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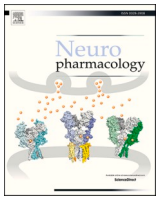

Psychedelics as potent anti-inflammatory therapeutics

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A B S T R A C T

Psychedelics have seen a resurgence of interest from both the scientific and lay community in recent years. Psychedelics are known for their ability to produce profound perceptual alterations, ego dissolution, and separation from reality in humans. Virtually all research into psychedelics and their mechanism of action has focused on examining effects in the brain, and on consciousness. Remarkably, we have discovered that psychedelics are also potent anti-inflammatories and immunomodulators in peripheral tissues. In this review, the discovery of this phenomenon, and the development of psychedelics as potential therapeutics for human inflammatory disease is presented. We believe that certain psychedelics represent a new class of small molecule, highly bioavailable, anti-inflammatory that is steroid sparing and efficacious at sub-behavioral levels that can be used to treat and prevent a variety of inflammatory-related diseases and conditions.

This article is part of the Special Issue on 'Psilocybin Research'.

When one thinks of psychedelics, images of the Grateful Dead and their fans dancing to trippy music may come to mind. Indeed, psychedelics came to prominence in the counterculture environment of the 1960's for their effects on the brain. They have the ability to elicit not only perceptual distortions such as geometric patterns and vivid colors, but to also produce peak ego-dissolution experiences and subjective effects of feeling at one with the universe (Nichols, 2016). With the passage of scheduling laws throughout the world in the late 1960's and early 1970's, these drugs were labeled as dangerous with no medical value. What is not generally appreciated was that during the counterculture of the 1960, legitimate scientific studies of these drugs were being performed to better understand brain function, and potential therapeutic value as medicines (Bonson, 2018). After scheduling, human studies halted, and access to these drugs by scientists was extremely restricted. Regardless, a handful of preclinical investigators continued the study of psychedelics, using them as tools to understand fundamental biological processes and brain circuitry in animal and cellular models. These investigations were instrumental in key discoveries in pharmacology and neuroscience. However, because psychedelics were known for their profound effects in the CNS, their study remained almost exclusively in models of CNS function. We discovered several years ago that certain psychedelics have potent anti-inflammatory activity in the periphery.

As serotonin receptor genes were beginning to be cloned in the 1990's and 2000's, it became possible to map expression and localization of the protein target of psychedelics, serotonin 2A (5-HT_{2A}) receptors (Willins et al., 1997). This receptor is one of the most widely expressed serotonin receptors in the body, and has been found in nearly every tissue and cell type examined. It has been known for several decades that at sites of inflammation in the body, serotonin levels are highly elevated, and that often applying serotonin within a model of inflammation exacerbated the inflammation (Arreola et al., 2015; Wu et al., 2019). As physiology and knowledge of receptor pharmacology and localization converged, the 5-HT_{2A} receptor was identified as a primary receptor mediating pro-inflammatory effects of serotonin (Shajib and Khan, 2015). Drugs that antagonize the 5-HT_{2A} receptor can block inflammation (Nishiyama, 2009). The mechanism of these antagonists is putatively to block the pro-inflammatory effects of serotonin at the 5-HT_{2A} receptor.

As research on 5-HT_{2A} receptors primarily focused on studying agonists for their psychoactive effects, we explored the ability of psychedelics to have anti-inflammatory activity in peripheral tissues. In our early experiments we used a common *in vitro* model used to study atherosclerosis, primary rat aortic smooth muscle (RASM) cells stimulated with the inflammatory cytokine tumor necrosis factor alpha (TNF-α). We found the drug (*R*)-2,5-dimethoxy-4-iodoamphetamine ((*R*)-DOI) had an EC_{50} of ~20 picomolor to prevent the inflammation induced by TNF- α, and block the induction of expression of several proinflammatory cytokines and adhesion protein genes (e.g. *IL (interleukin) 6*, *VCAM1* (vascular cell adhesion molecule 1), *ICAM1* (intracellular cell adhesion molecule 1) (Yu et al., 2008). (*R*)-DOI is widely believed to be selective for the 5-HT_2 family of receptors, with virtually no off-target affinity at low to moderate doses. In humans, DOI has been reported to alter perception and produce subjective 'psychedelic' behavioral effects for 24+ hours. In the RASM cell assay, the use of selective antagonists for 5-HT_{2B} and 5-HT_{2C} receptors demonstrated that the effect was

