JPET Fast Forward. Published on September 28, 2018 as DOI: 10.1124/jpet.118.250530
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Pharmacodynamic effects, pharmacokinetics, and metabolism of the synthetic cannabinoid AM-2201 in male rats

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a) Running Title AM-2201 pharmacodynamics and pharmacokinetics

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c) Manuscript features

Number of text pages: 25

Number of tables: 1

Number of figures: 4

Number of references: 32

Abstract: 249 words

Introduction: 701 words

Discussion: 1,583 words

d) Nonstandard abbreviations

AM-2201, 1-[(5-fluoropentyl)-1H-indol-3-yl]-(naphthalen-1-yl)methanone; AUC, area under the curve; CB_1R, cannabinoid-1 receptor; CB_2R, cannabinoid-2 receptor; C_{max}, maximum concentration; JHW-073, naphthalen-1-yl-(1-butylindol-3-yl)methanone; JWH-018, naphthalen-1-yl-(1-pentylindol-3-yl)methanone; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LOQ, limit of quantification; $t_{1/2}$, elimination half-life; T_{max}, time of maximum concentration time

e) Recommended section

Neuropharmacology

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ABSTRACT

Novel synthetic cannabinoids are appearing in recreational drug markets worldwide. Pharmacological characterization of these new drugs is needed to inform clinicians, toxicologists, and policy makers who monitor public health. [1-(5-Fluoropentyl)-1H-indol-3-yl](1-naphthyl)methanone (AM-2201) is an abused synthetic cannabinoid that was initially created as a research tool for investigating the endocannabinoid system. Here we measured pharmacodynamic effects of AM-2201 in rats, and simultaneously determined plasma pharmacokinetics for the parent drug and its metabolites. Male Sprague-Dawley rats were fitted with surgically-implanted temperature transponders and indwelling jugular catheters under pentobarbital anesthesia. One week later, rats received subcutaneous (sc) injection of AM-2201 (0.1, 0.3, and 1.0 mg/kg) or its vehicle, and serial blood specimens were withdrawn via catheters. Core temperatures and catalepsy were measured just prior to each blood withdrawal, and plasma was assayed for drug and metabolites using liquid chromatography-tandem mass spectrometry (LC-MS/MS). We found that AM-2201 produced dose-related hypothermia and catalepsy that peaked at 2 h and lasted up to 8 h. AM-2201 plasma concentrations rose linearly with increasing dose and ranged from 0.14 – 67.9 µg/L. Concentrations of three metabolites, AM-2201 *N*-(4-hydroxypentyl) (≤0.17 µg/L), naphthalen-1-yl-(1-pentylindol-3-yl)methanone (JWH-018) *N*-(5-hydroxypentyl) (≤1.14 µg/L), and JWH-018 *N*-pentanoic acid (≤0.88 µg/L) were detectable but much lower. Peak AM-2201, JWH-018 *N*-(5-hydroxypentyl), and JWH-018 *N*-pentanoic acid concentrations occurred at 1.3, 2.4 and 6.5 h, respectively. Concentrations of AM-2201, JWH-018 *N*-(5-hydroxypentyl), and JWH-018 *N*-pentanoic acid were negatively correlated with body temperature, but given the low concentrations of metabolites detected, AM-2201 is likely the major contributor to pharmacodynamic effects under our experimental conditions.

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INTRODUCTION

[1-(5-Fluoropentyl)-1H-indol-3-yl](1-naphthyl)methanone (AM-2201) is a synthetic cannabinoid that was first developed in 2000 as a pharmacological tool to study the endocannabinoid system (Figure 1)(Makriyannis and Deng, 2000). AM-2201 is a full agonist at cannabinoid-1 receptors (CB_1R) (Chimalakonda et al., 2012), producing psychoactive effects similar to the phytocannabinoid Δ^9-tetrahydrocannabinol (THC) (Huestis et al., 2001), but with a binding affinity 40 times higher (Makriyannis and Deng, 2000; Chimalakonda et al., 2012). Similarly, the binding affinity of AM-2201 at cannabinoid-2 receptors (CB_2R), responsible for cannabinoid-mediated peripheral effects, is 14 times higher than that of THC (Makriyannis and Deng, 2000). Smoking is the predominant route of AM-2201 consumption, and typical smoked doses of 250 µg to 2 mg are estimated from online drug forums (e.g., https://drugs-forum.com/wiki/AM-2201). By contrast, a 5 mg AM-2201 oral dose was reported as a “comfortable entry dose”, while 10 mg would be “powerful” (https://drugs-forum.com/wiki/AM-2201). These dose differences indicate potential AM-2201 degradation in the stomach, limited gastrointestinal absorption, or extensive gastrointestinal or hepatic metabolism. AM-2201 consumption induces typical cannabimimetic effects such as dry mouth, nausea, drowsiness, confusion, mydriasis, and tachycardia (Holm et al., 2013; Musshoff et al., 2013; Celofiga et al., 2014; Elian and Hackett, 2014; WHO, 2014). At higher doses, psychiatric and autonomic complications, such as extreme anxiety, acute psychosis, hyperemesis, and convulsions are reported (Patton et al., 2013; Celofiga et al., 2014).

