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Pharmacological and biotransformation studies of 1-acyl-substituted derivatives of *d*-lysergic acid diethylamide (LSD)

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ABSTRACT

The ergoline *d*-lysergic acid diethylamide (LSD) is one of the most potent psychedelic drugs. 1-Acetyl-LSD (ALD-52), a derivative of LSD containing an acetyl group on the indole nitrogen, also produces psychedelic effects in humans and has about the same potency as LSD. Recently, several other 1-acyl-substitued LSD derivatives, including 1-propanoyl-LSD (1P-LSD) and 1-butanoyl-LSD (1B-LSD), have appeared as designer drugs. Although these compounds are assumed to act as prodrugs for LSD, studies have not specifically tested this prediction. The present investigation was conducted to address the gap of information about the pharmacological effects and mechanism-of-action of 1-acyl-substituted LSD derivatives. Competitive binding studies and calcium mobilization assays were performed to assess the interaction of ALD-52, 1P-LSD, and 1B-LSD with serotonin 5-HT_2 receptor subtypes. A receptorome screening was performed with 1B-LSD to assess its binding to other potential targets. Head twitch response (HTR) studies were performed in C57BL/6J mice to assess *in vivo* activation of 5-HT_{2A} (the receptor thought to be primarily responsible for hallucinogenesis). Finally, liquid chromatography/ion-trap mass spectrometry (LC/MS) was used to quantify plasma levels of LSD in Sprague-Dawley rats treated with ALD-52 and 1P-LSD. 1-Acyl-substitution reduced the affinity of LSD for most monoamine receptors, including 5-HT_{2A} sites, by one to two orders of magnitude. Although LSD acts as an agonist at 5-HT_2 subtypes, ALD-52, 1P-LSD and 1B-LSD had weak efficacy or acted as antagonists in Ca^{2+}-mobilization assays. Despite the detrimental effect of 1-acyl substitution on 5-HT_{2A} affinity and efficacy, 1-acyl-substitued LSD derivatives induce head twitches in mice with relatively high potency. High levels of LSD were detected in the plasma of rats after subcutaneous administration of ALD-52 and 1P-LSD, demonstrating these compounds are rapidly and efficiently deacylated *in vivo*. These findings are consistent with the prediction that ALD-52, 1P-LSD and 1B-LSD serve as pro-drugs for LSD.

1. INTRODUCTION

The psychedelic (hallucinogenic) effects of *d*-lysergic acid diethylamide (LSD, Fig. 1) were discovered serendipitously by Dr. Albert Hofmann in 1943 (Hofmann 1979,1980). LSD is active at p.o. doses ranging from 60–200 μg (Shulgin and Shulgin 1997), making it a highly potent compound. The finding that minute doses of LSD can produce profound changes in consciousness inspired research across multiple disciplines and helped to launch the modern era of biological psychiatry. During the 1950s and 1960s, hundreds of clinical studies were conducted with LSD. Reports appeared indicating LSD may possess therapeutic efficacy in disorders such as depression, alcoholism, and chronic pain (Kast and Collins 1964; Pahnke et al. 1969; Krebs and Johansen 2012). By the early 1970s, however, most clinical work with LSD had ceased due to legal restrictions. Human trials have cautiously resumed in recent years to investigate the therapeutic efficacy of LSD and to explore its potential as a tool for neuroscience research (Gasser et al. 2015; Kraehenmann et al. 2017b; Müller et al. 2017; Preller et al. 2017; Preller et al. 2018b; Schmidt et al. 2018). In addition to its use in scientific studies, LSD was closely associated with the counterculture during the 1960s and it continues to be a popular recreational drug.

In addition to LSD, many other lysergamides have been synthesized in order to define the structure-activity relationships for this class of compounds (Nichols 2018). Various substitutions were made on the indole nitrogen (position N^1) of LSD, including the attachment of acyl groups. One of the earliest examples is 1□acetyl□LSD (ALD□52, Fig. 1). The preparation of ALD-52 was first reported by Troxler and Hofmann in 1957 (Troxler and Hofmann 1957). Tests of ALD-52 in human subjects revealed that it produces LSD-like effects and closely matches the time-

course of LSD (Rothlin 1957; Abramson 1959; Isbell et al. 1959; Malitz et al. 1960). According to Rothlin (1957), both LSD and ALD-52 are active at doses of 0.5–1 μg/kg, but further experimental details were not provided. Abramson (1959) found ALD-52 to be 91% as potent as LSD, whereas Isbell et al. (1959) reported that the two substances are equipotent. In another study conducted in normal subjects and psychotic patients, LSD and ALD-52 produced similar effects, but the latter compound seemed to alter cognition and body image to a greater extent than LSD (Malitz et al. 1960). Although claims have been made that some of the LSD distributed during the late 1960s was actually ALD-52 (Tendler and May 1984), the first confirmed detection of the drug on the illicit market occurred in April 2016 (EMCDDA 2018).

Similar to ALD-52, other N^1-acyl-substituted ergoline derivatives have been marketed online as recreational drugs. 1-Propanoyl-LSD (1P-LSD, Fig. 1) was first detected in 2015 (Brandt et al. 2016; EMCDDA 2018). 1-Butanoyl (1B-LSD) and 1-propanoyl-6-ethyl-6-nor-LSD (1P-ETH-LAD) have also appeared on the recreational drug market (Brandt et al. 2017b; Brandt et al. 2019). Notably, as far as we are aware, 1P-LSD, 1B-LSD, and 1P-ETH-LSD had not been reported in the scientific or patent literature prior to their appearance as recreational substances, making them true "designer drugs."

It is not clear whether ALD-52 and its homologues are the active pharmacological species, or alternatively whether they serve as prodrugs for LSD. According to recent studies, the psychedelic effects of LSD in humans are largely attributable to 5-HT_{2A} receptor activation (Barrett et al. 2017; Kraehenmann et al. 2017a; Kraehenmann et al. 2017b; Preller et al. 2017; Preller et al. 2018a). Although little is known about the pharmacological properties of ALD-52 and its homologues, substitution on the indole nitrogen typically reduces the affinity of ergolines for the human 5-HT_{2A} (h5-HT_{2A}) receptor by 20–30-fold (Johnson et al. 1994). For example, the

N^1-unsubstituted lysergamide LY193525 has sub-nanomolar affinity for the human 5-HT_{2A} receptor (K_i = 0.73 nM vs. [^{3}H]ketanserin), whereas its N^1-isopropyl derivative amersergide has ~20-fold lower affinity (K_i = 16.2 nM) (Johnson et al. 1994). According to mutagenesis experiments and molecular modeling studies, when tryptamines and ergolines bind to h5-HT_{2A}, the indole N^1 hydrogen interacts with residue 5.46 for agonist activation (McCorvy et al. 2018), and specifically with S242$^{5.46}$ in the human 5-HT_{2A} receptor (Nelson et al. 1993; Johnson et al. 1994; Almaula et al. 1996). Although studies to date have not characterized the biotransformation of ALD-52, 1P-LSD, or 1B-LSD *in vivo*, ergolines containing a methyl group on the indole nitrogen are metabolized by *N*-dealkylation (Enz et al. 1984; Tfelt-Hansen et al. 1985; Bredberg et al. 1986; Muller-Schweinitzer and Tapparelli 1986; Bredberg and Paalzow 1990). Experiments have confirmed that enzymes present in human serum and liver can deacylate ALD-52, 1P-LSD, 1B-LSD, and 1P-ETH-LAD (Brandt et al. 2016; Wagmann et al. 2019). A recent intoxication case involving 1P-LSD indicated formation of LSD *in vivo* with no detection of the parent molecule (Grumann et al. 2019). Similar to ALD-52, 1P-LSD and 1B-LSD also have about the same potency as LSD in humans, which is another factor indicating that they likely act as prodrugs.