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mediated through 5-HT_{2A} receptor activation (Yu et al., 2008). To put things in perspective, the anti-inflammatory potency of corticosteroids at their target (glucocorticoid receptors) is in the low nanomolar range, about 500x less potent than (*R*)-DOI at its target. We also tested several other psychedelics, and found that some were more potent than others (Yu et al., 2008). Our results hinted that the behavioral potency of a psychedelic was not related to its anti-inflammatory effects: Lysergic acid diethylamide (LSD), arguably the most potent psychedelic, was the weakest anti-inflammatory, with an IC_{50} ~30 nM. These results suggested that certain psychedelics represented a new and potent anti-inflammatory therapeutic class, with therapeutic efficacy at sub-behavioral levels. It should be noted here that the use of the term 'psychedelic' in this work refers to the modern pharmacological definition of psychedelic as a class of drug that induces perceptual and behavioral changes through activation of 5-HT_{2A} receptors. Although the potency of certain 5-HT_{2A} receptor agonists to block or reduce inflammation is sub-behavioral, there is no generic term for agonists of the 5-HT_{2A} receptor that produce an effect that is agnostic with respect to behavioral effects, or that specifically produces effects without behavioral alterations.

The next step was to translate these findings to an animal model. For this we again utilized (*R*)-DOI, and administered one of three low systemic doses (0.01 mg/kg, 0.1 mg/kg, 0.3 mg/kg; i.p.) 30 min prior to a systemic (i.p.) treatment with TNF-α. Five hours later, we collected tissues and measured proinflammatory biomarkers (including mRNA and protein for IL6, IL1b, VCAM1, ICAM1, MCP1 (monocyte chemoattractant protein 1)). In several tissues collected that included the aortic arch, small intestine, and blood plasma, we measured significant reductions to complete suppression of TNF-α-mediated inflammation (Nau et al., 2013). In some tissues, maximal effect was measured at the lowest dose, and for other tissues there were significant effects measured at the higher doses. Importantly, significant anti-inflammatory effects were measured at levels of (*R*)-DOI far below the known behavioral threshold. For example, a maximal anti-inflammatory effect was measured in the small intestine at a dose of 0.01 mg/kg and (*R*)-DOI has a published value of 0.3 mg/kg for the minimal dose to produce a head twitch response (Nau et al., 2013; Halberstadt et al., 2020). These experiments were crucial for demonstrating potent sub-behavioral anti-inflammatory efficacy in a whole animal, successfully making the jump from *in vitro* to *in vivo*.

The final step was to assess efficacy in animal models of disease. One model we utilized was a mouse model of cardiovascular and metabolic disease: ApoE (apolipoprotein E) knockout mice fed a high fat, high cholesterol diet. In this model, mice rapidly develop vascular inflammation, atherosclerosis, and symptoms of metabolic disease (high cholesterol, dysregulation of glucose homeostasis). Because feeding of the high fat "Western" diet protocol was 16 weeks, we elected to use implanted osmotic minipumps to slowly deliver low continuous amounts of (*R*)-DOI over this time period. The amount of drug in systemic circulation at any one time was sub-behavioral ($C_{ss} = 0.515 \pm 0.2$ ng/ml), and at no time during the treatment did we observe any abnormal behaviors in the mice (Flanagan et al., 2019a). In this model, after the 16 week feeding period, several proinflammatory biomarkers in the vascular tissue most prone to develop atherosclerosis (aortic arch) were completely suppressed (e.g. mRNA for IL6) (Flanagan et al., 2019a). An important aspect of psychedelics that we were noticing at this time, in combination with results from another model we were investigating, asthma, was that psychedelics were not acting as a broad-spectrum immunosuppressive like a corticosteroid, we were seeing some key proinflammatory biomarkers being potently suppressed, but several were not affected at all. For example, elevated mRNA levels of the proinflammatory chemokine Cx3cl1 were not affected. Further, for the biomarkers where suppression was evident, suppression was potent and to baseline levels, never suppressed below baseline levels, even at relatively high doses of drug. It appeared that psychedelics were only reducing expression of subsets of key inflammatory components, leaving the immune response largely intact, but affecting enough to normalize physiology. This type of mechanism of action is novel among known anti-inflammatories and immunomodulators, and advantageous as it is predicted to have fewer side effects such as opportunistic infections that are associated with broad immunosuppressants like corticosteroids. 5-HT_{2A} receptor agonists may therefore be beneficial to treat inflammatory disease where steroids are contraindicated, or the condition is steroid resistant. Also within the ApoE $^{-/-}$ model, we found that the (*R*)-DOI treatment group had significantly less total cholesterol in circulation, and that glucose homeostasis was normalized (Flanagan et al., 2019a). We do not, however, know the mechanisms for these two findings and they remain for future studies to address. Regardless, these two findings suggested that psychedelics at low doses could also have beneficial effects to treat metabolic disease and diabetes, in addition to inflammation.

