 1.0 mg/kg doses. Together, these results indicate that aside from absolute potencies the 0.5 mg/kg dose we chose for screening was likely a maximally efficacious dose across the panel of compounds tested and valid for meaningful comparisons of efficacy between drugs.

Remarkably, we found that a substitution at the 4-position was not even necessary to provide full efficacy. The compounds 2,5-DMA (Figure 7L) and 2C-H (Figure 7N) were both fully efficacious at 0.5 mg/kg. Interestingly, Dr. Alexander Shulgin has reported no behavioral effects of 2,5-DMA in humans up to a very high dose.[41] Whether or not the lack of behavioral effects in humans and rodents is due to potential rapid metabolism from first-pass metabolism after oral or i.p. administration that is not present following direct application to the lung remains to be tested. With all of our data taken together, we propose that 2C-H represents the pharmacophore anti-inflammatory structure for the phenylethylamine class of psychedelic. Modifications of the alkyl side chain, with the exception of the addition of an alpha-methyl, are not well tolerated. Furthermore, modifications or substitutions of the 2- and 5-methoxys are not well tolerated. However, substitutions at the 4-position are well-tolerated and can maintain anti-inflammatory activity, but perhaps only to a point where steric bulk exceeds some critical value.

Ergolines. LSD and ETH-LAD, which have significantly higher affinities than (*R*)-DOI for the 5-HT_{2A} receptor and are more potent for eliciting HTR in mice, demonstrate only partial normalization of PenH (Figure 7O,P). Lisuride, which has been proposed to be a nonhallucinogenic agonist at the 5-HT_{2A} receptor had no ability to normalize PenH at the tested dose (Figure 7Q). LSD is only a partial agonist with respect to canonical signaling pathways at the 5-HT_{2A} receptor. The closely related drug ETH-LAD (Figure 7P) has the same affinity for the receptor as LSD, however, is a full agonist at canonical signaling pathways. Because both LSD and ETH-LAD had only partial efficacy to prevent the development of allergic asthma symptoms, the degree of efficacy at the receptor by ergolines by canonical signaling is likely not the primary driver for efficacy in the asthma model. Together, our results indicate that ergolines as a class may simply be less efficacious at engaging anti-inflammatory pathways than phenethylamines, although several more would need to be tested to make this claim definitively.

Tryptamines. Psilocin, which has significantly weaker affinity for the 5-HT_{2A} receptor and less potency than (*R*)-DOI at activating canonical signaling pathways, is potent at normalizing PenH (Figure 7R), and a low sub-behavioral dose of 0.01 mg/kg (Figure 7S) inhaled is sufficient to completely prevent OVA-induced AHR to methacholine. The closely

https://dx.doi.org/10.1021/acsptsci.0c00063
ACS Pharmacol. Transl. Sci. 2021, 4, 488−502

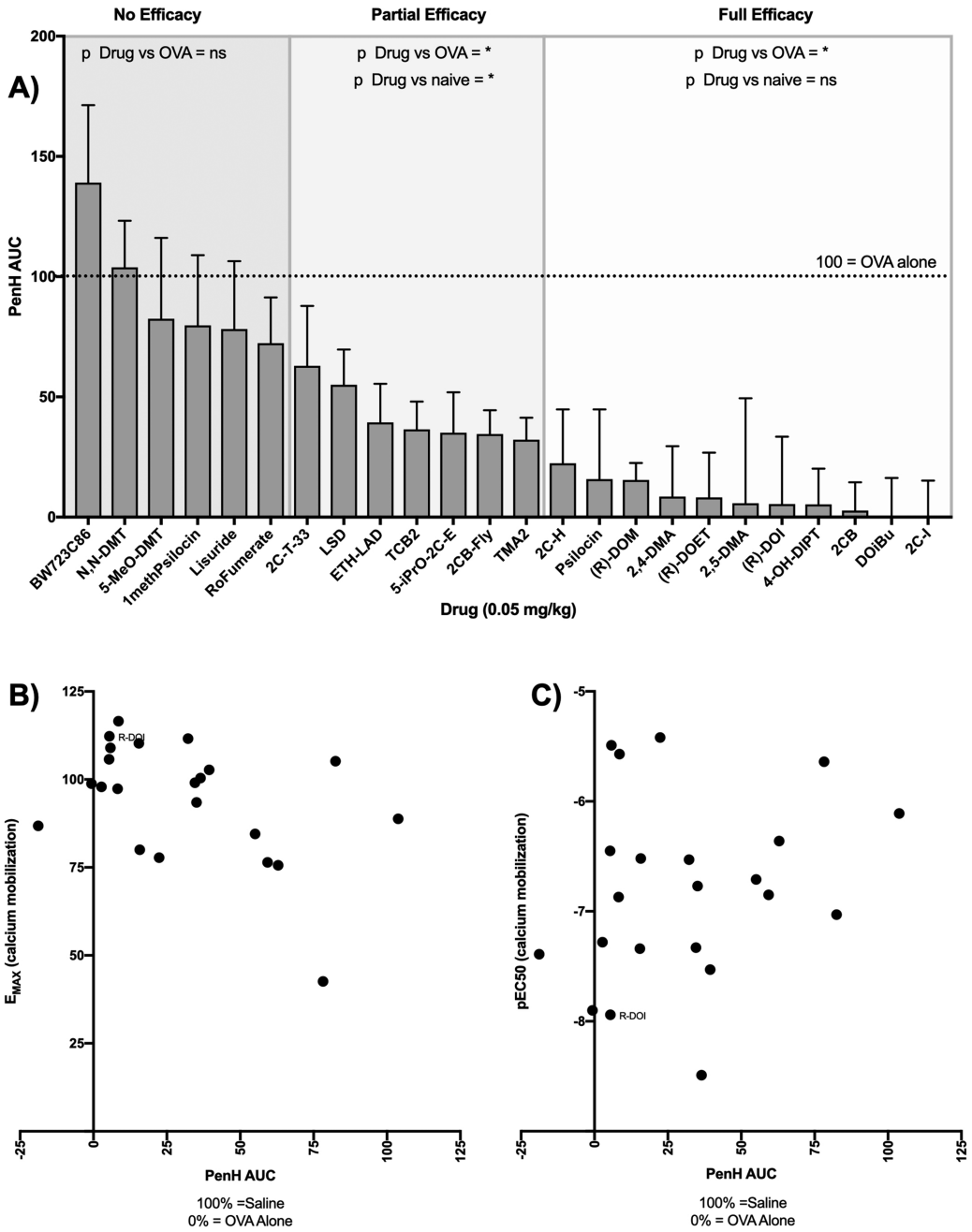


Figure 8. Efficacy comparisons. (A) Overall efficacy of individual drugs tested at 0.5 mg/kg (inhaled) to prevent methacholine induced AHR presented in rank order based on analysis of area under the curve (AUC), with values normalized to OVA-alone responses (*Y*-axis = 100), and saline control responses (*Y*-axis = 0). No efficacy: *p* Drug+OVA vs OVA = ns and *p* Drug+OVA vs saline < 0.05. Partial efficacy: *p* Drug+OVA vs OVA < 0.05 and *p* Drug + OVA vs saline < 0.05, or *p* Drug+OVA vs OVA = ns *and* *p* Drug+OVA vs saline = ns. Full efficacy: *p* Drug+OVA vs OVA < 0.05 and *p* Drug +OVA vs saline = ns. Statistical significance determined by 2-way ANOVA with Turkey post hoc analysis (complete tabular results shown in Table 1). (B) Comparison of PenH AUC values from (A) plotted against E_{MAX} of calcium mobilization for the same drugs tested in h5-HT_{2A} receptor-expressing HEK cells (see Table 1 for values). (C) Comparison of PenH AUC values from (A) plotted against the pEC_{50} of calcium mobilization for the same drugs tested in h5-HT_{2A} receptor-expressing HEK cells (see Table 1 for values). There is no significant correlation between efficacy of drugs to decrease PenH in response to methacholine and canonical signaling through calcium elicited by 5-HT_{2A} receptor activation (PenH AUC vs E_{MAX} calcium, Spearman $r = -0.387$, $p = 0.0754$; PenH AUC vs EC_{50} calcium, Spearman $r = 0.2422$, $p = 0.277$).