Considering the potential risks associated with AM-2201 intake, pharmacodynamic and pharmacokinetic studies are needed to document consumption in clinical and forensic cases. *In vitro* and *in vivo* animal and human experiments identified 6 major AM-2201 metabolites: AM-2201 *N*-(4-hydroxypentyl), AM-2201 6’-hydroxyindole, naphthalen-1-yl-(1-pentylindol-3-yl)methanone (JWH-018) *N*-(5-hydroxypentyl), JWH-018 *N*-pentanoic acid, naphthalen-1-yl-(1-butylindol-3-yl)methanone (JWH-

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073) *N*-(4-hydroxybutyl), and JWH-073 *N*-butanoic acid (Chimalakonda et al., 2012; Sobolevsky et al., 2012; Hutter et al., 2013; Elian and Hackett, 2014; Jang et al., 2014; Kim et al., 2014). AM-2201 and metabolite blood concentrations generally range from <0.1 – 12 μg/L in humans, with JWH-018 *N*-(5-hydroxypentyl) and JWH-018 *N*-pentanoic acid as principal metabolites (See Figure 1.) (Holm et al., 2013; Hutter et al., 2013; Kneisel et al., 2013; Musshoff et al., 2013; Patton et al., 2013; Wikstrom et al., 2013; Yeakel and Logan, 2013; Tuv et al., 2014; WHO, 2014). Importantly, AM-2201 is not detected in human urine. In a pre-clinical study, AM-2201 6'-hydroxyindole and JWH-018 *N*-pentanoic acid were the main AM-2201 metabolites in rat urine after repeated intraperitoneal (ip) injections of high drug doses (15 mg/kg) (Jang et al., 2014). To date, only one AM-2201 pharmacokinetic study is reported in the literature. AM-2201 kinetics were reported from a single case of human oral self-administration (0.07 mg/kg), where AM-2201 and metabolites elimination from blood and urine were followed for up to 267 h post-administration. However, AM-2201 and JWH-018 *N*-(5-hydroxypentyl) kinetic profiles were incomplete as maximum concentrations were not captured (Hutter et al., 2013).

Pharmacodynamic effects of cannabinoids in rodents can be evaluated by standard tests examining physiological parameters and behavioral endpoints. The "cannabinoid tetrad" is a set of tests to identify CB_1R activation that usually includes measurement of hypothermia, catalepsy, reduction in motor activity, and antinociceptive effects (Smith et al., 1994; Ovadia et al., 1995; McGregor et al., 1996; Stein et al., 1996; Rawls et al., 2002; Brents et al., 2011; Brents et al., 2012; Banister et al., 2015a; Banister et al., 2015b). In 2015, the first AM-2201 study in rats demonstrated that ip injection of 0.3, 1.0 and 3.0 mg/kg produced robust dose-related hypothermia (Banister et al., 2015b), and we confirmed these findings using subcutaneous (sc) administration of the drug to rats (Schindler et al., 2017). The metabolites AM-2201 *N*-(4-hydroxypentyl) and JWH-018 *N*-(5-hydroxypentyl) are known to be CB_1R full agonists (Brents et al., 2011; Chimalakonda et al., 2012), and these compounds may contribute to effects of AM-2201 consumption. The relationships between AM-2201 metabolite blood concentrations

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and cannabinoid effects has yet to be determined. Given the scarcity of pharmacological data for AM-2201, we investigated AM-2201 pharmacodynamics and pharmacokinetics after sc injections of AM-2201 (0.1, 0.3 and 1.0 mg/kg) in male rats fitted with temperature transponders and indwelling jugular catheters. We measured body temperature and catalepsy at timed intervals post-injection, while simultaneously obtaining serial blood specimens for analysis of AM-2201 and its metabolites using liquid chromatography with tandem mass spectrometry (LC-MS/MS) (Carlier et al., 2016).

MATERIALS AND METHODS

Chemical and reagents

Analytical standards were purchased from Cayman Chemical (Ann Arbor, MI, USA) and stored at –20°C until use. AM-2201 for administration to rats was provided by the National Institute on Drug Abuse (NIDA) Drug Supply Program (Rockville, MD, USA). LC-MS grade water, methanol and formic acid (Optima™ LC/MS) were obtained from Fisher Scientific (Fair Lawn, NJ, USA). LC-MS grade acetonitrile, HPLC grade *tert*-butyl methyl ether, dimethyl sulfoxide, sodium metabisulfite, and Tween 80® were acquired from Sigma-Aldrich® (St. Louis, MT, USA), while sterile 0.9 % NaCl (saline) was obtained from Hospira, Inc (Lake Forest, IL, USA). Heparin saline (1000 IU/mL) was purchased from Thomas Scientific (Swedesboro, NJ, USA), and sodium pentobarbital was furnished by the NIDA Intramural Research Program (IRP) Pharmacy, Baltimore, MD, USA. Distilled water was produced by an ELGA PURELAB® Ultra Analytic purifier (Siemens Water Technologies, Lowell, MA, USA). BG-100 Red abalone enzyme solution from KURA Biotec (Puerto Varas, Chile) was diluted in distilled water to contain 15,625 units/mL glucuronidase and 1,250 units/mL sulfatase. Ammonium acetate buffer was prepared with ammonium acetate salt (Sigma-Aldrich®; St. Louis, MO, USA) dissolved in distilled water; pH was subsequently adjusted with glacial acetic acid (Fisher Scientific; Fair Lawn, NJ, USA).

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Subjects

Male Sprague-Dawley rats (300 – 400 g) were purchased from Harlan Laboratories (Frederick, MD, USA). Subjects were double-housed under conditions of controlled temperature (22±2ºC) and humidity (45±5%) with *ad libitum* access to food and water. Lights were on between 7:00 AM and 7:00 PM. The NIDA, IRP Animal Care and Use Committee approved the animal experiments, and all procedures were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals. Vivarium facilities were fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. Experiments were designed to minimize the number of animals included in the study.