Given the difficulties associated with hallucinogen studies in humans, animal behavioral models have been widely used to investigate the pharmacology of serotoninergic hallucinogens (Halberstadt 2015; Halberstadt and Geyer 2018). LSD and other serotoninergic hallucinogens induce the head-twitch response (HTR), a paroxysmal side-to-side head rotation, in mice and rats via 5□HT_{2A} receptor activation (Halberstadt et al. 2011; Canal and Morgan 2012). The HTR is commonly used as a behavioural proxy in rodents for human hallucinogenic effects because it is one of only a few behaviours that can reliably distinguish hallucinogenic and non□

hallucinogenic 5□HT_{2A} receptor agonists (Gonzalez-Maeso et al. 2007; Halberstadt and Geyer 2013). Head twitches were traditionally assessed using direct observation but new methods have been developed to detect the behavior in a semi-automated fashion using a head-mounted magnet and a magnetometer coil (Halberstadt and Geyer 2013,2014). The HTR serves as a behavioral readout of 5-HT_{2A} activation and can be used to compare the *in vivo* potencies of 5-HT_{2A} receptor agonists (Nichols et al. 2015; Halberstadt et al. 2016; Brandt et al. 2017a; Brandt et al. 2018; Klein et al. 2018; Halberstadt et al. 2019b; Halberstadt et al. 2019c). Previous studies performed in our laboratory demonstrated that 1P-LSD and 1B-LSD induce head twitches in male C57BL/6J mice with approximately one-third and one-sixth of the potency of LSD, respectively (Brandt et al. 2016; Brandt et al. 2019). Surprisingly, according to Corne and Pickering (1967), ALD-52 does not induce head twitches in mice; however, it is difficult to interpret their findings due to the specific methods used in the experiments (i.e., HTR activity was assessed using quantal scoring).

The present investigation was conducted to address the gap of information about the pharmacological effects and mechanism-of-action of 1-acyl-substituted lysergamides. Interactions of ALD-52, 1P-LSD, and 1B-LSD with serotonin (5□HT) receptor subtypes were assessed using competitive binding and functional assays. Biotransformation studies were conducted to determine whether ALD-52 and 1P-LSD are metabolized to LSD after *in vivo* administration. HTR studies were also conducted to address the conflicting findings regarding the activity of ALD-52 in humans and mice. The results of these experiments support the prediction that the deacylation of ALD-52, 1P-LSD, and 1B-LSD to LSD likely plays a key role in their behavioral effects.

2. MATERIALS AND METHODS

2.1. Animals

Animals were housed in a vivarium at the University of California San Diego, an AAALAC-approved animal facility that meets all Federal and State requirements for care and treatment of laboratory animals. Male C57BL/6J mice (6-8 weeks old) obtained from Jackson Laboratories (Bar Harbor, ME, USA) and were housed up to four per cage in a climate-controlled room on a 12-h reverse-light cycle (lights on at 1900 h, off at 0700 h). Male Hsd:Sprague Dawley rats (ENVIGO, Indianapolis, IN, USA; initial weight 230–250 g) were housed in pairs in a climate-controlled room on a 12-h reverse-light cycle (lights on at 1900 h, off at 0700 h). Food and water were available *ad libitum* except during behavioral testing, which was conducted between 1000 and 1800 h. Animals were acclimatized for approximately 1 week after arrival prior to behavioral testing and maintained in American Association for Accreditation of Laboratory Animal Care-approved facilities that meet all federal and state guidelines. All animal experiments were carried out in accordance with NIH guidelines and were approved by the UCSD institutional animal care and use committee. Principles of laboratory animal care were followed as well as specific laws of the USA.

2.2. Drugs and Reagents

1-Acetyl-*d*-lysergic acid diethylamide (ALD-52), 1-propanoyl-*d*-lysergic acid diethylamide (1P-LSD), and 1-butanoyl-*d*-lysergic acid diethylamide (1B-LSD) were obtained

from Synex Synthetics BV (Maastricht, The Netherlands) as the hemitartrate (2:1) salts. M100,907 and *d*-lysergic acid diethylamide (LSD) hemitartrate (2:1) were available from previous studies. LSD-d_3 was obtained from LGC Standards (Wesel, Germany). For competitive binding and functional assays, test substances were dissolved in dimethyl sulfoxide. For studies in mice, ALD-52 was dissolved in isotonic saline and administered by the intraperitoneal (IP) route (5 mL/kg). M100907 was dissolved in water containing 2% Tween-80 and administered by the subcutaneous (SC) route (5 mL/kg). For studies in rats, test substances were dissolved in isotonic saline and injected subcutaneously (1 mL/kg).

2.3. Binding Studies

A screening at 27 receptor binding sites was performed by the NIMH Psychoactive Drug Screening Program (NIMH PDSP). LSD, ALD-52, 1P-LSD, and 1B-LSD were tested at 10 μM in competition assays against radioactive probe compounds; each primary binding assay was performed in quadruplicate. Sites exhibiting >50% inhibition at 10 μM were tested in secondary assays at the identified receptor using 11 concentrations of the lysergamide, measured in triplicate, to generate competition binding isotherms. K_i values were obtained from best-fit IC_{50} values (derived from nonlinear regression of the binding isotherms) using the Cheng-Prusoff equation (Cheng and Prusoff 1973). The experimental protocols are available from the NIMH PDSP website (Roth 2013).

2.4. 5-HT_2 Receptor Functional Assays

5-HT_2 functional experiments (measuring G_q-mediated calcium flux) were performed with Flp-In T-REx 293 cells (Invitrogen, Carlsbad, CA, USA) expressing either human 5-HT_{2A} (h5-HT_{2A}), mouse 5-HT_{2A} (m5-HT_{2A}), human 5-HT_{2B} (h5-HT_{2B}) or human 5-HT_{2C} INI (h5-HT_{2C}) receptor cDNA under the tetracycline repressor protein. In the T-Rex system, receptor expression was induced with 2 ! g/mL tetracycline, and cells were plated into black 384 clear-bottom, tissue culture plates in 40 ! L of DMEM containing 1% dialyzed foetal bovine serum (FBS) at a density of approximately 10,000 cells per well. After approximately 20-24 hours, media was decanted and replaced with 20 ! L per well of drug buffer (HBSS, 20 mM HEPES, pH 7.4) containing Fluo-4 Direct dye (Invitrogen) and incubated for 1 h at 37°C. Test substances were diluted in drug buffer (HBSS, 20 mM HEPES, 0.1% bovine serum albumin, 0.01% ascorbic acid, pH 7.4). Before the experiment, plates were allowed to equilibrate to room temperature and calcium flux was measured using a FLIPR^{TETRA} (Molecular Devices, Sunnyvale, CA, USA). Plates were read for fluorescence initially for 10 seconds (1 read per second) to establish a baseline, and then stimulated with drug dilutions or buffer and read for an additional 120 seconds. Peak fluorescence in each well was normalized to maximum-fold increase over baseline. Data were normalized to the maximum peak fold-over-basal fluorescence produced by 5-HT (100%) and baseline fluorescence (0%). Data were analyzed using the sigmoidal dose-response function of Prism 5.0 (GraphPad Software, San Diego, CA, USA).

2.5. Head-Twitch Response Studies

The head-twitch response (HTR) was assessed using a head-mounted magnet and a magnetometer detection coil (Halberstadt and Geyer 2013,2014; Nichols et al. 2015). Briefly,

mice were anesthetized, a small incision was made in the scalp, and a small neodymium magnet was attached to the dorsal surface of the cranium using dental cement. Following a one-week recovery period, HTR experiments were carried out in a well-lit room with at least 7 days between sessions to avoid carryover effects. Test compounds were injected immediately prior to testing. Mice (n = 5/group) were injected with drug or vehicle and then HTR activity was recorded in a glass cylinder surrounded by a magnetometer coil for 30 min. Coil voltage was low-pass filtered (2–10 kHz cutoff frequency), amplified, and digitized (20 kHz sampling rate) using a Powerlab/8SP with LabChart software ver. 7.3.2 (ADInstruments, Colorado Springs, CO, USA), then filtered off-line (40–200 Hz band-pass). Head twitches were identified manually based on the following criteria: 1) sinusoidal wavelets; 2) evidence of at least three sequential head movements (usually exhibited as bipolar peaks) with frequency " 40 Hz; 3) amplitude exceeding the level of background noise; 4) duration < 0.15 s; and 5) stable coil voltage immediately preceding and following each response. Head twitch counts were analyzed using one-way analyses of variance (ANOVA). *Post hoc* pairwise comparisons between selected groups were performed using Tukey's studentized range method. Significance was demonstrated by surpassing an #-level of 0.05. Median effective doses (ED_{50} values) and 95% confidence intervals (95% CI) for HTR dose-response experiments were calculated by nonlinear regression using a Gaussian distribution (Prism 7.00, GraphPad Software, San Diego, CA, USA).