related drug *N*,*N*-dimethyl tryptamine (*N*,*N*-DMT) had no measurable effect (Figure 7T), nor did 5-MeO−DMT (Figure 7U). However, 4-OH-DiPT (Figure 7V) does possess full efficacy to normalize PenH at 0.5 mg/kg. These results indicate that perhaps for tryptamines a 4-OH group is necessary for therapeutic efficacy and also that the requirements for the identity of *N*′-R groups are less stringent. Further testing of additional compounds will be necessary, however, to test this hypothesis fully. Our results with *N*,*N*-DMT having no efficacy in our asthma model, and by extrapolation no anti-inflammatory activity, are at odds with the work of others who have proposed it does have anti-inflammatory effects. Specifically, *N*,*N*-DMT has

https://dx.doi.org/10.1021/acsptsci.0c00063
ACS Pharmacol. Transl. Sci. 2021, 4, 488−502

been proposed to act on the sigma-1 receptor on cells of the immune system to prevent inflammation.[42] That we did not observe any measurable effects in our model system suggests sigma-1-receptor-mediated anti-inflammatory mechanisms may not be relevant for therapeutic efficacy in pulmonary inflammation, and/or that the anti-inflammatory activity of *N*,*N*-DMT is dependent on specific tissues that may or may not express sufficient sigma-1 receptor protein.[43]

Activation of Canonical Signaling Is Not Correlated with Efficacy to Prevent OVA-Induced AHR. Several, but not all, drugs we examined have known affinities for the 5-HT_{2A} receptor and known behavioral potencies.[8,41,44] In order to attempt to correlate canonical signaling downstream of the 5-HT_{2A} receptor to anti-asthma efficacy, we measured efficacy and potencies for each of the drugs used for calcium flux, which is induced by activation of the $G\alpha_q$ canonical signaling pathway, in heterologously expressed human 5-HT_{2A} receptors in HEK cells. In a comparison of PenH-AUC values determined for each drug as a proxy measure of anti-inflammatory efficacy (Figure 8A) to either EC_{50} or E_{Max} for calcium mobilization downstream of 5-HT_{2A} receptor activation (Table 1), we found no correlation between canonical signaling and anti-asthma efficacy (Figure 8B,C). As expected, our prototypical drug (*R*)-DOI, which is fully anti-inflammatory, was a potent and full activator of calcium mobilization. Drugs with similar receptor potencies such as 2C-B-Fly, however, were only partially efficacious as anti-inflammatories (Table 1; Figure 8A). These results are consistent with noncanonical signaling pathways linking anti-inflammatory activity to 5-HT_{2A} receptor activation that remain to be elucidated in future experiments. This uncoupling of traditional pharmacological properties of drug−receptor interaction and therapeutic effect is not unprecedented.[45] Therefore, it is likely that noncanonical signaling pathways yet to be identified underlie the anti-inflammatory/asthma effects of psychedelics.

Table 1

drug	calcium E_{MAX}	calcium pEC_{50}	PenH (AUC) relative to OVA alone	SEM
2,4-DMA	116.55	−5.53	8.53	20.97
2,5-DMA	108.96	−5.45	5.79	43.65
2C-B	97.89	−7.23	2.71	11.80
2C-B-Fly	99.05	−7.27	34.56	9.87
2C-H	77.76	−5.38	22.35	22.41
2C-I	86.80	−7.33	−18.81	15.21
2C-T-33	75.62	−6.31	62.93	24.89
4-OH-DIPT	105.72	−6.40	5.32	14.85
5-iPO-2CE	93.47	−6.72	35.12	16.80
5-MeO-DMT	105.17	−6.98	82.49	33.63
DMT	88.82	−6.06	103.85	19.38
DOiBu	98.82	−7.90	−0.66	16.27
ETH-LAD	102.71	−7.48	39.43	16.02
lisuride	42.61	−5.34	78.23	28.18
LSD	84.53	−6.66	55.06	14.63
psilocin	80.02	−6.47	15.80	29.0
R-DOET	97.37	−7.80	8.21	18.63
R-DOI	112.28	−7.88	5.43	28.05
R-DOM	110.26	−7.28	15.46	7.05
TCB-2	100.40	−8.43	36.46	11.56
TMA2	101.87	−5.73	32.22	9.14
BW723C86			139.10	32.21
1-methyl psilocin			79.73	29.16
Ro 60-0175			72.30	19.06

5-HT_{2B} and 5-HT_{2C} Receptors Are Not Involved in the Effects of (*R*)-DOI. Because (*R*)-DOI, as well as all other psychedelic drugs tested, also has significant affinity for and are agonists at 5-HT_{2B} and 5-HT_{2C} receptors, we sought to determine any potential involvement in the therapeutic effects to normalize PenH. We tested 0.5 mg/kg of a selective 5-HT_{2C} agonist and 5-HT_{2B} inverse agonist (1-methyl psilocin) (Figure 9A), and two mixed 5-$HT_{2B/2C}$ agonists (Ro 60−0175 fumarate and BW 723C86) (Figure 9B,C). There were no significant effects on PenH hyperresponsiveness to MeCh in the OVA treated animals for any of these three drugs. These results indicate that activity at 5-$HT_{2B/2C}$ receptors is not involved in the therapeutic effects of (*R*)-DOI to prevent allergic asthma in our model. Although these results are highly indicative of activity at the 5-HT_{2A} receptor being necessary and sufficient for anti-inflammatory/asthma activity, we performed a further experiment examining the ability of (*R*)-DOI to prevent allergic asthma in *HTR2A*$^{-/-}$ knockout mice lacking expression of the 5-HT_{2A} receptor (Figure S1). We followed protocols as previously described for our mouse model of acute OVA allergic asthma[11] and administered 0.5 mg/kg (*R*)-DOI nose only prior to each OVA exposure. We found that there was no effect of (*R*)-DOI to prevent the development of AHR. Utilizing our models, we have demonstrated that agonism at serotonin 5-HT_{2B} and 5-HT_{2C} receptors does not have therapeutic efficacy. Combined with our data demonstrating no anti-asthma effects in the *HTR2A*$^{-/-}$ mouse, we conclude that the activation of 5-HT_{2A} receptors by select psychedelics is necessary and sufficient to confer anti-inflammatory activity and to prevent the symptoms of asthma in our model. This finding is in agreement with our previous *in vitro*[9] and *in vivo* studies.[10−12] Regardless, a possibility we cannot rule out without further testing is that some of these drugs may be interacting with off-target receptors to produce their effects. The most common off-target receptors for psychedelics include 5-HT_{1A} receptors (tryptamines and ergolines) and α2 adrenergic receptors (phenakylamines).[8] We do not, however, believe that this is the case. There is little to no evidence in the literature of 5-HT_{1A} receptors having any anti-inflammatory or therapeutic effects in the lung relevant to asthma, and in human clinical studies testing the effects of the α2 agonist clonidine on methacholine-induced airway reactivity, no significant differences between placebo and clonidine groups were measured.[46]

Summary. Our previous *in vitro* work demonstrated that different 5-HT_{2A} receptor agonists have disparate anti-inflammatory properties.[9] We hypothesized that structural differences between ligands gave rise to differential anti-inflammatory potencies and efficacy through functionally selective (ligand biased) mechanisms. Here, we examined the structure−activity relationships between agonist and anti-inflammatory/asthma efficacy in a new rat model of allergic asthma. We first validated our asthma model, and then used it to examine structure−activity relationships. During this process, we demonstrated the utility of our rat model as an *in vivo* platform that is robust and long-lasting for the analysis and/or screening of compounds for therapeutic efficacy to treat symptoms associated with pulmonary inflammation and asthma. Parameters measured were more consistent between individual rats than we have found for mice, and once sensitized to OVA, the rats maintain a stable hyper-allergic state and have a robust testing period of ∼1 year, compared to only ∼6 months in

https://dx.doi.org/10.1021/acsptsci.0c00063
ACS Pharmacol. Transl. Sci. 2021, 4, 488−502