Surgical procedures

Rats were anesthetized with sodium pentobarbital (60 mg/kg ip), and catheters constructed of Silastic® (Dow Corning; Midland, MI, USA) and vinyl tubing were surgically implanted into the right jugular vein as previously described (Concheiro et al., 2014). Briefly, the proximal Silastic end of the catheter was advanced to the atrium whereas the distal vinyl end was exteriorized on the nape of the neck and plugged with a metal stylet. Immediately after catheter implantation, while still under anesthesia, rats received surgically-implanted temperature transponders (model IPTT-300, Bio Medic Data Systems, Seaford, DE, USA) to allow for the non-invasive measurement of body temperature (Elmore and Baumann, 2018). The temperature transponder emits radio frequency signals that are received by a compatible hand-held reader system (DAS-7006/7r, Bio Medic Data Systems). Transponders are cylindrical in shape, 14 x 2 mm, and were implanted sc along the midline of the back posterior to the shoulder blades via a pre-packaged sterile guide needle delivery system. Rats were single-housed post-operatively and given at least 1 week to recover from surgery.

Blood collection procedures and AM-2201 injections

On the day of an experiment, rats were brought into the laboratory in their home cages and allowed 1 h to acclimate to the surroundings. Polyethylene extension tubes were attached to 1 mL tuberculin syringes, filled with sterile saline, and connected to the vinyl end of the catheters. The extension tubes were threaded outside the cage to facilitate blood sampling by an investigator remote from the animal. Catheters were flushed with 0.3 mL of 48 IU/mL heparin saline to facilitate blood withdrawal. To prepare drug solutions, each mg of AM-2201 was diluted into 50 µL dimethyl sulfoxide and 50 µL of Tween 80®, then sonicated for 1 min to dissolve. To this solution, 900 µL of sterile saline was added to yield a 1 mg/mL stock solution of AM-2201. Aliquots of stock solution were diluted with vehicle consisting of dimethyl sulfoxide:Tween 80®:saline 1:1:18 (*v/v/v*) to yield drug concentrations of 0.3 and 0.1 mg/mL. Groups of 5 rats received sc injection of vehicle (control), 0.1, 0.3, or 1.0 mg/kg AM-2201 on the lower back between the hips. The sc route of administration was chosen because our previous studies used this route when examining the effects of AM-2201 and other cannabinoids in rats (see Elmore and Baumann, 2018; Schindler et al., 2017). Additionally, similar to the smoked route of administration, the sc route largely bypasses first pass metabolism in the liver. Rats were randomly assigned to each dose group. Blood specimens (300 µL) were withdrawn via catheters immediately before (t_0) and at 0.25, 0.5, 1, 2, 4, 8 and 24 h following sc injection. Specimens were collected into 1 mL tuberculin syringes, then transferred to 1.5 mL plastic tubes containing 5 µL of 250 mM sodium metabisulfite as a preservative and 5 µL of 1,000 IU/mL heparin as an anticoagulant. Blood was centrifuged at 1000g for 10 min at 4ºC. Plasma was decanted into cryovials and stored at – 80°C until analysis. After each blood withdrawal, an equal volume of saline solution was infused via the intravenous catheter to maintain volume and osmotic homeostasis. Rats were free to move around the cage during the sampling procedure.

Measurement of catalepsy and body temperature

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Catalepsy scores and body temperature were determined at each blood withdrawal. Rat behaviors were observed by an experienced rater for 1 min just prior to measurement of body temperature via a handheld reader sensitive to signals emitted by the surgically-implanted transponder. The behavioral rater was blind to treatment conditions. On each test day, one investigator prepared drug solutions and administered the drug to rats, whereas another investigator performed the behavioral scoring without knowing the dose administered to each subject. During the 1 min observation period, catalepsy behaviors were scored, as previously described, based on three overt symptoms: immobility, flattened body posture, and splayed limbs (Elmore and Baumann, 2018). Each symptom was scored as 1=absent, 2=present, or 3=continuous or intense, at each time point. For each rat, catalepsy scores at each time point were summed, yielding a minimum score of 3 and a maximum score of 9. Blood samples were withdrawn immediately after temperature recording.

AM-2201 and metabolites quantification

Plasma specimens were assayed using a fully validated analytical method capable of detecting and quantifying AM-2201 and 13 of its metabolites, AM-2201 *N*-(4-hydroxypentyl), AM-2201 6'-hydroxyindole, AM-2201 7'-hydroxyindole, JWH-018 *N*-(2-hydroxypentyl), JWH-018 *N*-(3-hydroxypentyl), JWH-018 *N*-(4-hydroxypentyl), JWH-018 *N*-(5-hydroxypentyl), JWH-018 *N*-pentanoic acid, JWH-018 *N*-propanoic acid, JWH-073 *N*-(2-hydroxybutyl), JWH-073 *N*-(3-hydroxybutyl), JWH-073 *N*-(4-hydroxybutyl), and JWH-073 *N*-butanoic acid (Carlier et al., 2016). Briefly, 75 µL aliquots were hydrolyzed in 400 mM ammonium acetate buffer, pH 4.0, with β-glucuronidase and sulfatase enzymes. Samples were diluted in acetonitrile and ammonium acetate buffer then poured onto supported liquid extraction cartridges (1 mL ISOLUTE® SLE+ cartridges from Biotage®; Charlotte, NC, USA). Analytes were eluted with *tert*-butyl methyl ether, and solvent was evaporated under a nitrogen stream. Residues were reconstituted in mobile phase before injection onto the chromatographic system.