2.6. Biotransformation Studies

Rats (n = 5-6/group, 29 total) were treated with vehicle, ALD-52 (0.1 or 0.3 mg/kg), or 1P-LSD (0.1 or 0.3 mg/kg). The animals were anesthetized (isoflurane) and decapitated 15 min

post-injection, and trunk blood was collected in cooled heparinized tubes. Plasma was isolated by centrifugation and stored at –80°C.

2.6.1. Sample preparation by solid-phase extraction. Sample preparation was performed in accordance with Maurer et al. (2016) with minor modifications. Ten μL of methanolic LSD-d_3 (as internal standard, final plasma concentration 2 ng/mL) were added to 0.5 mL of rat plasma, diluted with 2 mL of purified water, mixed for 15 s on a rotary shaker, and loaded on a HCX cartridge (130 mg, 3 mL; Biotage, Uppsala, Sweden) previously conditioned with 1 mL of methanol and 1 mL of purified water. After extraction, the cartridge was washed with 1 mL of purified water, 1 mL of 0.01 M aqueous hydrochloric acid, and 2 mL of methanol. Reduced pressure was applied until the cartridge was dry and the analytes were eluted with 1 mL of a freshly prepared mixture of methanol-aqueous ammonia (98:2, v/v) into a reaction tube. The eluate was evaporated to dryness under a stream of nitrogen at 70°C and the residue was dissolved in 50 μL of a mixture of 10 mM aqueous ammonium formate-acetonitrile (1:1, v/v) containing 0.1% formic acid. The limit of detection (LOD) of the method was 2 ng/mL LSD.

2.6.2. LC- ion trap MS instrumentation for LSD quantification. Samples were analyzed using a ThermoFisher Scientific (TF, Dreieich, Germany) LXQ linear ion trap MS, coupled to a TF Accela ultra high performance LC (UHPLC) system consisting of a degasser, a quaternary pump, and an autosampler. Gradient elution was performed on a TF Hypersil GOLD C18 column (100 mm × 2.1 mm inner diameter, 1.9 ! m particle size). The mobile phase consisted of 10 mM aqueous ammonium formate plus 0.1% formic acid (pH 3.4, eluent A) and acetonitrile plus 0.1% formic acid (eluent B). The flow rate was set to 0.5 mL/min and the following gradient was used: 0–2.0 min 2% B, 2.0–4.0 min to 80% B, 4.0–6.0 min hold 80% B, 6.0–6.5 min to 90% B, 6.5–7.0 min hold 90% B, 7.0-10.0 min hold 80% B, 10.0-17.0 hold 2% B.

Analyses were performed in a targeted acquisition mode with an inclusion list, where MS^2 spectra of given precursor ions (LSD and LSD-d_3) were recorded. The injection volume was 10 μL each. The MS was equipped with a heated electrospray ionization II (HESI-II) source, other conditions were as follows: positive ionization mode; sheath gas, nitrogen at flow rate of 34 arbitrary units (AU); auxiliary gas, nitrogen at flow rate of 11 AU; vaporizer temperature, 250 °C; source voltage, 3.00 kV; ion transfer capillary temperature, 300 °C; capillary voltage, 38 V; tube lens voltage, 110 V; automatic gain control (AGC) target, 5,000 ions for MS^2; data type, centroid; normalized collision energy, 35.0; wideband activation, enabled; isolation width, *m/z* 1.5. TF Xcalibur Qual Browser software version 2.0.7 was used for data evaluation and LSD concentration was determined comparing the peak areas of LSD and LSD-d_3 within the same run.

2.6.3. LC-high resolution MS/MS instrumentation for detection of metabolites.

Analyses were performed in accordance to Wagmann et al. (2019). A TF Dionex UltiMate 3000 Rapid Separation (RS) UHPLC system with a quaternary UltiMate 3000 RS pump and an UltiMate 3000 RS autosampler was used, controlled by the TF Chromeleon software version 6.80, and coupled to a TF Q-Exactive Plus equipped with a HESI-II source. Mass calibration was performed prior to analysis according to the manufacturer's recommendations using external mass calibration. Gradient elution was performed on a TF Accucore PhenylHexyl column (100 mm × 2.1 mm inner diameter, 2.6 ! m particle size). The mobile phases consisted of 2 mM aqueous ammonium formate containing formic acid (0.1%, v/v) and acetonitrile (1%, v/v, pH 3, eluent A) and 2 mM ammonium formate in acetonitrile/methanol (50:50, v/v) containing formic acid (0.1%, v/v) and water (1%, v/v, eluent B). The gradient and flow rate were programmed as follows: 0–10 min 10% B to 50% B, 10–12 min hold 98% B, and 12–14 min hold 10% B,

constantly at a flow rate of 0.5 mL/min. HESI-II source conditions were as follows: heater temperature, 438 °C; ion transfer capillary temperature, 269 °C; sheath gas, 53 AU; auxiliary gas, 14 AU; sweep gas, 3 AU; spray voltage, 3.50 kV, and S-lens RF level, 60.0. Mass spectrometric analysis was performed in positive full-scan mode and targeted MS^2 mode using an inclusion list containing accurate masses of expected metabolites. Pick others mode was activated to ensure the recording of MS^2 spectra of precursor ions not in the inclusion list. The settings for full-scan data acquisition were as follows: resolution, 35,000; AGC target, 1e6; maximum injection time (IT), 120 ms; scan range, m/z 100–700. The settings for the targeted MS^2 were as follows: resolution, 17,500; AGC target, 2e5; maximum IT, 250 ms; isolation window, *m/z* 1.0; high-collision dissociation cell with stepped normalized collision energy 17.5, 35.0, 52.5. TF Xcalibur Qual Browser software version 2.2 SP1.48 was used for data evaluation.

3. RESULTS

3.1. Receptor binding studies

Table 1 shows the affinities of LSD and N^1-acyl-substituted homologues for recombinant human 5□HT_{1A}, 5□HT_{2A}, and 5-HT_{2C} receptors (labelled with [^{3}H]8□OH□DPAT, [^{3}H]ketanserin, and [^{3}H]mesulergine, respectively). Consistent with previous studies (Nichols et al. 2002; Rickli et al. 2015; Nichols 2018), LSD has nanomolar affinity for 5□HT_{1A} (K_i = 9.5 nM), 5□HT_{2A} (K_i = 14.7 nM), and 5□HT_{2C} (K_i = 45.3 nM) receptors. N^1-Acyl substitution reduced the affinity of LSD for the 5-HT_{1A} receptor and the magnitude of the effect was dependent on the length of the acyl group. 1B-LSD had 36-fold lower affinity (K_i = 345 nM)

than LSD, 1P-LSD had 67-fold lower affinity (K_i = 637 nM), and ALD-52 had 111-fold lower affinity (K_i = 1,054 nM). 5-HT_{2A} receptor affinity was also reduced by N^1-acyl substitution. Substitution with an acetyl group or a propanoyl group on the indole nitrogen of LSD reduced 5-HT_{2A} receptor affinity more than tenfold (ALD-52: K_i = 174 nM; 1P-LSD: K_i = 196 nM), whereas the reduction in 5-HT_{2A} affinity produced by a butanoyl group was closer to fivefold (1B-LSD: K_i = 87.7 nM). In contrast to the effect on 5-HT_{1A} and 5-HT_{2A} receptor affinity, N^1-acyl substitution increased the affinity of LSD for the 5-HT_{2C} receptor by approximately 2–4-fold (see Table 1).