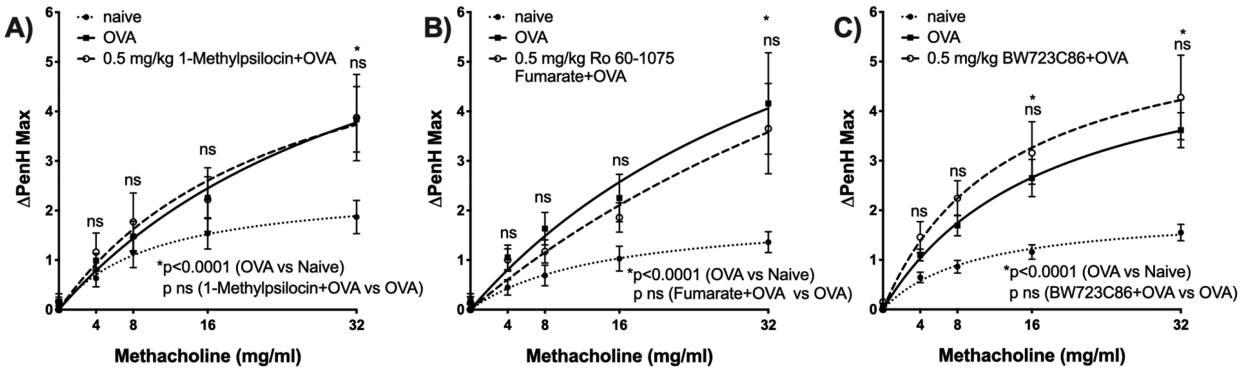


Figure 9. 5-$HT_{2B/C}$ receptors are not implicated. Animals were exposed to 5-HT_{2C} and 5-HT_{2B} selective drugs prior to OVA exposure. (A) 5-HT_{2C} receptor selective agonist 1-methylpsilocin (0.5 mg/kg; inhaled) did not prevent AHR. (B) 5-HT_{2C} receptor selective agonist 60−1075 Fumarate (0.5 mg/kg; inhaled) did not prevent AHR. (C) 5-$HT_{2B/C}$ receptor agonist BW723C86 (0.5 mg/kg; inhaled) did not prevent AHR. n = 5−6 animals/treatment group. *, $p < 0.0001$ OVA vs Naïve; ns = no significance drug vs OVA. Error bars represent ± SE; 2-way ANOVA with Bonferonni post hoc test.

BALB/c mice. Results showing equipotency for nebulized compared to intraperitoneal injection for (*R*)-DOI indicate that potential therapies in the clinic need not be limited to inhaled formulations. SAR analysis indicates that 2C-H represents the pharmacophore for phenalkylamine anti-inflammatory efficacy, and it also highlights certain modifications that are permissive or detrimental to efficacy for phenalkylamines and tryptamines and that ergolines as a class may not be fully efficacious. *In vitro* data with regard to potency and efficacy at canonical signaling pathways indicate that relevant anti-inflammatory pathways do not correlate with canonical signaling pathways. These results, together with no apparent correlation between behavioral effects, support the notion that anti-inflammatory and behavioral effects may be separable through functionally selective mechanisms in drug discovery efforts to identify nonbehavioral 5-HT_{2A} receptor agonists with anti-inflammatory activity for the treatment of inflammation-related diseases like asthma in the clinic.

The data in the Supporting Information further support our findings in rats that activation of 5-HT_{2A} receptors is necessary and sufficient for the anti-asthma effects of psychedelics. In summary, treatment with a high dose of the 5-HT_2 receptor agonist (*R*)-DOI does not reduce or affect OVA-induced AHR in *HTR2A*$^{-/-}$ knockout mice.

■ METHODS

Drugs and Reagents. (*R*)-DOI was synthesized and provided by Dr. Bruce Blough at Research Triangle Institute (Research Triangle Park, NC) and by Eleusis Therapeutics (New York, NY). LSD; ETH-LAD, 2,4-DMA, 5-iPrO-2CE, 2C-B, TCB-2, (*R*)-DOB, (*R*)-DOM, (*R*)-DOiBu, (*S*)-DOI, (*R*)-DOET, 5-MeO-DMT, psilocin, and mescaline were synthesized and provided by Dr. David Nichols (Purdue University). 2C-T-33 was synthesized and provided by Dr. Daniel Traschel (ReseaChem Gmbh, Burgdorf, CH). 2C-H, 2C-I, 4-OH-DIPT, 2,5-DMA, and TMA-2 were purchased from Cayman Chemical (MI). Lisuride; 1-methylpsilocin; Ro-0175 fumarate; and BW723C86 were purchased from Tocris Bioscience (Bristol, UK). *N*,*N*-DMT, 5-HT, ovalbumin (OVA), methacholine (MeCh), and DL-isoproterenol hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO). See Figure 1 for the structures of each compound.

Animals. For all experiments described, respiratory-pathogen-free Brown Norway (RijHsd-BN) rats were obtained from Envigo (Somerset, NJ) and Charles River (Raleigh, NC). All animals used in these studies were male and 6−8 weeks of age on arrival with a body weight of 170−220 g. Animals were allowed to acclimate at least 1 week prior to initiation of OVA sensitization. For the duration of the study, rats were housed singly in the animal care facility at the Louisiana State University Health Sciences Center (New Orleans, LA) in ventilated cages housed in a pathogen-free animal facility with *ad libitum* access to food and water on a 12 h/12 h light/dark cycle. All protocols were prepared in accordance with the *Guide for the Care and Use of Laboratory Animals*[47] and approved by the Institutional Animal Care and Use Committee at Louisiana State University Health Sciences Center.

OVA-Induced Acute Allergic Airway Inflammation. Sensitization-challenge protocols used in this study are summarized in Figure 2. For sensitization, Brown Norway rats (7−9 weeks old) were i.p. injected with (500 μL) of 2.0 mg of chicken OVA (Sigma-Aldrich) emulsified in 2.0 mL of Imject Alum [$Al(OH)_3/Mg(OH)_2$; Pierce, Rockford, IL] on days 0 and 7, as described by Elwood et al.[25] OVA exposure methods were based on our previously described mouse model of acute asthma.[11] OVA-alone treated rats were exposed to 3 times weekly exposure of 10.0 mg of OVA slowly dissolved in 10.0 mL of 0.9% sterile saline solution (Baxter Healthcare Corp., Deerfield, IL) in a 15 L (38.00 × 19.05 × 19.7 cm) acrylic induction chamber. No more than 6 animals were exposed in the chamber per challenge. OVA aerosol was generated using an ultrasonic nebulizer (Pari Proneb nebulizer, Midlothian, WA) in conjunction with a Pari Proneb pump at a 1.0% OVA concentration for a total duration of 30 min, as described in Palmans et al.[26]

Drug Exposure. For nose-only exposures, rats were exposed in groups of 3−4 rats/group to the appropriate concentration of drug dissolved in a total volume of 4.5 mL of sterile saline using an inExpose nose-only inhalation system (SCIREQ, Montreal, QC, Canada) 30 min prior to each OVA challenge. Each 4.5 mL of sample was aerosolized using a VixOne nebulizer (Westmed, Inc., Tucson, AZ) in conjunction with a Pari Proneb pump. Exposures lasted 15 min. All respiratory parameters were measured 48 h after the final OVA exposure (Figure 2). For i.p. administration, the appropriate drug was dissolved in sterile saline at a concentration of 1.0 mg/kg and injected at the appropriate weight/volume ratio.