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Analysis was performed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) with a Shimadzu system consisting of an LC-30AD HPLC coupled to a LC-MS-8050 mass spectrometer (Shimadzu Corp; Columbia, MD, USA). MS transitions were monitored as follows (quantification transition in bold): **360 > 127** and 360 > 155 for AM-2201; **376 > 127.1** and 376 > 155.1 for AM-2201 *N*-(4-hydroxypentyl); **376 > 127** and 376 > 155 for AM-2201 6'-hydroxyindole; **376 > 127** and 376 > 155 for AM-2201 7'-hydroxyindole; **358 > 155** and 358 > 127 for JWH-018 *N*-(2-hydroxypentyl); **358 > 155** and 358 > 127 for JWH-018 *N*-(3-hydroxypentyl); **358 > 155** and 358 > 127 for JWH-018 *N*-(4-hydroxypentyl); **358 > 155** and 358 > 127 for JWH-018 *N*-(5-hydroxypentyl); **372 > 155** and 372 > 127 for JWH-018 *N*-pentanoic acid; **344 > 155** and 344 > 127 for JWH-018 *N*-propanoic acid; **344 > 155** and 344 > 127 for JWH-073 *N*-(2-hydroxybutyl); **344 > 127** and 344 > 155 for JWH-073 *N*-(3-hydroxybutyl); **344 > 127** and 344 > 155 for JWH-073 *N*-(4-hydroxybutyl); and **358 > 155** and 358 > 127 for JWH-073 *N*-butanoic acid. Data were processed with ASCENT software from Indigo BioAutomation (Indianapolis, IN, USA). Gradient elution was performed at 700 µL/min on a Raptor™ LC biphenyl column (Restek®; Bellefonte, PA, USA) with mobile phase A) 0.1% formic acid in water and B) 0.1% formic acid in methanol:acetonitrile 50:50 (*v/v*). Two multiple reaction monitoring (MRM) mass transitions were monitored for each analyte and internal standard.

Each sample batch was accompanied by 7 calibrators, a blank, a blank fortified with internal standards, and low, medium, and high quality controls (see Carlier et al., 2016). Lowest limits of quantification (LOQ) were 0.1 µg/L for AM-2201 *N*-(4-hydroxypentyl), JWH-018 *N*-propanoic acid and JWH-073 *N*-(2-hydroxybutyl), and 0.05 µg/L for other analytes. Analyte recoveries and matrix effects were 58.4 – 84.4% and -62.1 to -15.6%, respectively (n = 10). Inter-assay bias and imprecision were 88.8 – 110.1% and 0.3 – 11.9% coefficients of variation, respectively (n= 10).

Data analysis and statistics

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Pharmacodynamic and pharmacokinetic findings were statistically evaluated with GraphPad Prism version 7.04 (GraphPad Software; La Jolla, CA, USA). Body temperature and catalepsy data were evaluated with two-way analysis of variance (ANOVA) (dose x time) followed by Bonferroni post-hoc tests. Pharmacokinetic data were analyzed using WinNonlin version 5.2 (Pharsight; Mountain View, CA, USA) to calculate pharmacokinetic constants including maximum concentration (C_{max}), time of maximum concentration (T_{max}), area under the curve ($AUC_{(0\text{-}24\ h)}$), and elimination half-life ($t_{1/2}$) for each analyte. We examined AUC from 0 to 24 h post-injection for AM-2201 and its metabolites. AUC is the integral of the time-concentration profile for a given analyte and represents the total analyte exposure over time. The pharmacokinetic constants were subjected to one-way ANOVA (dose) followed by Bonferroni post-hoc tests to determine differences between dose groups. At least three data points on the terminal elimination phase were required for $t_{1/2}$ determination. Relationships between analyte plasma concentrations and body temperature were assessed using a Pearson's correlation analysis. $p < 0.05$ was employed as the minimum statistical significance threshold for all comparisons.

RESULTS

Pharmacodynamic effects

The time-course effects for body temperature and catalepsy after sc AM-2201 (0.1, 0.3, and 1.0 mg/kg) are depicted in Figure 2. Temperature was significantly affected by AM-2201 dose ($F_{3,16}$=13.96, $p<0.0001$) and time ($F_{7,112}$=65.00, $p<0.0001$), with a significant dose x time interaction ($F_{21,112}$=14.18, $p<0.0001$). Specifically, AM-2201 produced a dose-related reduction in body temperature compared to vehicle control that lasted up to 8 h post-injection. The maximum temperature drop occurred at 2 h post-injection for all doses, with 1.4, 2.0, and 4.0ºC decreases at 0.1, 0.3, and 1.0 mg/kg, respectively. After the 0.1 mg/kg dose of AM-2201, temperature decreased significantly compared to control only at 2 h post-injection, whereas

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after the 1.0 mg/kg dose, temperature decreased for 8 h but returned to baseline by 24 h. Catalepsy scores were significantly affected by AM-2201 dose ($F_{3,16}$=119.30, p<0.0001) and time after drug administration ($F_{7,112}$ = 32.47, p<0.0001), with a significant dose x time interaction ($F_{21,112}$=8.36, p<0.0001). AM-2201 induced a dose-related increase in catalepsy scores that lasted up to 4 h post-injection. The maximum increase in catalepsy occurred at 1 h post-injection for all doses. After 0.1 mg/kg AM-2201, catalepsy increased above control levels only at the 1 h time point, while after the 1.0 mg/kg dose, catalepsy increased at all time points for 4 h post-injection.