The inflammatory disease model we have used the most has been the sensitization model of allergic asthma. In this model we have used standard, and developed new, paradigms for generating allergic asthma in rodents (e.g. mice and rats). They essentially rely on initial sensitization to an allergen, chicken egg white protein ovalbumin (OVA), followed by an exposure phase. The sensitization phase involves injecting OVA into the animal (i.p.) once each on Day 0 and 7 or 14 to generate an IgE immune response. The exposure phase consists of placing the animal in a sealed chamber and exposing it to nebulized OVA for 20 min on three consecutive days at least 14 days after the final OVA sensitization to initiate an allergic IgE-mediated response in the lungs. The responses include pulmonary inflammation, goblet cell hyperplasia and mucus overproduction, and airway hyperresponsiveness that together result in difficulty breathing. The physiological responses closely resemble what occurs in a human asthmatic patient after exposure to an allergen like pollen. These models allow us to probe several aspects of immunity and the immune system simultaneously that include responses in adaptive immunity (e.g. T Helper 2 (Th2) cells), innate immunity (e.g. macrophages), eosinophils, and non-bone marrow-derived cells including bronchial smooth muscle and endothelial cells, which all contribute to the outcome measure phenotypes of pulmonary inflammation and airway hyperresponsiveness.

In the acute prophylactic models we have used, drug is administered 30 min prior to OVA exposure in order to block the effects of OVA to stimulate immune responses. Drug is administered via nebulization through a nose cone, similar to an asthmatic patient using an inhaler, to deliver directly to the lung. Although BALB/c (Th2 biased) mice are often used in this type of study, we have found that brown Norway rats provide for more consistent and robust results and can be used for screening purposes for almost a year following our modified protocols. Using this platform we have performed dose response, route of administration, and comparative studies for different psychedelics. In our early experiments, we demonstrated that (*R*)-DOI was effective in completely preventing pulmonary inflammation, mucus overproduction, and normalized breathing mechanics (Nau et al., 2015). Underlying these effects were prevention of Th2 cell recruitment to the lungs, and prevention of eosinophila. Consistent with our previous observations, we found that psychedelics were not acting as broad immunosuppressants: only subsets of key proinflammatory molecules were blocked in expression, with others left intact and no molecule's expression reduced below control baseline. For example, OVA-increased expression of the mRNAs for IL6, IL1b, GMCSF (granulocyte-macrophage colony-stimulating factor), and IL5 were all significantly blocked by (*R*)-DOI, but mRNA encoding IL4 was not, and for those whose expression was blocked they were not repressed below baseline levels (Nau et al., 2015).

Remarkably, the IC_{50} dose for (*R*)-DOI in this prophylactic paradigm is ~0.005 mg/kg, administered via nebulization or by intraperitoneal injection (Flanagan et al., 2021). This is > 50x less than the behavioral threshold dose. We have also shown that the drug psilocin, the active form of the prodrug psilocybin, has virtually the same potency as

(*R*)-DOI (Flanagan et al., 2021), indicating that the effects are not limited to (*R*)-DOI or are chemotype dependent. In our rat screening platform, we examined 25 different psychedelics to block the effects of OVA exposure, with representatives from the three major classes of psychedelics (phenethylamines, tryptamines, ergolines). Our primary outcome measure was whole body plethysmography (WBP). This measures hyperresponsiveness to the bronchoconstrictor methacholine in sealed chambers for awake freely moving animals. The measure of enhanced pause (PenH) in WBP is an excellent proxy measure for pulmonary inflammation, as the primary outcome measure, enhanced pause (PenH), will not be normal if inflammation is present and will instead show hyperresponsiveness to methacholine, with the more inflammation present the higher the change in PenH. Further, WBP is not an endpoint measure so cohorts of animals can be repeatedly used for screening purposes.