https://dx.doi.org/10.1021/acsptsci.0c00063
ACS Pharmacol. Transl. Sci. 2021, 4, 488−502

Noninvasive Whole-Body Plethysmography (WBP) and Methacholine Challenge. To minimize the impact of circadian influences, all respiratory recordings were performed between 10 am and 3 pm.[48−50] For measurement of airway responsiveness to MeCh, a noninvasive bias flow ventilated whole body plethysmography system (EMKA Technologies, Falls Church, VA) was used in spontaneously breathing, unrestrained rodents. The plethysmograph (PLY3215; diameter 10 in Buxco Electronics, Troy, NY) was ventilated by a continuous flow of 2.5 L/min (Bias Flow Regulator, Vent2, EMKA Technologies, Paris, FR).[51] A differential pressure transducer (EMKA Technologies, FR) was connected on one pole to the main chamber and on the second pole to a reference chamber. The transducer measures pressure differences between both chambers as caused by the respiratory cycle, mainly inhalation and exhalation.[52] The IOX2 Software System (IOX 2.8.2.13; EMKA Technologies, FR) provides a breath-by-breath analysis of pressure signals and transforms these pressure differences via computerized calculations to a dimensionless empirically established value, enhanced pause or PenH. Numerous experiments from our lab[11,12] and others[53,54] have shown PenH to be a reliable and sensitive measure of bronchoconstriction and a superior measure in assessing the degree of bronchoconstriction compared to other derived parameters such as box pressure or box flow,[54] and it faithfully reproduces the results of forced respiratory techniques such as flexiVent (flexiVent, SCIREQ, Montreal, CA).[11] Despite these findings, it must be acknowledged that the use of PenH as a measure of airway mechanics is a matter of debate.[55] A large portion of this controversy stems from interpretation of pulmonary responsiveness using older plethysmography equipment, in which fluctuations to any ambient parameter (i.e., temperature and humidity) impact airways resistance calculations.[56] As such, the type of WBP is an important factor to consider. Although any data collected from a sealed chamber plethysmograph (PressureWBP) does not represent pulmonary resistance, data gathered from a WBP chamber with a pneumotachograph in its wall (FlowWBP) is correlative with pulmonary resistance,[57] which is the type of WBP performed in our studies. Accordingly, with appropriate mathematical analysis and a properly calibrated FlowWBP system, PenH does quantitatively measure fluctuations in airway resistance regardless of variations in ambient parameters. Although a degree of caution should be exercised using PenH data from a properly calibrated FlowWBP system as a proxy for direct measurement using forced ventilation to measure pulmonary airways resistance (FlexiVent), FlexiVent is a terminal assay, whereas FlowWBP can be reliably repeated in the same cohort of animals, necessitating its use for evaluating large numbers of drugs and doses such as we have performed.

For the assay, the chamber pressure signal is calibrated by dynamic injection of 5 mL of room air via syringe (BD; 10 mL syringe with luer-lok tip; Franklin Lakes, NJ).[51] Animals are then placed in the chamber, where baseline data are recorded for 5 min following a 10 min habituation period in the plethysmograph.[54] After measurement of baseline PenH, either aerosolized saline (0.9% NaCl Solution) or an aqueous solution of MeCh in increasing concentrations (4, 8, 16, 32 mg/mL) were nebulized through an inlet of the plethysmography chamber for 3 min, followed by measurements of PenH values for 3 min.[52,58] An Aeroneb Pro vibrating-mesh nebulizer (Aerogen Ltd., Galway, Ireland) was used to generate aerosol. Following recordings, to prevent a MeCh gradient there was a wash-out period of 7 min in which the animal was provided with fresh air.[59−61] For studies involving isoproterenol, the above protocol was utilized; however, following the 32 mg/mL MeCh challenge animals were exposed to 30 mg/mL isoproterenol for 3 min, and PenH values were measured for 3 min. The dosage of isoproterenol was extrapolated from Vogin et al.[62] and deemed by Kondo et al.[63] not to have lasting myocardial effects. Data from the IOX2 software were analyzed using Datanalyst software (Datanalyst v2.6.1.14; EMKA Technologies, France) and expressed as the mean SEM of maximal PenH values per group.

For testing, we maintained six cohorts of rats: two saline-only control groups (n = 6/group), and four OVA-sensitized and exposed groups (n = 6/group). These cohorts were further divided into two sets, each set with one saline-only group, and two OVA-exposed and -sensitized groups. In any given testing round, we used one set of treatment groups with one OVA-treated group used as an OVA-only control that was exposed only to OVA, another OVA-treated group was used as our experimental group for drug pretreatment prior to OVA exposure, and one saline-only control group that was exposed only to aerosolized saline. The following round we utilized the other set with the same group designations. On the third round, we returned to the first set; however, we utilized the previously drug-exposed OVA-treated group as the OVA-only group (to ensure resetting of the immune response following drug treatments) and the previously used OVA-only group as the OVA + drug group. This staggering allowed at least 2 weeks between experimental manipulation on any given rat, so we could ensure the previously drug-treated animal's responses returned to normal prior to another drug treatment and validated that there were no cumulative drug effects between trials to confound results on an ongoing basis. At this rate we could test one experimental drug/dose per week, every week. The same cohorts of rats were used for testing drugs over periods of about 3−9 months. During this time, the measured PenH in the control groups, as well as the OVA-only treated group measurements, did not significantly differ between trials, indicating that there were no age-related confounding effects on pulmonary response to OVA over time and that repeated drug exposures did not affect subsequent responses to OVA exposures and/or drug treatments. As a final test, the terminal round of testing for all rats after completion of experimental testing was to reassess the effects of inhaled (R)-DOI (0.1 mg/kg) to ensure animals were still equally responsive at the end of testing as they were at the beginning several months earlier. The first cohort of experimental groups was used to develop and validate the model using (R)-DOI only and to generate lung tissue for gene expression analysis. The second cohort was used to validate further the model with (R)-DOI, to collect BALF, and to generate lung tissues for histopathological analysis. The third cohort was used for compound screening and the route of administration experiments.

Histopathology. Lungs were isolated and prepared 48 h after the final OVA exposure. Animals were humanely sacrificed by exsanguination under anesthesia (ketamine/xylazine mixture; 100 and 10 mg/kg, respectively). A 14-guage angiocatheter (Excel Safelet Catheter, Excel International, Los Angeles, CA) was inserted into the trachea, and a 4−0 silk suture (Oasis, Mettawa, IL) was tied around the trachea. The catheter was withdrawn, and the lungs were removed en bloc. A gentle infusion of 10 mL of Zinc Formal-Fixx Concentrate (Thermo Scientific, Shandon, Inc., Pittsburgh, PA) was used to inflation-fix the lungs, which were immersed in Zinc Formal-Fixx at room

https://dx.doi.org/10.1021/acsptsci.0c00063
ACS Pharmacol. Transl. Sci. 2021, 4, 488−502