AM-2201 and metabolite pharmacokinetics

The time-concentration profiles for AM-2201 and its metabolites are depicted in Figure 3. It is noteworthy that the assay method we employed for this study was designed to detect and quantify AM-2201 and 13 of its identified metabolites (Carlier et al., 2016). Nevertheless, only 3 metabolites - JWH-018 *N*-(5-hydroxypentyl), JWH-018 *N*-pentanoic acid, and AM-2201 *N*-(4-hydroxypentyl)- were detected in rat plasma specimens from our studies. Furthermore, AM-2201 *N*-(4-hydroxypentyl) was detected in only five samples at 2, 4, and 8 h following administration of 0.3 and 1.0 mg/kg AM-2201, respectively. Given the small number of AM-2201 *N*-(4-hydroxypentyl) positive specimens, this metabolite was excluded from pharmacokinetic statistical analysis. Pharmacokinetic constants are reported in Table 1. Plasma time-concentration profiles for AM-2201 were significantly affected by dose ($F_{2,11}$=15.67, p<0.001) and time ($F_{6,66}$=12.69, p<0.0001), with concentrations rising linearly as dose increased. AM-2201 concentrations after 0.3 mg/kg were significantly greater than those after 0.1 mg/kg at 0.5 and 1 h post-injection, whereas concentrations after 1.0 mg/kg were greater than those after 0.1 mg/kg for 8 h post-injection. AM-2201 was still detectable 24 h after injection of 1.0 mg/kg, with a value of 3.22 ± 0.40 µg/L. AM-2201 C_{max} was significantly altered by the dose administered ($F_{2,11}$=6.39, p<0.01), as was AUC ($F_{2,11}$=34.84, p<0.001). Post-hoc tests revealed that AM-2201 C_{max} and AUC values after 1.0 mg/kg were

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significantly greater than those observed for the 0.3 and 0.1 mg/kg doses. AM-2201 T_{max} was achieved at ~1.3 h and not affected by dose administered ($F_{2,11}$=0.03, ns). By contrast, AM-2201 $t_{1/2}$ was influenced by dose ($F_{2,11}$=5,54, p<0.02), such that half-life after 1.0 mg/kg was significantly greater than that observed after the 0.1 mg/kg dose.

JWH-018 *N*-(5-hydroxypentyl) plasma time-concentration profiles were significantly affected by dose ($F_{2,11}$=67.29, p<0.0001) and time ($F_{6,66}$=48.53, p<0.0001), with concentrations rising as dose increased (Figure 3). JWH-018 *N*-(5-hydroxypentyl) concentrations after 0.3 mg/kg AM-2201 were significantly greater than those after 0.1 mg/kg at 1, 2, and 4 h post-injection whereas, concentrations after 1.0 mg/kg were greater than those after 0.1 mg/kg for all time points up to 8 h post-injection. JWH-018 *N*-(5-hydroxypentyl) was measurable 24 h after 1.0 mg/kg AM-2201, but concentrations were close to the limits of detection. JWH-018 *N*-(5-hydroxypentyl) C_{max} was significantly augmented as dose increased ($F_{2,11}$=51.57, p<0.001) and AUC was influenced in a similar manner ($F_{2,11}$=104.90, p<0.001). Post-hoc tests revealed that *N*-(5-hydroxypentyl) C_{max} and AUC values after 1.0 mg/kg were significantly above those observed for 0.1 and 0.3 mg/kg. JWH-018 *N*-(5-hydroxypentyl) T_{max} occurred at ~2 h and was not affected by dose administered ($F_{2,11}$=1.63, ns), while $t_{1/2}$ for this metabolite could not be determined due to lack of data from the descending limb of the elimination curve. JWH-018 *N*-pentanoic acid concentration-time profiles were significantly affected by dose ($F_{2,11}$=39.42, p<0.0001) and time ($F_{6,66}$=73.86, p<0.0001), with concentrations rising as dose increased. JWH-018 *N*-pentanoic acid concentrations after 0.3 mg/kg AM-2201 were significantly greater than those after 0.1 mg/kg at 2, 4, and 8 h post-injection, whereas concentrations after 1.0 mg/kg were greater than those after 0.1 mg/kg for all time points up to 8 h post-injection. JWH-018 *N*-pentanoic acid was detectable 24 h after 1.0 mg/kg AM-2201, with a concentration of 0.31±0.06 µg/L. JWH-018 *N*-pentanoic acid C_{max} was significantly greater as dose increased ($F_{2,11}$=27.54, p<0.001) as was AUC ($F_{2,11}$=147.02, p<0.001). JWH-018 *N*-pentanoic

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acid T_{max} was delayed compared to JWH-018 *N*-(5-hydroxypentyl) and was not affected by dose ($F_{2,11}$=1.99, ns), and $t_{1/2}$ for this metabolite could not be determined.

Correlation analyses

Because we measured pharmacodynamic and pharmacokinetic endpoints from the same rats, we were able to examine relationships between temperature and analyte concentrations in plasma. The correlation findings are depicted in Figure 4. Body temperature was negatively correlated to AM-2201 (Pearson's r = -0.608, p<0.0001), JWH-018 *N*-(5-hydroxypentyl) (Pearson's r = -0.778, P <0.0001), and JWH-018 *N*-pentanoic acid (Pearson's r = -0.379, P <0.001) plasma concentrations.

DISCUSSION

AM-2201 is an example of a synthetic compound that was initially developed as a pharmacological tool to investigate the endocannabinoid system but was subsequently diverted for recreational use in humans. The compound was one of the most common synthetic cannabinoids found in plant-based "spice" products available in the clandestine market during 2011-2013 in the United States (https://www.deadiversion.usdoj.gov/nflis/spec_rpt_CathCan_2013.pdf). Here we report pharmacodynamic effects of AM-2201 in rats, along with the first plasma pharmacokinetic profiles for the parent compound and its metabolites *in vivo*. Our study has three main findings. First, AM-2201 produced robust dose-dependent hypothermia and catalepsy in rats, consistent with the effects of the compound reported in the literature (Banister et al, 2015b; Schindler et al., 2017). Second, only 3 of the previously identified metabolites of AM-2201 were found in plasma after sc administration of the drug to rats, where JWH-018 *N*-(5-hydroxypentyl) and JWH-018 *N*-pentanoic acid were the major compounds detected. Finally, the plasma concentrations of AM-2201 metabolites were far below those of the parent

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compound, indicating metabolites are unlikely to contribute to the pharmacodynamic effects of the drug under the conditions of the present study.