In addition to focusing on the interaction of N^1-acyl-substituted LSD derivatives with 5-HT_{1A} and 5-$HT_{2A/2C}$ receptors, the binding affinity of 1B-LSD was determined for 24 other monoamine receptors (Table 2). Based on the data previously reported for LSD (see Table 3), N^1-butanoyl substitution appears to have a detrimental effect on binding to most of those sites. Compared to LSD, 1B-LSD has 10–100-fold lower affinity for most monoamine receptor subtypes. However, there were a few exceptions. In addition to the 5-HT_{2C} receptor (see above), 1B-LSD also retains high affinity for the 5-HT_{2B} receptor. In a study using [^{3}H]LSD as the radioligand, LSD bound to the human 5-HT_{2B} receptor with K_i = 3.7 nM (Wacker et al. 2013), which is very similar to the affinity of 1B-LSD in the present investigation (K_i = 3.5 nM).

3.2. Functional assays at selected serotonin receptors

Experiments also assessed whether ALD-52, 1P-LSD and 1B-LSD can activate 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors by measuring G_q-mediated Ca^{2+} flux in HEK cells (Table 4). As shown in Fig. 2, ALD-52 (EC_{50} = 38 nM; E_{max} = 25%), 1P-LSD (EC_{50} = 11 nM; E_{max} = 11%) and

1B-LSD (EC_{50} = 85 nM; E_{max} = 23%) act as very weak partial agonists at the human 5-HT_{2A} receptor compared to LSD (EC_{50} = 1.52 nM; E_{max} = 94%) when evaluated under similar experimental conditions. ALD-52, 1P-LSD and 1B-LSD were also tested at the murine 5-HT_{2A} receptor (Table 4); those compounds had slightly higher potency and efficacy at m5-HT_{2A} compared to h5-HT_{2A}. Notably, ALD-52, 1P-LSD, and 1B-LSD showed no agonist activity at h5-HT_{2B} and h5-HT_{2C} (Table 4), despite showing relatively high affinity for those sites (Tables 1 and 2), indicative of antagonist activity at these receptors. By contrast, LSD acts as an agonist at recombinant h5-HT_{2B} and h5-HT_{2C} receptors, albeit with slightly lower potency compared to h5-HT_{2A} and m5-HT_{2A} receptors (Table 4).

3.3. Head-twitch response

ALD-52 induced the HTR when tested in mice ($F(4,20) = 16.74$, $p<0.0001$; Fig. 3). Analysis of the dose-response data by nonlinear regression showed that ALD-52 induces the behavior with an ED_{50} = 297.2 nmol/kg (Table 5). The effects of LSD, 1P-LSD, and 1B-LSD on HTR, assessed in previous studies using similar methods (Halberstadt and Geyer 2013; Brandt et al. 2016; Brandt et al. 2019), are included in Table 5 for comparison. Interestingly, the rank order of potency of the four lysergamides is inversely proportional to the length of the substituent present on the indole nitrogen. LSD, with an ED_{50} value of 132.8 nmol/kg (Halberstadt and Geyer 2013), is the most potent compound in this series; ALD-52 and 1P-LSD have about half and one-third of the potency of LSD, respectively, whereas 1B-LSD is even less potent, acting with ~14% of the potency of LSD. An extra-sum-of-squares *F*-test confirmed that ALD-52 ($F(1,22) = 12.08$, $p=0.0021$) is less potent than LSD in mice; similar results were

reported previously for 1P-LSD and 1B-LSD (Brandt et al. 2016; Brandt et al. 2019). Nevertheless, although the N^1-acyl substituted derivatives are less potent than LSD, the HTR studies confirm these compounds are capable of activating 5-HT_{2A} receptors *in vivo* with relatively high potency compared to many other hallucinogens (Klein et al. 2018; Halberstadt et al. 2019a; Halberstadt et al. 2019b).

A blockade experiment with the selective 5-HT_{2A} antagonist M100,907 was performed to confirm that ALD-52 induces the HTR by activating the 5-HT_{2A} receptor. As was shown previously for 1P-LSD (Brandt et al. 2016), ALD-52 (0.3 mg/kg) did not induce head twitches in mice pretreated with 0.1 mg/kg M100,907 ($t(8) = 7.65$, $p < 0.0001$; Fig. 3).

3.4. Biotransformation of ALD-52 and 1P-LSD

To determine whether ALD-52 and 1P-LSD are metabolized to LSD, plasma samples were collected 15 minutes after SC administration of those compounds to rats. LSD was quantified using LSD-d_3 as an internal standard. As shown in Table 6, the plasma samples contained high levels of LSD. In comparison, according to Aghajanian and Bing (1964), plasma levels in humans peak at 9.5 ng/mL after intravenous administration of 2 μg/kg LSD. Another study detected 4.5 ng/mL LSD in the plasma of participants who had ingested 200 μg of the drug (Dolder et al. 2016). Similar levels have been reported in LSD intoxication cases (Smith and Robinson 1985). Conversely, the peak plasma concentrations in three rats injected IP with 0.5 mg/kg LSD (Nakahara et al. 1996) are compatible with our findings for 0.3 mg/kg ALD-52 and 0.3 mg/kg 1P-LSD. Higher plasma concentrations (>0.25 μg/mL) were detected in monkeys (*Macaca mulatta*) after i.v. administration of 0.2 mg/kg LSD (Axelrod et al. 1957). It appears

that ALD-52 and 1P-LSD are deacylated at roughly the same rate because rats treated with either compound had almost identical plasma levels of LSD.

In addition to LSD, the parent compounds were also detected in plasma, as well as several other metabolites (Table 7). The biotransformation was almost identical with the pattern observed previously when ALD-52 and 1P-LSD were incubated with human liver homogenates (Wagmann et al. 2019). Possible metabolic pathways for ALD-52 and 1P-LSD are shown in Fig. 4. Both ALD-52 and 1P-LSD were metabolized by *N*-deethylation and N^6-demethylation; ALD-52 was also hydroxylated to two phenolic metabolites. As shown in Table 7, various metabolites of LSD were present in the plasma samples. Dihydroxy-LSD is probably identical to 2-oxo-3-hydroxy-LSD (O-H-LSD), which is believed to be the main metabolite of LSD in humans (Poch et al. 1999; Johansen and Jensen 2005; Favretto et al. 2007; Dolder et al. 2015; Steuer et al. 2017). *N*-Deethyl-LSD (lysergic acid monoethylamide, LAE-32) and N^6-demethyl-LSD (nor-LSD) have been identified as metabolites of LSD in rats (Niwaguchi et al. 1974; Siddik et al. 1979b) and humans (Lim et al. 1988; Cai and Henion 1996; Canezin et al. 2001; Steuer et al. 2017). Rats and humans are also known to metabolize LSD to 13- and 14-hydroxy-LSD and to the corresponding glucuronides (Siddik et al. 1975,1979b,a; Canezin et al. 2001; Steuer et al. 2017). Hydroxy LSD isomers 1 and 2 are probably identical to 13- and 14-hydroxy-LSD but it was not possible to determine the position of the hydroxy groups based on fragmentation patterns. The third hydroxy LSD isomer is probably lysergic acid ethyl-2-hydroxyethylamide (LEO), which was previously detected as a human metabolite (Cai and Henion 1996; Canezin et al. 2001; Dolder et al. 2018).