Table 2. qRT-PCR Primer Sequences

gene	sequence		amplicon length
IL-1β	sense	5′ TGT-GAT-GAA-AGA-CGG-CAC-AC 3′	70 nt
	antisense	5′ CTT-CTT-TGG-GTA-TTG-TTT-GG 3′	
IL-4	sense	5′ CCT-CCG-TGA-GCT-GTC-TGA-TT 3′	63 nt
	antisense	5′ CAC−CCA-GGA-CCT-TGA-TGC 3′	
IL-5	sense	5′ TAT-GGG-AGC-TTC-GGC-AAC 3′	72 nt
	antisense	5′ TTG-ACA-GGT-GGC-AGA-AGT-AAA-A 3′	
IL-6	sense	5′ CCT-GGA-GTT-TGT-GAA-GAA-CAA-CT 3′	142 nt
	antisense	5′ GGA-AGT-TGG-GGT-AGG-AAG-GA 3′	
IL-13	sense	5′ GGC−CCT-CAG-GGA-GCT-TAT 3′	70 nt
	antisense	5′ GCT-GTT-GCA-CAG-GGA-AGT-CT 3′	
Gm-csf	sense	5′ CAT-CTC-TAA-TGA-GTT-CTC-CAT-CCA-G 3′	77 nt
	antisense	5′ CCC-GTA-GAC−CCT-GCT-TGT-AT 3′	
MUC5AC	sense	5′ GGT-GAC-TGC-GAG-TGC-TTC-T 3′	127 nt
	antisense	5′ CCT-CTG-GGT-TGT-AGT-AGT-CAC-AGA	
TNFα	sense	5′ CAG-AGA-AGA-AGC-AGA-CCA-AGG 3′	92 nt
	antisense	5′ AGG-GAC-TCG-AGG-GGT-CTC 3′	
GusB	sense	5′ CTC-TGG-TGG-CCT-TAC-CTG-AT 3′	79 nt
	antisense	5′ CAG-ACT-CAG-GTG-TTG-TCA-TCG 3′	

temperature overnight and dehydrated in a graded series of ethanol solutions. Fixed lungs were embedded in paraffin and sectioned at 4 μm thickness. Each lung section was stained with periodic acid−Schiff (PAS) for examination of mucus cell metaplasia and evaluated as previously described.[64] Adjacent sections were stained with hematoxylin and eosin (H&E) to assess severity of interstitial and peribronchial inflammation. Five trained individuals blinded to the background of the samples assigned a total inflammation score for each lung section as follows: 0, normal; 1, inflammatory infiltration comprises less than 25% of entire section; 2, inflammatory infiltrate 25−50% entire section; and 3, more than 50% of entire section exhibits inflammatory infiltration.

Cytokine and Chemokine Analysis by qRT-PCR. Lungs were harvested 48 h after the final OVA exposure and frozen until processing at −80 °C. Total RNA was extracted from the left lobe of each rat with TRIzol reagent, purchased from Life Technologies (Carlsbad, CA), following the manufacturer's instruction. The RNA pellet was resuspended in nuclease-free H_2O, and RNA was quantified by spectrophotometer (NanoDrop ND-1000; NanoDrop Technologies, Inc., Wilmington, DE). RNA was quantified at A260 and A280, with no RNA utilized that was below a 1.6 260/280 nm ratio. Suitable RNA was processed into first-strand cDNA using the ImProm-II cDNA synthesis kit (Promega, Madison, WI) following the manufacturer's instructions. Gene expression levels of cytokines were determined using reverse transcription and quantitative real-time PCR (qRT-PCR). The input cDNA for each reaction was 1000 ng of total RNA. Primers were designed to be compatible with the Universal ProbeLibrary system using the Universal ProbeLibrary Assay Design Center (Roche Diagnostics, Indianapolis, IN) and synthesized by Integrated DNA Technologies (Coralville, IA). Primer sequences used in this study are listed in Table 2. Probes utilized in this report were from the Universal ProbeLibrary (Roche Diagnostics, Indianapolis, IN) and are listed with the following universal probe numbers: U78, U21, U2, U106, U17, U124, U5, U68, U89, U15, and U42 for *IL-1β*, *IL-4*, *IL-5*, *IL-6*, *IL-13*, *Gm-csf*, *MUC5AC*, *TNFα*, and *GusB*. Triplicate amplification reactions using the first-strand cDNA sample from each rat were performed on a Roche 480 LightCycler II LC (Roche Diagnostics) using the qPCR PerfeCTa FastMix II (Quanta Biosciences, Inc., Gaithersburg, MD) following the manufacturer's directions. Amplification of *β-glucuronidase* (*GUSB*), a housekeeping gene consistently expressed among all samples, was used as the reference standard. Relative expression was determined using the ΔΔ-Ct method. Significance of expression was measured using a one-way analysis of variance (ANOVA), with all genes marked as significant exhibiting at least a $p < 0.05$.

Calcium Flux Assay. $G\alpha_q$-mediated calcium flux downstream of 5-HT_{2A} receptor activation was determined using HEK293 cells stably expressing the human 5-HT_{2A} receptor.[65] Cells were seeded in DMEM supplemented with 10% fetal bovine serum, 100 units/mL penicillin, 100 mg/mL streptomycin, and 100 mg/mL Zeocin onto 96-well poly-D-lysine plates with clear bottoms (12 000 cells/well) and cultured at 37 °C. The following day, media was aspirated and replaced with serum-free DMEM for 12 h. On the day of the experiment, the cells were washed once with HBSS supplemented with 20 mM HEPES, loaded with 75 μL of 3 μM Fluo-2 AM HA (Ion Indicators, LLC) diluted in HBSS−HEPES buffer, incubated for 1 h at 37 °C, washed again with HBSS−HEPES, and maintained in 50 μL HBSS−HEPES at 25 °C. The plates of dye-loaded cells were placed into a FlexStation 3 microplate reader (Molecular Devices, LLC) to monitor fluorescence (excitation, 485 nm; emission, 525 nm; cutoff, 515 nm). Plates were read for 20 s (2 s interval) to establish baseline fluorescence and then challenged with compounds diluted in a range of 10 pM to 10 μM or buffer and read for an additional 80 s. After obtaining a calcium mobilization trace for each sample, the mean baseline fluorescence was subtracted from peak fluorescence in each well. E_{MAX} values were determined by normalization to the maximum 5-HT response (100%) on the same plate. The data were analyzed using nonlinear regression curve-fitting routines in Graph-Pad Prism 8.0 (GraphPad Software, Inc.) to generate EC_{50} values. These data for test compounds were then normalized to 5-HT by calculating a ratio of mean EC_{50} value across all test plates versus same plate response and multiplying it by individual test compound EC_{50} values.

Statistics. All statistical analysis was performed using GraphPad Prism (GraphPad Software, La Jolla, CA).

https://dx.doi.org/10.1021/acsptsci.0c00063
ACS Pharmacol. Transl. Sci. 2021, 4, 488−502

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsptsci.0c00063.

Data and methods for testing (*R*)-DOI to prevent AHR in *HTR2A*$^{-/-}$ knockout mice (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Charles D. Nichols − *Department of Pharmacology and Experimental Therapeutics, Louisiana Stat University Health Sciences Center, New Orleans, Louisiana 70112, United States;* orcid.org/0000-0002-0615-0646; Email: cnich1@lsuhsc.edu

Authors

Thomas W. Flanagan − *Department of Pharmacology and Experimental Therapeutics, Louisiana Stat University Health Sciences Center, New Orleans, Louisiana 70112, United States*

Gerald B. Billac − *Department of Pharmacology and Experimental Therapeutics, Louisiana Stat University Health Sciences Center, New Orleans, Louisiana 70112, United States*

Alexus N. Landry − *Department of Pharmacology and Experimental Therapeutics, Louisiana Stat University Health Sciences Center, New Orleans, Louisiana 70112, United States*

Melaine N. Sebastian − *Department of Pharmacology and Experimental Therapeutics, Louisiana Stat University Health Sciences Center, New Orleans, Louisiana 70112, United States*

Stephania A. Cormier − *Department of Biological Sciences, Louisiana State University, Baton Rouge, Louisiana 70803, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsptsci.0c00063

Notes

The authors declare the following competing financial interest(s): C.D.N. is supported by a sponsored research contract from Eleusis Therapeutics and is a member of its Scientific Advisory Board.

■ ACKNOWLEDGMENTS

Funding was provided by the American Asthma Foundation and Eleusis Therapeutics. The authors thank Dr. David T. Siefker for histology staining and scoring; Dr. Javier Gonzalez-Maeso for the *HTR2A*$^{-/-}$ mice; Dr. Meghan Hibicke, B. DeVellis, and Dr. Sahika Guner for histology analysis and scoring; and F. Lorio for construction of exposure chambers. This paper is dedicated to the memory of Melaine N. Sebastian.

■ REFERENCES

(1) Nichols, D. E., and Nichols, C. D. (2008) Serotonin receptors. *Chem. Rev. 108* (5), 1614−41.