Consistent with our temperature data, Banister et al. (2015b) demonstrated that ip injection of AM-2201 causes transient hypothermia in rats fitted with indwelling biotelemetry transmitters. In their study, ip administration of 0.3, 1.0, and 3.0 mg/kg AM-2201 decreased temperature 1-1.5ºC, with a maximum effect at 1 h post-injection. A second less pronounced temperature drop occurred between 4 and 5 h after administration (≤0.5ºC). The authors hypothesized that the second phase of AM-2201 hypothermia might be due to combined effects of the parent compound and its bioactive metabolites (Banister et al., 2015b). In our study, sc administration of AM-2201 to rats produced a dose-dependent drop in body temperature that reached maximum at 2 h post-injection, without any indication of a secondary delayed decrease. The sc dose of 1.0 mg/kg AM-2201 induced a hypothermic response that reached 4ºC, which is more robust than the effects observed after an equivalent ip dose in rats (see Banister et al., 2015b). The present findings agree with our previous results in rats where sc injections of AM-2201 (Schindler et al., 2017) or JWH-018 (Elmore and Baumann, 2018) induce hypothermia that is maximal after 1-2 h and slowly returns to normal in a monophasic manner.

The absence of two-stage hypothermia in our study might be related to the sc route of administration which would be expected to minimize AM-2201 metabolism when compared to the ip route. We found that plasma concentrations of AM-2201, JWH-018 *N*-(5-hydroxypentyl), and JWH-018 *N*-pentanoic acid exhibited significant negative correlations with body temperature, suggesting a role for these compounds in the observed hypothermic effects. AM-2201 and JWH-018 *N*-(5-hydroxypentyl) are both potent and efficacious CB_1R agonists (Makriyannis and Deng, 2000; Brents et al., 2011; Chimalakonda et al., 2012), whereas JWH-018 *N*-pentanoic acid is inactive (Brents et al., 2011). However, the low concentrations of JWH-018 *N*-(5-hydroxypentyl) found in the present study demonstrate this metabolite is unlikely to contribute to pharmacodynamic effects of sc administered AM-

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2201. It is noteworthy that human users normally self-administer AM-2201 by smoking or vaping, but we were unable to employ either of these routes. The sc route was chosen because our previous studies used this route to characterize the pharmacological effects of AM-2201 and other cannabinoids in rats (see Elmore and Baumann, 2018; Schindler et al., 2017). Similar to the smoked and vaped routes of administration, the sc route largely bypasses first pass metabolism in the liver. Nevertheless, our use of the sc route limits the clinical relevance of our findings. Future studies in rodents should examine pharmacodynamic effects and pharmacokinetics of AM-2201, and other synthetic cannabinoids, after smoked or vaporized routes of administration (see Lefever et al., 2017).

Our behavioral findings show for the first time that AM-2201 induces immobility, flattened body posture, and splayed limbs, consistent with a profound cataleptic effect lasting for 4 h post-injection. These findings are similar to the robust catalepsy observed after administration of JWH-018 to rats (Elmore and Baumann, 2018). In a similar manner, JWH-018 and JWH-073 induce catalepsy and locomotor suppression in mice, with maximum effects at 1 h post-administration (3 and 10 mg/kg ip) (Brents et al., 2011; Brents et al., 2012). Taken together, the available findings agree that synthetic cannabinoids like AM-2201 and JWH-018 induce catalepsy in both rats and mice.

We present the first plasma pharmacokinetic profiles for AM-2201 and its metabolites in rats. Importantly, only 3 of the 13 metabolites of AM-2201 previously identified *in vitro* were detected: AM-2201 *N*-(4-hydroxypentyl), JWH-018 *N*-(5-hydroxypentyl), and JWH-018 *N*-pentanoic acid. The analytical method we employed can detect and quantify 10 other metabolites with limits of detection ranging from 0.025 to 0.100 µg/L, but none were detected. Our findings are consistent with most *in vivo* experiments that identified AM-2201 *N*-(4-hydroxypentyl), JWH-018 *N*-(5-hydroxypentyl), and JWH-018 *N*-pentanoic acid as major metabolites of AM-2201 in serum, urine, and hair samples of rats and humans (Chimalakonda et al., 2012; Sobolevsky et al., 2012; Hutter et al., 2013; Elian and Hackett, 2014; Jang et al., 2014; Kim et al., 2014). AM-2201 plasma concentrations and AUC values increased in

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proportion to the dose of AM-2201 administered, indicating linear kinetics, at least for the doses tested here. Linearity and $t_{1/2}$ values could not be determined for the AM-2201 metabolites because too few data points on the terminal elimination curve were included for accurate measurement. We found that AM-2201 $t_{1/2}$ after 1.0 mg/kg was significantly longer than $t_{1/2}$ after 0.1 mg/kg, suggesting reduced clearance of the drug at high doses. Further studies are warranted to determine the precise $t_{1/2}$ for AM-2201 metabolites in rats and other species.