4. DISCUSSION

LSD is a prototypical hallucinogen. Similar to LSD, derivatives containing an acyl substituent on the indole nitrogen are also potent psychedelic drugs. ALD-52, one of the earliest members of this class, was evaluated in humans in several published trials (Rothlin 1957; Abramson 1959; Isbell et al. 1959; Malitz et al. 1960). More recent additions include 1P-LSD and 1B-LSD, which have been marketed online as "legal" alternatives to LSD. One unresolved question is whether ALD-52, 1P-LSD, and 1B-LSD are active agents or alternatively serve as pro-drugs for LSD. Although substitution at the indole nitrogen generally has detrimental effects on the binding of ergolines to human and monkey 5-HT_{2A} receptors (Johnson et al. 1993; Johnson et al. 1994), ALD-52, 1P-LSD and 1B-LSD are only slightly less potent than LSD in humans, indicating they may act as prodrugs. Indeed, in the present studies, N^1-acyl substitution reduced the affinity of LSD for most monoamine receptors, including 5-HT_{2A} sites. In addition, although LSD acts as an agonist at 5-HT_2 subtypes, ALD-52, 1P-LSD, and 1B-LSD have low efficacy or act as antagonists when tested in Ca^{2+}-mobilization assays. Despite their weak activity at 5-HT_{2A}, all three compounds are relatively potent in mice, inducing the HTR with ~15% to ~50% of the molar potency of LSD. In the biotransformation experiment, ALD-52 and 1P-LSD were rapidly and efficiently metabolized to LSD, which likely explains why they are behaviorally active via the 5-HT_{2A} receptor despite having very low agonist efficacy. In summary, the present findings support the hypothesis that ALD-52, 1P-LSD, and 1B-LSD act as pro-drugs for LSD.

Consistent with published *in vitro* evidence (Brandt et al. 2016; Wagmann et al. 2019), ALD-52 and 1P-LSD were rapidly metabolized to LSD after administration to rats, supporting the prediction that the latter agent is the active species. As far as we are aware, this is the first

demonstration under controlled conditions that ergolines are metabolized by deacylation after *in vivo* administration. The deacylation of 1P-LSD to LSD reported in this study is consistent with a recent report about an intoxication case (Grumann et al. 2019). N^1-Demethylation, by contrast, is a known route of biotransformation for ergolines. For example, methysergide is metabolized to methylergometrine in humans (Tfelt-Hansen et al. 1985; Bredberg et al. 1986), rats (Bredberg and Paalzow 1990), and dogs (Muller-Schweinitzer and Tapparelli 1986). Mesulergine has also been shown to undergo N^1-demethylation to active metabolites (Enz et al. 1984). Based on the level of LSD in plasma detected 15 min after administration of ALD-52 and 1P-LSD, it appears the N^1-acyl group is rapidly hydrolyzed. The rapid metabolism of ALD-52 and 1P-LSD likely explains why those compounds have roughly the same time-course as LSD in humans (Isbell et al. 1959) and mice (Brandt et al. 2016). The plasma concentration of LSD in rats treated SC with 0.3 mg/kg of ALD-52 or 0.3 mg/kg 1P-LSD is actually slightly higher than was observed in a previous study where rats received a larger dose of LSD (0.5 mg/kg) by the IP route (Nakahara et al. 1996), indicating a large percentage of the total dose is metabolized to LSD.

LSD and N^1-unsubstituted analogs have submicromolar affinity for a variety of serotonergic, dopaminergic, and adrenergic receptors (Nichols et al. 2002; Rickli et al. 2015; Halberstadt et al. 2019c). The addition of an acyl group to the indole nitrogen of LSD reduces its affinity for most of those sites, including the 5-HT_{2A} receptor. Consistent with these findings, N^1-alkyl substitution has also been shown to reduce the affinity of ergolines for the 5-HT_{2A} receptor in humans and monkeys (Johnson et al. 1993), likely because the indole N-H requires a hydrogen bond with the sidechain of $S242^{5.46}$. Notably, in contrast to the effect on 5-HT_{2A} affinity, N^1-acyl substitution had little effect on the binding of LSD to 5-HT_{2B} and 5-HT_{2C} receptors, indicating different molecular interactions are involved when LSD binds to these

receptors. Based on the published crystal structure of LSD bound to 5-HT_{2B} (Wacker et al. 2017), the indole nitrogen engages the backbone oxygen of $G221^{5.42}$, also present in the 5-HT_{2C} receptor, instead of the proposed 5-HT_{2A} $S242^{5.46}$ residue, found deeper in the pocket. Crystal structures of other ergolines such as methylergonovine have shown that the indole N-H interaction with transmembrane (TM) 5 residue 5.46 is critical for agonist activation (McCorvy et al. 2018), whereby longer indole N^1-alkyl chains on ALD-52, 1P-LSD, and 1B-LSD would sterically inhibit movement of TM5 for activation, thus acting as antagonists or very weak partial agonists at 5-HT_2 receptors.

The present studies focused on the interaction of ALD-52, 1P-LSD, and 1B-LSD with the 5-HT_{2A} receptor, which is thought to be the primary molecular target of LSD and other serotonergic hallucinogens. 5-HT_{2A} receptor antagonists such as ketanserin and risperidone can block the characteristic effects of LSD, psilocybin, and *ayahuasca* (a hallucinogenic decoction or tea containing *N*,*N*-dimethyltryptamine) (Vollenweider et al. 1998; Valle et al. 2016; Kraehenmann et al. 2017a; Kraehenmann et al. 2017b; Preller et al. 2017; Preller et al. 2018a). Although ALD-52, 1P-LSD, and 1B-LSD likely act as prodrugs for LSD, they do retain some affinity and efficacy at the 5-HT_{2A} receptor, which makes it difficult to completely exclude the possibility that some of their *in vivo* effects occur because they interact directly with 5-HT receptors. It seems unlikely, however, that direct receptor interactions play a role in the mechanism of action of ALD-52 and 1P-LSD. First, ALD-52 and 1P-LSD have considerably lower affinity and functional potency compared to LSD at 5-HT_{2A}, so they would have to compete with LSD for access to the receptor. Although the extent to which LSD occupies 5-HT_{2A} binding sites in the brain has not been reported, 5-HT_{2A} occupation levels range from 43% to 72% in subjects given 3-30 mg. p.o. doses of psilocybin (Madsen et al. 2019). If LSD

occupies the 5-HT_{2A} receptor to a similar extent and is primarily responsible for the psychedelic effects produced by ALD-52 and 1P-LSD, then the binding of LSD may limit the ability of the parent compounds to interact with the 5-HT_{2A} receptor. Furthermore, LSD has a relatively long residence time at 5-HT_{2A} and only slowly dissociates once it binds (Bennett and Aghajanian 1974; Burris and Sanders-Bush 1992; Wacker et al. 2017), further reducing the likelihood that ALD-52 and 1P-LSD can occupy the receptor to an appreciable extent after *in vivo* administration.

ALD-52 induced the HTR in mice, as was found for 1P-LSD and 1B-LSD in previous studies (Brandt et al. 2016; Brandt et al. 2019). Blockade studies with the selective antagonist M100,907 confirmed that the HTR induced by ALD-52 and 1P-LSD is mediated by the 5-HT_{2A} receptor. Although ALD-52, 1P-LSD, and 1B-LSD have submicromolar affinity for the 5-HT_{2A} receptor, those molecules are probably not the active species in the HTR experiments. The number of head twitches induced by hallucinogens depends on the particular compound being tested and is probably determined by their efficacy at the 5-HT_{2A} receptor. Specifically, according to Vickers et al. (2001), the magnitude of the HTR is correlated with 5-HT_{2A} agonist efficacy. Mice also emit head twitches spontaneously due to 5-HT_{2A} receptor activation by serotonin (Dursun and Handley 1996). Notably, the baseline level of head twitches can mask the response induced by very weak 5-HT_{2A} receptor agonists in mice (unpublished observations). The magnitude of the HTR induced by the 1-acyl LSD derivatives, however, exceeds the baseline level of head twitches, which is not consistent with their very low efficacy at the 5-HT_{2A} receptor. Hence, LSD is likely the active species in the HTR experiments shown in Figure 3, although we cannot completely exclude the possibility that ALD-52 contributes to the response.

In addition to LSD, several other metabolites of ALD-52 and 1P-LSD were detected in rats. The same metabolites were observed when ALD-52 and 1P-LSD were incubated with pooled human liver microsomes (Wagmann et al. 2019), indicating these compounds are probably metabolized in a similar manner when they are administered to humans. Based on these findings, positive screening results for LSD or its metabolites should be interpreted with caution because these results may not always reflect LSD intake (which may be important in forensic cases where 1-acetylated lysergamides might not be controlled substances). Although ALD-52 and 1P-LSD have several unique metabolites that are not detected after intake of LSD (Dolder et al. 2018; Wagmann et al. 2019), all of the unique metabolites of ALD-52 and 1P-LSD are likely susceptible to N^1-deacylation, so their concentration in plasma and tissues may decrease to undetectable levels if there is a relatively long interval between intake and sample collection.