(2) Flanagan, T. W., and Nichols, C. D. (2018) Psychedelics as anti-inflammatory agents. *Int. Rev. Psychiatry 30*, 363−375.

(3) Duerschmied, D., Suidan, G. L., Demers, M., Herr, N., Carbo, C., Brill, A., Cifuni, S. M., Mauler, M., Cicko, S., Bader, M., Idzko, M., Bode, C., and Wagner, D. D. (2013) Platelet serotonin promotes the recruitment of neutrophils to sites of acute inflammation in mice. *Blood 121* (6), 1008−15.

(4) Shajib, M. S., and Khan, W. I. (2015) The role of serotonin and its receptors in activation of immune responses and inflammation. *Acta Physiol. 213* (3), 561−74.

(5) Nichols, C. D. (2009) Serotonin 5-HT(2A) Receptor Function as a Contributing Factor to Both Neuropsychiatric and Cardiovascular Diseases. *Cardiovasc. Psychiatry Neurol. 2009*, 475108.

(6) Kyzar, E. J., Nichols, C. D., Gainetdinov, R. R., Nichols, D. E., and Kalueff, A. V. (2017) Psychedelic Drugs in Biomedicine. *Trends Pharmacol. Sci. 38* (11), 992−1005.

(7) Stefulj, J., Jernej, B., Cicin-Sain, L., Rinner, I., and Schauenstein, K. (2000) mRNA expression of serotonin receptors in cells of the immune tissues of the rat. *Brain, Behav., Immun. 14* (3), 219−24.

(8) Nichols, D. E. (2016) Psychedelics. *Pharmacol. Rev. 68* (2), 264−355.

(9) Yu, B., Becnel, J., Zerfaoui, M., Rohatgi, R., Boulares, A. H., and Nichols, C. D. (2008) Serotonin 5-hydroxytryptamine(2A) receptor activation suppresses tumor necrosis factor-alpha-induced inflammation with extraordinary potency. *J. Pharmacol. Exp. Ther. 327* (2), 316−23.

(10) Nau, F., Jr., Yu, B., Martin, D., and Nichols, C. D. (2013) Serotonin 5-HT2A receptor activation blocks TNF-alpha mediated inflammation in vivo. *PLoS One 8* (10), No. e75426.

(11) Nau, F., Jr., Miller, J., Saravia, J., Ahlert, T., Yu, B., Happel, K. I., Cormier, S. A., and Nichols, C. D. (2015) Serotonin 5-HT(2) receptor activation prevents allergic asthma in a mouse model. *American journal of physiology. Lung cellular and molecular physiology 308* (2), L191−8.

(12) Flanagan, T. W., Sebastian, M. N., Battaglia, D. M., Foster, T. P., Cormier, S. A., and Nichols, C. D. (2019) 5-HT2 receptor activation alleviates airway inflammation and structural remodeling in a chronic mouse asthma model. *Life Sci. 236*, 116790.

(13) Flanagan, T. W., Sebastian, M. N., Battaglia, D. M., Foster, T. P., Maillet, E. L., and Nichols, C. D. (2019) Activation of 5-HT2 Receptors Reduces Inflammation in Vascular Tissue and Cholesterol Levels in High-Fat Diet-Fed Apolipoprotein E Knockout Mice. *Sci. Rep. 9* (1), 13444.

(14) Hope, D. B., and Smith, A. D. (1960) Distribution and activity of monoamine oxidase in mouse tissues. *Biochemical journal 74*, 101−7.

(15) Shen, H. W., Jiang, X. L., Winter, J. C., and Yu, A. M. (2010) Psychedelic 5-methoxy-N,N-dimethyltryptamine: metabolism, pharmacokinetics, drug interactions, and pharmacological actions. *Curr. Drug Metab. 11* (8), 659−66.

(16) Koerner-Rettberg, C., Doths, S., Stroet, A., and Schwarze, J. (2008) Reduced lung function in a chronic asthma model is associated with prolonged inflammation, but independent of peribronchial fibrosis. *PLoS One 3* (2), No. e1575.

(17) Locke, N. R., Royce, S. G., Wainewright, J. S., Samuel, C. S., and Tang, M. L. (2007) Comparison of airway remodeling in acute, subacute, and chronic models of allergic airways disease. *Am. J. Respir. Cell Mol. Biol. 36* (5), 625−32.

(18) Haczku, A., Moqbel, R., Elwood, W., Sun, J., Kay, A. B., Barnes, P. J., and Chung, K. F. (1994) Effects of prolonged repeated exposure to ovalbumin in sensitized brown Norway rats. *Am. J. Respir. Crit. Care Med. 150* (1), 23−7.

(19) Elwood, W., Lötvall, J. O., Barnes, P. J., and Chung, K. F. (1992) Effect of dexamethasone and cyclosporin A on allergen-induced airway hyperresponsiveness and inflammatory cell responses in sensitized Brown-Norway rats. *Am. Rev. Respir. Dis. 145* (6), 1289−94.

(20) Tarayre, J. P., Aliaga, M., Barbara, M., Tisseyre, N., Vieu, S., and Tisne-Versailles, J. (1992) Model of bronchial allergic inflammation in the brown Norway rat. Pharmacological modulation. *Int. J. Immunopharmacol. 14* (5), 847−55.

(21) Wagner, E. M., Jenkins, J., Schmieder, A., Eldridge, L., Zhang, Q., Moldobaeva, A., Zhang, H., Allen, J. S., Yang, X., Mitzner, W., Keupp, J., Caruthers, S. D., Wickline, S. A., and Lanza, G. M. (2015) Angiogenesis and airway reactivity in asthmatic Brown Norway rats. *Angiogenesis 18* (1), 1−11.

(22) Namovic, M. T., Walsh, R. E., Goodfellow, C., Harris, R. R., Carter, G. W., and Bell, R. L. (1996) Pharmacological modulation of eosinophil influx into the lungs of Brown Norway rats. *Eur. J. Pharmacol. 315* (1), 81−8.

(23) Zosky, G. R., and Sly, P. D. (2007) Animal models of asthma. *Clin. Exp. Allergy 37* (7), 973−88.