In 2013, Hutter et al. reported the first AM-2201 pharmacokinetic investigation in humans, based on a single case of oral consumption of the drug (5 mg, 0.07 mg/kg) (Hutter et al., 2013). In their study, AM-2201 serum concentrations decreased from 0.6 to <0.02 μg/L (LOQ) during the 1.5 to 125 h post-ingestion. Only 4 AM-2201 metabolites were detected in serum, including JWH-018 *N*-pentanoic acid, JWH-018 *N*-(5-hydroxypentyl), AM-2201 6'-hydroxyindole, and AM-2201 *N*-(4-hydroxypentyl). Importantly, serum concentrations of JWH-018 *N*-pentanoic acid exceeded that of AM-2201 concentrations in all samples. JWH-018 *N*-(5-hydroxypentyl) and JWH-018 *N*-pentanoic acid maximum concentrations occurred at 1.5 and 4.1 h post-administration, respectively. JWH-018 *N*-pentanoic acid was detectable up to 57 h post-ingestion (≥0.05 μg/L). In our rat study, AM-2201 was the major analyte in all plasma specimens after sc AM-2201 injection, while JWH-018 *N*-(5-hydroxypentyl) and JWH-018 *N*-pentanoic acid were found at >10-fold lower concentrations. AM-2201, JWH-018 *N*-(5-hydroxypentyl), and JWH-018 *N*-pentanoic acid T_{max} values were 1.3, 2.4, and 6.5 h, respectively, confirming early AM-2201 hydroxylation followed by later carboxylation. The high AM-2201 concentrations we measured in rats, when compared to those in the Hutter study, are likely the result of inter-species or route of administration differences. In particular, Hutter et al. employed oral administration of AM-2201 where gastrointestinal and hepatic metabolism would be substantial, whereas we employed the sc route which largely bypasses such metabolism.

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AM-2201 blood concentrations in authentic human cases of AM-2201 exposure generally did not exceed 5 µg/L (Holm et al., 2013; Hutter et al., 2013; Kneisel et al., 2013; Musshoff et al., 2013; Wikstrom et al., 2013; Yeakel and Logan, 2013; Tuv et al., 2014; WHO, 2014). In 2013, a man died from a self-inflicted stab wound to the neck following psychiatric complications related to AM-2201 intake; the postmortem AM-2201 blood concentration from this victim was 12 µg/L (Patton et al., 2013). In our rat study, AM-2201 plasma C_{max} was 8 µg/L after a 0.1 mg/kg dose, which is in the range of blood concentrations reported in human users. AM-2201 plasma C_{max} reached 42 µg/L after a 1.0 mg/kg dose, with an average concentration >20 µg/L during the 8 h following AM-2201 injection; all animals fully recovered. In 2014, Jang et al. reported that 3 daily ip injections of 15 mg/kg AM-2201 to rats led to formation of the metabolites AM-2201 *N*-(4-hydroxypentyl), AM-2201 6'-hydroxyindole, JWH-018 *N*-(5-hydroxypentyl), JWH-018 *N*-pentanoic acid, and JWH-073 *N*-butanoic acid in urine. We failed to detect AM-2201 6'-hydroxyindole or JWH-073 *N*-butanoic acid in rat plasma, which could indicate rapid elimination of these metabolites into urine after formation. On the other hand, Jang et al. administered repeated injections of a much higher dose of AM-2201 than we administered here. Since we observed sustained hypothermia and catalepsy after 1.0 mg/kg AM-2201 (see Figure 2), along with high circulating concentrations of the drug, it seems that a 15 mg/kg dose of AM-2201 in rats is not relevant to doses ingested by humans.

In summary, we report the first pharmacokinetic profiles for AM-2201 and its metabolites following sc administration of the drug to rats. JWH-018 *N*-(5-hydroxypentyl) and JWH-018 *N*-pentanoic acid were the main metabolites detected, in accordance with previous preclinical, clinical, and forensic studies. In contrast to the single human case of AM-2201 ingestion, we found that AM-2201 plasma concentrations in rats were much higher than those of its metabolites in all samples, possibly due to interspecies differences or route of administration differences. Importantly, the AM-2201 plasma concentrations and AUC values rose linearly with increasing dose, indicating simple linear kinetics, at

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least for the doses tested here. Plasma concentrations of AM-2201 and its metabolites were significantly correlated with hypothermia, but the extraordinarily low concentrations of JWH-018 *N*-(5-hydroxypentyl) and JWH-018 *N*-pentanoic acid indicate these compounds do not contribute to cannabimimetic effects under our experimental conditions. Because of the paucity of human data with synthetic cannabinoid compounds, future animal studies should compare the effects of dose and route of administration on the pharmacodynamics and pharmacokinetics of these compounds as they emerge in the recreational drug marketplace.

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ACKNOWLEDGMENTS

The authors would like to acknowledge Andrew Peabody and Matt Lambing of Indigo BioAutomation (Indianapolis, IN, USA) for providing access to ASCENT software and assistance processing plasma data, and Shimadzu Corporation (Columbia, MD, USA) for providing LC-MS/MS instrumentation via a NIH Materials Transfer Agreement.

JPET Fast Forward. Published on September 28, 2018 as DOI: 10.1124/jpet.118.250530
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AUTHORSHIP CONTRIBUTION

Participated in research design: Baumann, Wohlfarth, Scheidweiler, and Huestis.

Conducted experiments: Carlier, Baumann, Salmeron, and Wohlfarth.

Performed data analysis: Carlier, Baumann and Scheidweiler.

Wrote or contributed to the writing of the manuscript: Carlier, Baumann, Salmeron, Wohlfarth, Scheidweiler, and Huestis.

JPET Fast Forward. Published on September 28, 2018 as DOI: 10.1124/jpet.118.250530
This article has not been copyedited and formatted. The final version may differ from this version.