In summary, the hallucinogenic effects produced by ALD-52, 1P-LSD, and 1B-LSD are likely dependent on their metabolism to LSD. To our knowledge, this analysis is the first to quantify the pharmacological properties and biotransformation of LSD derivatives containing an acyl group on the indole nitrogen. Although the first human studies with ALD-52 were conducted in the late-1950s, virtually nothing has been published about its pharmacological properties. The present studies therefore answer longstanding questions about the mechanism of action of ALD-52 and other N^1-substituted LSD derivatives. Nevertheless, clinical trials are required to understand the mechanism of action of these compounds in humans. Given the high probability that additional N^1-substituted LSD derivatives will appear in the future, these results provide important insights into the action of this class of ergoline hallucinogens.

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Table 1. Receptor binding data for lysergamides at cloned human 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} receptors.

		LSD	ALD-52	1P-LSD	1B-LSD
Receptor	Radioligand	K_i (nM) ± SEM[a]	K_i (nM) ± SEM[a]	K_i (nM) ± SEM[a]	K_i (nM) ± SEM[a]
5-HT_{1A}	[^{3}H]8-OH-DPAT	9.5 ± 3.3 (3)	1,054 ± 367 (5)	637 ± 97 (4)	345 ± 118 (3)
5-HT_{2A}	[^{3}H]ketanserin	14.7 ± 4.2 (5)	174 ± 43 (3)	196 ± 19 (5)	87.7 ± 9.4 (3)
5-HT_{2C}	[^{3}H]mesulergine	45.3 ± 16.3 (3)	10.2 ± 3.5 (3)	13.0 ± 1.7 (3)	20.8 ± 3.4 (3)

[a]The number of independent determinations (performed in triplicate) is indicated in parentheses.

Table 2. Receptor binding data for 1B-LSD

Receptor	Species[a]	Radioligand	K_i (nM) ± SEM[b,c]
5-HT_{1B}	Human	[^{3}H]GR125743	1,463 ± 338 (3)
5-HT_{1D}	Human	[^{3}H]GR125743	295 ± 77 (5)
5-HT_{2B}	Human	[^{3}H]LSD	3.5 ± 0.6 (3)
5-HT_{5a}	Human	[^{3}H]LSD	158 ± 42 (3)
5-HT_6	Human	[^{3}H]LSD	607 ± 112 (3)
5-HT_{7A}	Human	[^{3}H]LSD	554 ± 158 (3)
#$_{1A}$	Human	[^{3}H]prazosin	>10,000[c]
#$_{1B}$	Human	[^{3}H]prazosin	>10,000[c]
#$_{1D}$	Human	[^{3}H]prazosin	>10,000[c]
#$_{2A}$	Human	[^{3}H]rauwolscine	537 ± 85 (3)
#$_{2B}$	Human	[^{3}H]rauwolscine	2,656 ± 741 (3)
#$_{2C}$	Human	[^{3}H]rauwolscine	812 ± 267 (3)
$$_1$	Human (heart)	[^{125}I]pindolol	>10,000[c]
$$_2$	Human	[^{3}H]CGP12177	>10,000[c]
$$_3$	Human	[^{3}H]CGP12177	>10,000[c]
D_1	Human	[^{3}H]SCH23390	1,167 ± 175 (3)
D_2	Human	[^{3}H]*N*-methylspiperone	>10,000[c]
D_3	Human	[^{3}H]*N*-methylspiperone	352 ± 64 (3)
D_4	Human	[^{3}H]*N*-methylspiperone	2,271 ± 417 (3)
D_5	Human	[^{3}H]SCH23390	>10,000[c]
H_1	Human	[^{3}H]pyrilamine	>10,000[c]
H_2	Human	[^{3}H]tiotidine	842 ± 201 (4)
H_3	Guinea pig	[^{3}H]#-methylhistamine	>10,000[c]
H_4	Human	[^{3}H]histamine	>10,000[c]

[a]Cloned receptors were used unless noted otherwise.
[b]The number of independent determinations (performed in triplicate) is indicated in parentheses.
[c]Values of >10,000 nM are listed when there was <50% displacement at 10 μM in the primary binding assay.

Table 3. Previously published receptor binding data for LSD.

Receptor	Species[a]	Radioligand	K_i (nM)	Reference
5-HT_{1A}	Human	[^{3}H]8-OH-DPAT	3.0	(Rickli et al. 2015)
	Human	[^{3}H]8-OH-DPAT	1.1	(Nichols et al. 2002)
5-HT_{1B}	Human	[^{3}H]5-HT	12	(Peroutka 1994)
	Rat	[^{3}H]GR125743	3.9	(Nichols et al. 2002)
5-HT_{1D}	Human	[^{3}H]5-HT	2.4	(Peroutka 1994)
5-HT_{2A}	Human	[^{3}H]ketanserin	4.2	(Rickli et al. 2015)
	Rat	[^{3}H]ketanserin	5.2	(Egan et al. 2000)
	Rat	[^{3}H]ketanserin	12	(Leonhardt et al. 1992)
5-HT_{2B}	Human	[^{3}H]LSD	3.7	(Wacker et al. 2013)
5-HT_{2C}	Human	[^{3}H]mesulergine	15	(Rickli et al. 2015)
	Rat	[^{3}H]mesulergine	10	(Egan et al. 1998)
5-HT_{5a}	Rat	[^{3}H]LSD	9.0	(Nichols et al. 2002)
5-HT_6	Human	[^{3}H]LSD	2.4	(Hirst et al. 2003)
	Rat	[^{3}H]LSD	6.9	(Nichols et al. 2002)
5-HT_{7A}	Rat	[^{3}H]LSD	6.6	(Nichols et al. 2002)
α_{1A}	Human	[^{3}H]prazosin	670[b]	(Rickli et al. 2015)
α_{2A}	Human	[^{3}H]rauwolscine	12	(Rickli et al. 2015)
β_1	Rat	[^{125}I]pindolol	140	(Nichols et al. 2002)
β_2	Rat	[^{125}I]pindolol	740	(Nichols et al. 2002)
D_1	Human	[^{3}H]SCH23390	310[b]	(Rickli et al. 2015)
	Rat	[^{3}H]SCH23390	180	(Nichols et al. 2002)
D_2	Human	[^{3}H]spiperone	25	(Rickli et al. 2015)
	Rat	[^{3}H]*N*-methylspiperone	120	(Nichols et al. 2002)
D_3	Human	[^{3}H]spiperone	96	(Rickli et al. 2015)
	Rat	[^{3}H]*N*-methylspiperone	27	(Nichols et al. 2002)
D_4	Rat	[^{3}H]*N*-methylspiperone	56	(Nichols et al. 2002)
D_5	Rat	[^{3}H]SCH23390	340	(Nichols et al. 2002)
H_1	Human	[^{3}H]pyrilamine	1,100[b]	(Rickli et al. 2015)
	Rat (brain)	[^{3}H]pyrilamine	1,540	(Nichols et al. 2002)

[a]Cloned receptors were used unless noted otherwise.

[b]Data were originally reported in μM; trailing zeroes were added to facilitate conversion to nM.

Table 4. Functional activity of ALD-52, 1P-LSD and 1B-LSD at 5-HT_2 receptor subtypes.