(24) Kucharewicz, I., Bodzenta-Lukaszyk, A., and Buczko, W. (2008) Experimental asthma in rats. *Pharmacol. Rep. 60* (6), 783−788.
(25) Elwood, W., Lotvall, J. O., Barnes, P. J., and Chung, K. F. (1991) Characterization of allergen-induced bronchial hyperresponsiveness and airway inflammation in actively sensitized brown-Norway rats. *J. Allergy Clin. Immunol. 88* (6), 951−60.
(26) Palmans, E., Kips, J. C., and Pauwels, R. A. (2000) Prolonged allergen exposure induces structural airway changes in sensitized rats. *Am. J. Respir. Crit. Care Med. 161*, 627−635.
(27) Nagata, A., Yamada, Y., Nakamura, A., Asano, T., Yamada, T., Isaka, M., and Itoh, M. (1999) Alteration of endogenous corticosteroids and catecholamines in allergen- induced eosinophilic inflammation in Brown Norway rats. *Allergol. Int. 48* (3), 209−215.
(28) Du, T., Sapienza, S., Wang, C. G., Renzi, P. M., Pantano, R., Rossi, P., and Martin, J. G. (1996) Effect of nedocromil sodium on allergen-induced airway responses and changes in the quantity of airway smooth muscle in rats. *J. Allergy Clin. Immunol. 98* (2), 400−7.
(29) Pini, L., Torregiani, C., Martin, J. G., Hamid, Q., and Ludwig, M. S. (2006) Airway remodeling in allergen-challenged Brown Norway rats: distribution of proteoglycans. *American journal of physiology. Lung cellular and molecular physiology 290* (6), L1052−8.
(30) Curths, C., Wichmann, J., Dunker, S., Windt, H., Hoymann, H. G., Lauenstein, H. D., Hohlfeld, J., Becker, T., Kaup, F. J., Braun, A., and Knauf, S. (2014) Airway hyper-responsiveness in lipopolysaccharide-challenged common marmosets (Callithrix jacchus). *Clin. Sci. 126* (2), 155−62.
(31) Krane, C. M., Fortner, C. N., Hand, A. R., McGraw, D. W., Lorenz, J. N., Wert, S. E., Towne, J. E., Paul, R. J., Whitsett, J. A., and Menon, A. G. (2001) Aquaporin 5-deficient mouse lungs are hyperresponsive to cholinergic stimulation. *Proc. Natl. Acad. Sci. U. S. A. 98* (24), 14114−9.
(32) Pranzatelli, M. R. (1990) Evidence for involvement of 5-HT2 and 5-HT1C receptors in the behavioral effects of the 5-HT agonist 1-(2,5-dimethoxy-4-iodophenyl aminopropane)-2 (DOI). *Neurosci. Lett. 115* (1), 74−80.
(33) Schreiber, R., Brocco, M., Audinot, V., Gobert, A., Veiga, S., and Millan, M. J. (1995) (1-(2,5-dimethoxy-4 iodophenyl)-2-amino-propane)-induced head-twitches in the rat are mediated by 5-hydroxytryptamine (5-HT) 2A receptors: modulation by novel 5-HT2A/2C antagonists, D1 antagonists and 5-HT1A agonists. *J. Pharmacol. Exp. Ther. 273* (1), 101−112.
(34) Hawkins, M. F., Uzelac, S. M., Baumeister, A. A., Hearn, J. K., Broussard, J. I., and Guillot, T. S. (2002) Behavioral responses to stress following central and peripheral injection of the 5-HT(2) agonist DOI. *Pharmacol., Biochem. Behav. 73* (3), 537−44.
(35) Elmore, J. S., Decker, A. M., Sulima, A., Rice, K. C., Partilla, J. S., Blough, B. E., and Baumann, M. H. (2018) Comparative neuro-pharmacology of N-(2-methoxybenzyl)-2,5-dimethoxyphenethylamine (NBOMe) hallucinogens and their 2C counterparts in male rats. *Neuropharmacology 142*, 240−250.
(36) Braun, U., Shulgin, A. T., Braun, G., and Sargent, T. (1977) 3rd, Synthesis and body distribution of several iodine-131 labeled centrally acting drugs. *J. Med. Chem. 20* (12), 1543−6.
(37) Sargent, T., Kalbhen, D. A., Shulgin, A. T., Braun, G., Stauffer, H., and Kusubov, N. (1975) In vivo human pharmacodynamics of the psychodysleptic 4-BR-2,5-dimethoxyphenylisopropylamine labelled with ʻBRor ʻBR. *Neuropharmacology 14* (3), 165−74.
(38) Beránková, K., Szkutová, M., and Balíková, M. (2007) Distribution profile of 2,5-dimethoxy-4-bromoamphetamine (DOB) in rats after oral and subcutaneous doses. *Forensic Sci. Int. 170* (2−3), 94−9.
(39) Nichols, D. E. (2017) Chemistry and Structure-Activity Relationships of Psychedelics. *Curr. Top. Behav. Neurosci. 36*, 1−43.
(40) Nichols, D. E. (2012) Structure-activity relationships of serotonin 5-HT2A agonists. *WIRES Membrane Transport and Signaling 1*, 559−579.
(41) Shulgin, A., Shulgin, A., and Nichols, D. (1991) *PIHKAL (Phenethylamines I have known and loved): a chemical love story*, Transform Press, Berkley, CA.
(42) Szabo, A., Kovacs, A., Frecska, E., and Rajnavolgyi, E. (2014) Psychedelic N,N-dimethyltryptamine and 5-methoxy-N,N-dimethyl-tryptamine modulate innate and adaptive inflammatory responses through the sigma-1 receptor of human monocyte-derived dendritic cells. *PLoS One 9* (8), No. e106533.
(43) Mei, J., and Pasternak, G. W. (2001) Molecular cloning and pharmacological characterization of the rat sigma1 receptor. *Biochem. Pharmacol. 62* (3), 349−55.
(44) Shulgin, A. T., and Shulgin, A. (1997) *Tihkal: the continuation*, Transform Press, Berkeley, CA.
(45) McCorvy, J. D., and Roth, B. L. (2015) Structure and function of serotonin G protein-coupled receptors. *Pharmacol. Ther. 150*, 129−42.
(46) Foxworth, J. W., Reisz, G. R., Pyszczynski, D. R., and Knudson, S. M. (1995) Oral clonidine in patients with asthma: no significant effect on airway reactivity. *Eur. J. Clin. Pharmacol. 48* (1), 19−22.
(47) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. (2011) *Guide for the Care and Use of Laboratory Animals*, 8th ed., National Academies Press, Washington, DC.
(48) Lai, Y.-T., and Huang, Y.-S. (2017) Modification and Application of a Commercial Whole-body Plethysmograph to Monitor Respiratory Abnormalities in Neonatal Mice. *Bio-protocol 7* (12), No. e2343.
(49) Lai, Y. T., Su, C. K., Jiang, S. T., Chang, Y. J., Lai, A. C., and Huang, Y. S. (2016) Deficiency of CPEB2-Confined Choline Acetyltransferase Expression in the Dorsal Motor Nucleus of Vagus Causes Hyperactivated Parasympathetic Signaling-Associated Bron-choconstriction. *J. Neurosci. 36* (50), 12661−12676.
(50) Pazhoohan, S., Raoufy, M. R., Javan, M., and Hajizadeh, S. (2017) Effect of Rho-kinase inhibition on complexity of breathing pattern in a guinea pig model of asthma. *PLoS One 12* (10), No. e0187249.
(51) Kirschvink, N., Vincke, G., Fievez, L., Onclinx, C., Wirth, D., Belleflamme, M., Louis, R., Cataldo, D., Peck, M. J., and Gustin, P. (2005) Repeated cadmium nebulizations induce pulmonary MMP-2 and MMP-9 production and emphysema in rats. *Toxicology 211* (1−2), 36−48.
(52) Michielsen, C. P., Leusink-Muis, A., Vos, J. G., and Bloksma, N. (2001) Hexachlorobenzene-induced eosinophilic and granulomatous lung inflammation is associated with in vivo airways hyperresponsive-ness in the Brown Norway rat. *Toxicol. Appl. Pharmacol. 172* (1), 11−20.
(53) Hamelmann, E., Schwarze, J., Takeda, K., Oshiba, A., Larsen, G. L., Irvin, C. G., and Gelfand, E. W. (1997) Noninvasive measurement of airway responsiveness in allergic mice using barometric plethysmog-raphy. *Am. J. Respir. Crit. Care Med. 156*, 766−775.
(54) Djuric, V. J., Cox, G., Overstreet, D. H., Smith, L., Dragomir, A., and Steiner, M. (1998) Genetically transmitted cholinergic hyper-responsiveness predisposes to experimental asthma. *Brain, Behav., Immun. 12* (4), 272−84.
(55) Bates, J., Irvin, C., Brusasco, V., Drazen, J., Fredberg, J., Loring, S., Eidelman, D., Ludwig, M., Macklem, P., Martin, J., Milic-Emili, J., Hantos, Z., Hyatt, R., Lai-Fook, S., Leff, A., Solway, J., Lutchen, K., Suki, B., Mitzner, W., Pare, P., Pride, N., and Sly, P. (2004) The use and misuse of Penh in animal models of lung disease. *Am. J. Respir. Cell Mol. Biol. 31* (3), 373−4.
(56) Enhorning, G., van Schaik, S., Lundgren, C., and Vargas, I. (1998) Whole-body plethysmography, does it measure tidal volume of small animals? *Can. J. Physiol. Pharmacol. 76* (10−11), 945−951.
(57) Lomask, M. (2006) Further exploration of the Penh parameter. *Exp. Toxicol. Pathol. 57*, 13−20.
(58) Pauluhn, J. (2003) Respiratory hypersensitivity to trimellitic anhydride in Brown Norway rats: analysis of dose-response following topical induction and time course following repeated inhalation challenge. *Toxicology 194* (1−2), 1−17.
(59) Chong, B. T., Agrawal, D. K., Romero, F. A., and Townley, R. G. (1998) Measurement of bronchoconstriction using whole-body plethysmograph: comparison of freely moving versus restrained guinea pigs. *J. Pharmacol. Toxicol. Methods 39* (3), 163−8.
(60) Hirt, R. A., Vondrakova, K., de Arespacochaga, A. G., Gutl, A., and van den Hoven, R. (2007) Effects of cadmium chloride inhalation

on airflow limitation to histamine, carbachol and adenosine 5′-monophosphate assessed by barometric whole body plethysmography in healthy dogs. *Vet. J. 173* (1), 62−72.