From these experiments, we found that the efficacy of a psychedelic to prevent changes in PenH induced by OVA treatment (essentially prevent pulmonary inflammation), was not correlated with calcium mobilization or β-arestin2 recruitment at the 5-HT_{2A} receptor, or behavioral potency (Flanagan et al., 2021). The extremely potent psychedelic LSD was only partially efficacious, even at relatively high doses, and the powerful psychedelics DMT (N,N-dimethyltryptamine) and 5-MeO-DMT had no measurable anti-inflammatory activity (Flanagan et al., 2021). From these studies, we determined the pharmacophore for phenethylamine 5-HT_{2A} receptor agonists as anti-inflammatory to be 2, 5-dimethoxyamphetamine (2C–H), and gained knowledge of how different substitutions of that molecule affects efficacy. We believe that functional selectivity underlies these differential effects (Urban et al., 2007), and that certain ligands engage necessary subsets of amino acid residues in the binding pocket of the receptor that stabilize conformational states that couple to anti-inflammatory signal transduction pathway effectors. We have leveraged this knowledge to generate new chemical entities maintaining anti-inflammatory potency and efficacy with reduced behavioral effects in next generation psychedelic anti-inflammatory molecules currently under study (*unpublished data*). Ultimately, we believe that it may be possible to engineer a molecule that acts as an agonist for anti-inflammatory effector engagement at 5-HT_{2A} receptors that does not engage behavioral-related effector pathways, and thus be devoid of behavioral effects, or possible effects due to activation of these pathways in peripheral tissues. Another approach for a less behavioral drug would be to devise a 5-HT_{2A} receptor agonist with anti-inflammatory properties that does not cross the blood brain barrier.

In addition to these experimental paradigms that are designed to probe prevention of inflammation, we have also investigated the ability of (*R*)-DOI to rescue pre-existing inflammation. In one protocol, we employed the standard acute paradigm but administered drug (nose only) 6 h prior to WBP testing, 42 h after the final OVA exposure when pulmonary inflammation is at its maximal levels. In this system, (*R*)-DOI had significant and specific anti-inflammatory effects on T-cells within the lung, and normalized hyperresponsiveness to methacholine in WBP experiments (*unpublished data*). In another system we developed, one for inducing chronic asthma-like symptoms in mice, we induced chronic pulmonary inflammation, mucus overproduction, and airway hyperresponsiveness lasting at full levels at least 14 days past the final OVA exposure (Flanagan et al., 2019b). Significantly, this model also induces a strong degree of pulmonary fibrosis and airway remodeling, similar to what is found in patients with severe asthma. Utilizing this system we found that (*R*)-DOI (nose only) was fully able to rescue mucus overproduction, pulmonary inflammation, and to normalize hyperresponsiveness to methacholine (Flanagan et al., 2019b). Also, treatment with (*R*)-DOI was able to produce a 70% reversal of collagen deposition and pulmonary fibrosis (Flanagan et al., 2019b). These data together demonstrate that in models of allergic asthma, psychedelics like (*R*)-DOI are not only able to prevent inflammation, but to also rescue pre-existing and severe symptoms.

In summary, we have shown in several experimental systems *in vitro* and *in vivo*, including different models of human inflammatory disease, that psychedelics can potently prevent and ameliorate several types of inflammation. Anti-inflammatory effects have been demonstrated in several cell and tissue types across several species, suggesting it is a broad-based phenomenon. Experiments using selective antagonists and the *HTR2A*$^{-/-}$ knockout mice have shown that the effects are due to 5-HT_{2A} receptor activation. Significantly, psychedelics are not broad-spectrum immunosuppressants and only target subsets of key proinflammatory molecule expression. Further, psychedelics may have therapeutic utility in treating aspects of metabolic disease. If successfully translated to human disease therapeutics, psychedelics at subbehavioral levels represent a new class of orally available anti-inflammatory with steroid-sparing properties potentially effective in several inflammatory related diseases including but not limited to asthma, atherosclerosis, cardiovascular disease, and inflammatory bowel disease.

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Declaration of interests

The author declares that he has a financial interest in Eleusis Therapeutics.

Data availability

No data was used for the research described in the article.

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Abbreviations

2C–H	(2,5-dimethoxyamphetamine)
5-HT2A	(5-hydroxytryptamine receptor 2A)
ApoE	(apolipoprotein E)
DMT	(N,N-dimethyltryptamine)
i.p.	(intraperitoneal)
IC50	(concentration for 50% inhibitory effect)
ICAM1	(intracellular cell adhesion molecule 1)
IL	(interleukin)
LSD	(lysergic acid diethylamide)
MCP1	(monocyte chemoattractant protein 1)
OVA	(ovalbumin)
PenH	(enhanced pause)
RASM	(rat aortic smooth muscle)
(R)-DOI	((*R*) 2,5-dimethoxy-4-iodoamphetamine)
Th2	(T helper 2)
TNF-a	(tumor necrosis factor alpha)
VCAM1	(vascular cell adhesion molecule 1)
WBP	(whole body plethysmography)

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