	ALD-52		**1P-LSD**		**1B-LSD**		**LSD**		**5-HT**	
	EC_{50}, nM (pEC_{50} ± SEM)	**E_{max} % 5-HT**	**EC_{50}, nM (pEC_{50} ± SEM)**	**E_{max} % 5-HT**	**EC_{50}, nM (pEC_{50} ± SEM)**	**E_{max} % 5-HT**	**EC_{50}, nM (pEC_{50} ± SEM)**	**E_{max} % 5-HT**	**EC_{50}, nM (pEC_{50} ± SEM)**	**E_{max} % 5-HT**
h5-HT_{2A}	38 (7.45 ± 0.07)	25 ± 2	11 (7.99 ± 0.21)	11 ± 1	85 (7.07 ± 0.14)	23 ± 2	1.52 (8.82 ± 0.05)	94 ± 1	0.38 (9.42 ± 0.05)	100
m5-HT_{2A}	12 (7.94 ± 0.04)	29 ± 1	28 (7.55 ± 0.08)	23 ± 1	45 (7.35 ± 0.07)	35 ± 2	4.25 (8.37 ± 0.10)	86 ± 3	0.54 (9.27 ± 0.02)	100
h5-HT_{2B}	*N.A.*	*N.A.*	*N.A.*	*N.A.*	*N.A.*	*N.A.*	15.3 (7.81 ± 0.07)	78 ± 2	0.53 (9.27 ± 0.05)	100
h5-HT_{2C}	*N.A.*	*N.A.*	*N.A.*	*N.A.*	*N.A.*	*N.A.*	35.1 (7.46 ± 0.11)	82 ± 3	0.25 (9.60 ± 0.06)	100

Data represent the mean and SEM from between three and five independent experiments performed in triplicate. E_{max} is defined as percent 5-HT maximum response. *N.A.*, No Activity

Table 5. Summary of head twitch response (HTR) data for LSD and 1-acyl-substituted derivatives.

Drug[a]	Dose (mg/kg)	*N*	HTR Counts (mean ± SEM)	ED_{50} μg/kg (95% CI)	ED_{50} nmol/kg (95% CI)	Molar potency (relative to LSD)
LSD[b]				52.9 (38.9–72.0)	132.8 (97.6–180.7)	1
1-Acetyl-LSD	0	5	6.4 ± 1.3	130.9 (93.9–182.3)	297.2 (213.2–413.9)	0.45
	0.03	5	8.4 ± 1.6			
	0.1	5	12.6 ± 1.1			
	0.3	5	25.4 ± 3.2 *			
1-Propanoyl-LSD[c]				158.9 (65.0–388.8)	349.6 (143.0–855.4)	0.38
1-Butanoyl-LSD[d]				457.6 (334.9–625.5)	976.7 (714.8–1335)	0.14

*p<0.01 vs. vehicle control group (Tukey's test)
[a]All of the compounds were hemitartrate (2:1) salts
[b]Data previously reported by Halberstadt and Geyer (2013)
[c]Data previously reported by Brandt et al. (2016)
[d]Data previously reported by Brandt et al. (2019).

Table 6. Plasma concentration of LSD in rats treated with ALD-52 and 1P-LSD.

	Treatment Drug	
Dose	ALD-52 (LSD concentration, mean±SEM)	1P-LSD (LSD concentration, mean±SEM)
0.1 mg/kg	35.5±7.7 ng/mL	36.2±7.2 ng/mL
0.3 mg/kg	103.8±9.7 ng/mL	98.8±17.3 ng/mL

Plasma samples were collected from male Sprague-Dawley rats (n = 5-6/group) 15 min after SC administration of ALD-52 or 1P-LSD.

Table 7. Metabolites of ALD-52 and 1P-LSD that were detected in rats.

Parent drug and metabolites detected	Treatment Drug			
	ALD-52		1P-LSD	
	0.1 mg/kg	0.3 mg/kg	0.1 mg/kg	0.3 mg/kg
ALD-52				
ALD-52	+	+	-	-
N-Deethyl ALD-52	+	+	-	-
N^6-Demethyl ALD-52	+	+	-	-
Hydroxy ALD-52 isomer 1 (indole ring)	-	+	-	-
Hydroxy ALD-52 isomer 2 (ethylamide function)	+	+	-	-
1P-LSD				
1P-LSD	-	-	+	+
N-Deethyl 1P-LSD	-	-	-	+
N^6-Demethyl 1P-LSD	-	-	+	+
N-Deethyl-N^6-demethyl 1P-LSD	-	-	-	+
LSD metabolites				
N-Deethyl LSD (lysergic acid monoethylamide)	+	+	+	+
N^6-Demethyl LSD (nor-LSD)	+	+	+	+
Hydroxy LSD isomer 1 (indole ring)	-	-	+	+
Hydroxy LSD isomer 2 (indole ring)	-	-	+	+
Hydroxy LSD isomer 3 (ethylamide function)	+	+	+	+
Dihydroxy LSD (2-oxo-3-OH-LSD)	+	+	+	+
Hydroxy LSD glucuronide (indole ring)	+	+	-	+

Table S1. Individual data points for the two panels in Figure 3.

Experiment	Drug treatment	Head-twitch counts for the entire 30-min test sessions
1	Vehicle	3, 5, 6, 7, 11
	0.03 mg/kg ALD-52	3, 7, 10, 10, 12
	0.1 mg/kg ALD-52	10, 11, 12, 14, 16
	0.3 mg/kg ALD-52	19, 20, 22, 32, 34
	1 mg/kg ALD-52	13, 15, 16, 19, 20
2	Vehicle + 0.3 mg/kg ALD-52	10, 17, 18, 19, 23
	0.1 mg/kg M100907 + 0.3 mg/kg ALD-52	0, 0, 0, 0, 3

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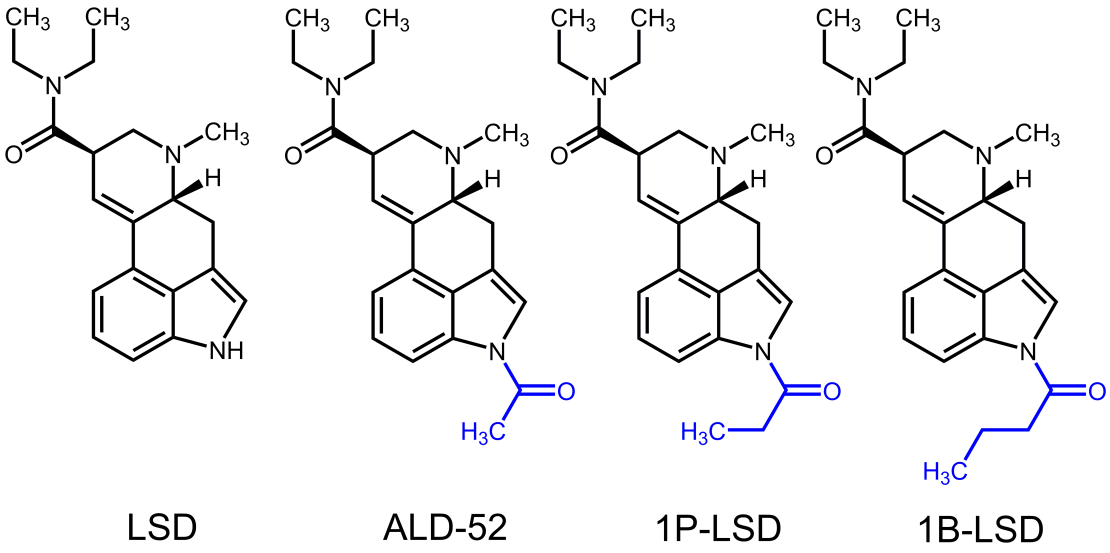

Figure 1. Chemical structures of LSD and N^1-substituted derivatives. Structural changes in comparison to LSD are indicated in blue.