(61) Arts, J. H., Bloksma, N., Leusink-Muis, T., and Kuper, C. F. (2003) Respiratory allergy and pulmonary irritation to trimellitic anhydride in Brown Norway rats. *Toxicol. Appl. Pharmacol. 187* (1), 38−49.

(62) Vogin, E.E., Goldhamer, R.E., Scheimberg, J., Carson, S., and Boxill, G.C. (1970) Teratology studies in rats and rabbits exposed to an isoproterenol aerosol. *Toxicol. Appl. Pharmacol. 16* (2), 374−381.

(63) Kondo, T., Ogawa, Y., Sugiyama, S., Ito, T., Satake, T., and Ozawa, T. (1987) Mechanism of isoproterenol induced myocardial damage. *Cardiovasc. Res. 21* (4), 248−54.

(64) You, D., Ripple, M., Balakrishna, S., Troxclair, D., Sandquist, D., Ding, L., Ahlert, T. A., and Cormier, S. A. (2008) Inchoate CD8+ T cell responses in neonatal mice permit influenza-induced persistent pulmonary dysfunction. *J. Immunol. 181* (5), 3486−94.

(65) Braden, M. R., Parrish, J. C., Naylor, J. C., and Nichols, D. E. (2006) Molecular Interaction of Serotonin 5-HT $_{2A}$ Receptor Residues Phe339 $^{(6.51)}$ and Phe340 $^{(6.52)}$ with Superpotent 6N-Benzyl Phenethylamine Agonists. *Mol. Pharmacol. 70*, 1956−1964.

https://dx.doi.org/10.1021/acsptsci.0c00063
ACS Pharmacol. Transl. Sci. 2021, 4, 488−502

Structure-Activity Relationship Analysis of Psychedelics in a Rat Model of Asthma Reveals the Anti-Inflammatory Pharmacophore

Thomas W. Flanagan[1], Gerald B. Billac[1], Alexus N. Landry[1], Melaine N. Sebastian[1], Stephania A. Cormier[2], and *Charles D. Nichols[1]

1. Department of Pharmacology and Experimental Therapeutics
   Louisiana State University Health Sciences Center
   New Orleans, LA 70112
2. Department of Biological Sciences
   Louisiana State University
   202 Life Sciences Building
   Baton Rouge, LA 70803

S1

Supporting Information

Table of Contents

-Supplementary Figure

Figure S1. (R)-DOI fails to prevent OVA-induced AHR in HTR2A-/- knockout mice.

-Supplementary Materials and Methods

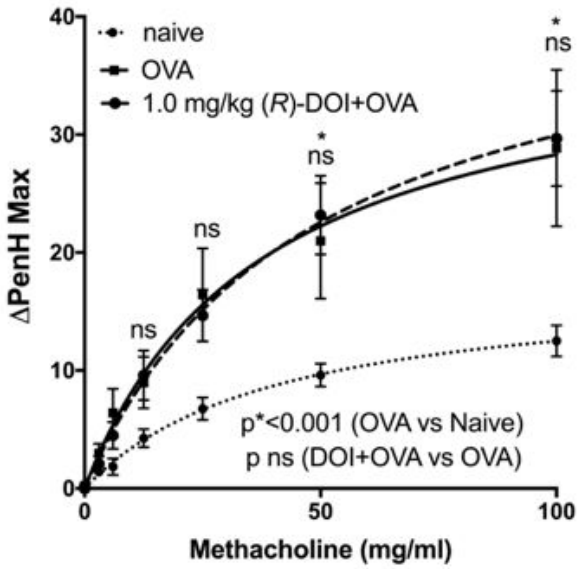


Figure S1. (*R*)-DOI fails to prevent OVA-induced AHR in *HTR2A*$^{-/-}$ knockout mice. Results from whole body plethysmography on awake, freely moving 5-HT_{2A} receptor knockout mice exposed to 0.5 mg/kg (*R*)-DOI (inhaled). n=7-8 mice/treatment group; *p<0.001 OVA vs Naïve; ns = no significance Drug vs OVA. Error bars represent ± SEM; 2-way ANOVA with Bonferonni

S2

Supplemental Materials and Methods

Animals

Generation of null-mutation mice lacking 5-HT_{2A} receptor expression was previously described [54]. Breeder *HTR2A*$^{-/-}$ mice in a 129Sv background (129v5HT2a-/-) [55] were generously provided by Dr. Javier González-Maeso (Virginia Commonwealth University School of Medicine, Richmond, VA). Animal care and experimental procedures were approved by the Institutional Animal Care and Use Committee at Louisiana State University Health Sciences Center. All animals were maintained in the animal care facility at the Louisiana State University Health Sciences Center (New Orleans, LA) in a pathogen-free environment with *ad libitum* access to food and water on a 12 hr light/dark cycle (lights out at 7:00 P.M.).

OVA-induced acute allergic airway inflammation and drug exposure

Twenty-four male 6-8 wk old male mice weighing between 20-25 gm were sensitized and challenged with chicken OVA Grade V (Sigma-Aldrich), as previously described [11]. Sensitization occurred via intraperitoneal injection (100 μl) of 20 μg OVA emulsified in 2 mL Imject Alum [$Al(OH)_3/Mg(OH)_2$; Pierce] on days *0* and *7*. On days *14-16* OVA and DOI+OVA mice were placed in a 7 liter (21 x 22 x 15 cm) clear plexiglass induction chamber (Vet Equip, Inc., Pleasanton, CA) and challenged for 20 min with a 1% (wt/vol) OVA solution in sterile saline (Baxter Healthcare Corp.) generated using a VixOne reusable nebulizer (Westmed) powered by a PARI PRONEB Ultra compressor (Pari Proneb). Naïve animals were challenged as above with sterile saline. Thirty min. prior to saline/OVA challenge, each animal was placed in a nylon-coated stainless-steel wire

restraint (SoftRestraints;SciReq, Montreal, PQ, Canada) and treated with 1.0 mg/kg (*R*)-DOI or saline control (nose-only inhalation) once daily using an ultrasonic nebulizer (Aeroneb Pro).

Non-invasive whole-body plethysmography and methacholine challenge

48 h post final OVA challenge airway function was measured in a single chamber, WBP system (Buxco Electronics and EMKA Technologies) in unrestrained, conscious mice. PenH was used as a proxy for AHR as previously described [42]. For our studies all animals were placed in a single chamber for a 10 min acclimatization period; animals were subsequently measured for baseline PenH 3 min prior to challenge. Mice were exposed for 1 min to the bronchoconstrictor MeCh (Sigma-Aldrich) at increasing concentrations (0, 3.125, 6.25, 12.5, 50, and 100 mg/mL in isotonic saline) and PenH was recorded for 3 min. The maximal PenH was analyzed using the Datanlyst software

(Datanalyst v2.6.1.14), averaged for each dose, and plotted as percent change from vehicle controls, as described [12].

Statistics

All statistical analysis was performed using GraphPad Prism (GraphPad Software, La Jolla, CA).