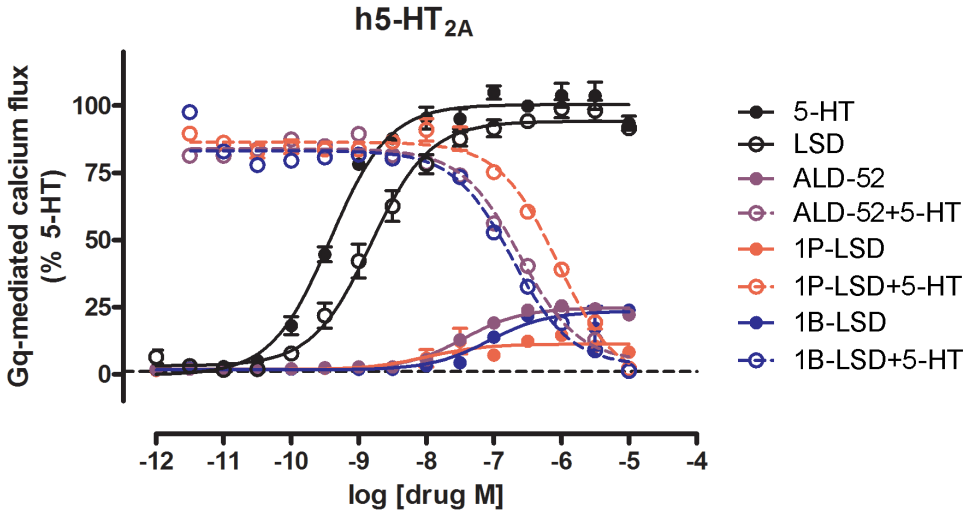

Figure 2. Agonist and antagonist potencies of N^1-substituted LSD derivatives at the human 5-HT_{2A} (h5-HT_{2A}) receptor. Receptor activation was assessed using Ca^{2+} mobilization. Concentration-response curves are shown for 5-HT, LSD, ALD-52, 1P-LSD, and 1B-LSD. Antagonist assays were performed in the presence of 3 nM 5-HT. Values are the mean ± SEM of between three and five independent experiments performed in triplicate.

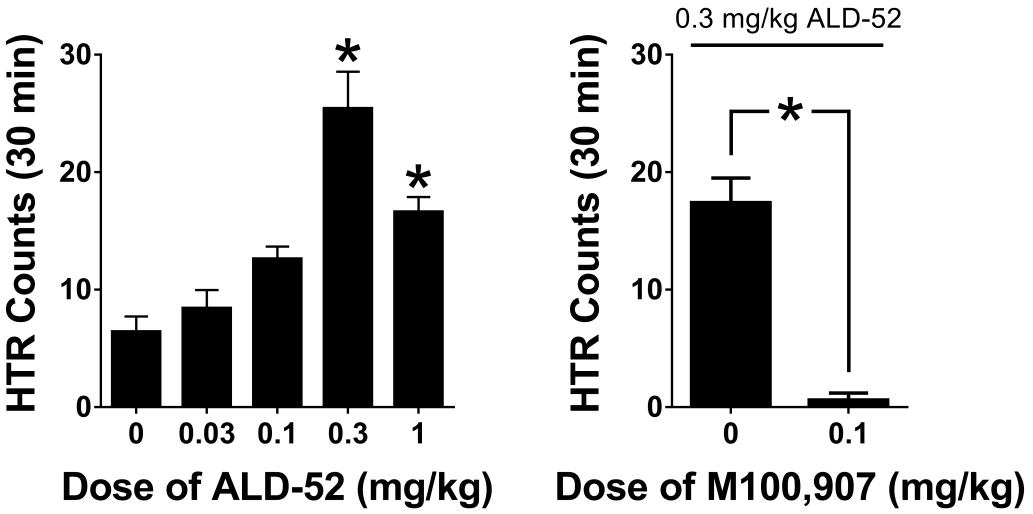

Figure 3. Effect of ALD-52 on the head twitch response (HTR) in male C57BL/6J mice. (*Left panel*) ALD-52 produces a dose-dependent increase in HTR counts. Mice (n = 5/group, 25 total) were treated with ALD-52 and HTR was assessed for 30 min. (*Right panel*) The HTR induced by ALD-52 is blocked by pretreatment with the selective 5-HT_{2A} receptor antagonist M100,907. Mice (n = 5/group, 10 total) were pretreated with vehicle or 0.1 mg/kg M100907 and then treated with 0.3 mg/kg ALD-52. Data are presented as group means ± SEM for the entire 30□min test sessions. The individual data points for the two panels are included in Table S1. *$p < 0.01$, significant difference from the vehicle control group.

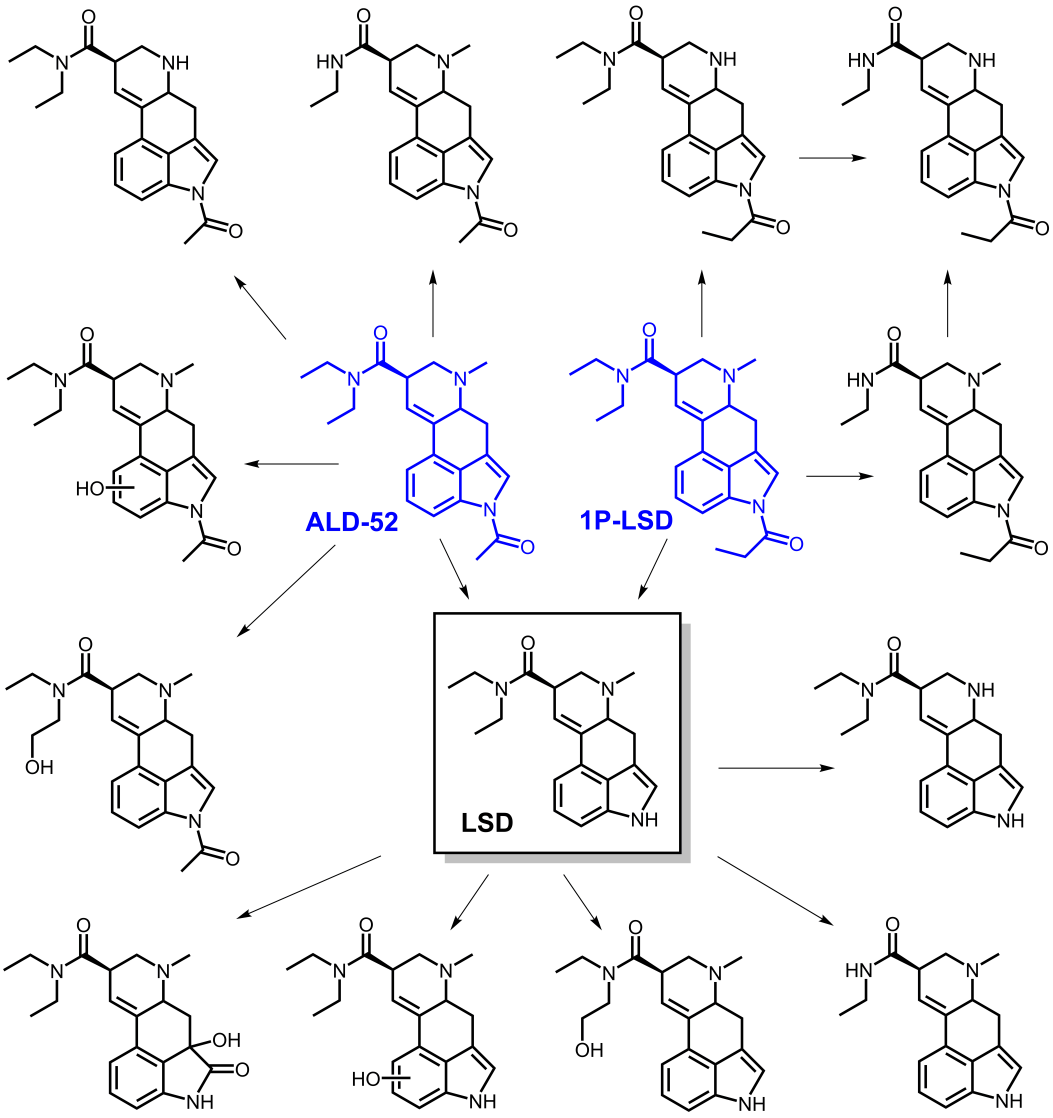

Figure 4. Postulated metabolic pathways for ALD-52 and 1P-LSD after subcutaneous administration to Sprague-Dawley rats. As indicated in the diagram, both ALD-52 and 1P-LSD are metabolized to LSD.

- N^1-Substituted LSD derivatives such as ALD-52 are potent hallucinogens in humans
- N^1-Substitution markedly reduces the efficacy of LSD at the 5-HT_{2A} receptor
- ALD-52 and 1P-LSD are metabolized to LSD in rats
- N^1-Substituted LSD derivatives likely act as prodrugs