REFERENCES

Aung MM, Griffin G, Huffman JW, Wu M, Keel C, Yang B, Showalter VM, Abood ME and Martin BR (2000) Influence of the N-1 alkyl chain length of cannabimimetic indoles upon CB(1) and CB(2) receptor binding. *Drug Alcohol Depend* **60**:133-140.

Banister SD, Moir M, Stuart J, Kevin RC, Wood KE, Longworth M, Wilkinson SM, Beinat C, Buchanan AS, Glass M, Connor M, McGregor IS and Kassiou M (2015a) Pharmacology of Indole and Indazole Synthetic Cannabinoid Designer Drugs AB-FUBINACA, ADB-FUBINACA, AB-PINACA, ADB-PINACA, 5F-AB-PINACA, 5F-ADB-PINACA, ADBICA, and 5F-ADBICA. *ACS Chem Neurosci* **6**:1546-1559.

Banister SD, Stuart J, Kevin RC, Edington A, Longworth M, Wilkinson SM, Beinat C, Buchanan AS, Hibbs DE, Glass M, Connor M, McGregor IS and Kassiou M (2015b) Effects of bioisosteric fluorine in synthetic cannabinoid designer drugs JWH-018, AM-2201, UR-144, XLR-11, PB-22, 5F-PB-22, APICA, and STS-135. *ACS Chem Neurosci* **6**:1445-1458.

Brents LK, Gallus-Zawada A, Radominska-Pandya A, Vasiljevik T, Prisinzano TE, Fantegrossi WE, Moran JH and Prather PL (2012) Monohydroxylated metabolites of the K2 synthetic cannabinoid JWH-073 retain intermediate to high cannabinoid 1 receptor (CB1R) affinity and exhibit neutral antagonist to partial agonist activity. *Biochem Pharmacol* **83**:952-961.

Brents LK, Reichard EE, Zimmerman SM, Moran JH, Fantegrossi WE and Prather PL (2011) Phase I Hydroxylated Metabolites of the K2 Synthetic Cannabinoid JWH-018 Retain In Vitro and In Vivo Cannabinoid 1 Receptor Affinity and Activity. *PLoS One* **6**:e21917.

Carlier J, Scheidweiler KB, Wohlfarth A, Salmeron BD, Baumann MH, Huestis MA (2016) Quantification of [1-(5-fluoropentyl)-1H-indol-3-yl](naphthalene-1-yl)methanone (AM-2201) and 13 metabolites in human and rat plasma by liquid chromatography-tandem mass spectrometry. *J Chromatogr A* **1451**:97-106.

JPET Fast Forward. Published on September 28, 2018 as DOI: 10.1124/jpet.118.250530
This article has not been copyedited and formatted. The final version may differ from this version.

Celofiga A, Koprivsek J and Klavz J (2014) Use of synthetic cannabinoids in patients with psychotic disorders: case series. *J Dual Diagn* **10**:168-173.

Chimalakonda KC, Seely KA, Bratton SM, Brents LK, Moran CL, Endres GW, James LP, Hollenberg PF, Prather PL, Radominska-Pandya A and Moran JH (2012) Cytochrome P450-mediated Oxidative Metabolism of Abused Synthetic Cannabinoids Found in "K2/Spice": Identification of Novel Cannabinoid Receptor Ligands. *Drug Metab Dispos* **40**:2174-2184.

Concheiro M, Baumann MH, Scheidweiler KB, Rothman RB, Marrone GF, Huestis MA (2014) Nonlinear pharmacokinetics of (+/-)3,4-methylenedioxymethamphetamine (MDMA) and its pharmacodynamic consequences in the rat. *Drug Metab Dispos* **42**:119-125.

Elian AA and Hackett J (2014) Analysis of AM-2201 and metabolites in a drugs and driving case. *Drug Test Anal* **6**:389-395.

Elmore JS and Baumann MH (2018) Repeated exposure to the "Spice" cannabinoid JWH-018 induces tolerance and enhances responsiveness to 5-HT1A receptor stimulation in male rats. *Front Psychiatry* **9**:55.

Holm NB, Pineda RS, Andersen DW, Rasmussen BS, Dalsgaard PW, Hoegberg LCG, Johansen SS and Linnet K (2013) Screening of Danish traffic cases for synthetic cannabinoids in whole blood by LC-MS/MS. *Scand J Forensic Sci* **19**:45-51.

Huestis MA, Gorelick DA, Heishman SJ, Preston KL, Nelson RA, Moolchan ET and Frank RA (2001) Blockade of effects of smoked marijuana by the CB1-selective cannabinoid receptor antagonist SR141716. *Arch Gen Psychiatry* **58**:322-330.

Hutter M, Moosmann B, Kneisel S and Auwarter V (2013) Characteristics of the designer drug and synthetic cannabinoid receptor agonist AM-2201 regarding its chemistry and metabolism. *J Mass Spectrom : JMS* **48**:885-894.

JPET Fast Forward. Published on September 28, 2018 as DOI: 10.1124/jpet.118.250530
This article has not been copyedited and formatted. The final version may differ from this version.

Jang M, Shin I, Yang W, Shin I, Choi H, Chang H and Kim E (2014) Determination of AM-2201 metabolites in urine and comparison with JWH-018 abuse. *Int J Legal Med* **128**:285-294.

Kim J, Park Y, Park M, Kim E, Yang W, Baeck S, Lee S and Han S (2014) Simultaneous determination of five naphthoylindole-based synthetic cannabinoids and metabolites and their deposition in human and rat hair. *J Pharmaceutical Biomed Anal* **102C**:162-175.

Kneisel S, Speck M, Moosmann B, Corneillie TM, Butlin NG and Auwarter V (2013) LC/ESI-MS/MS method for quantification of 28 synthetic cannabinoids in neat oral fluid and its application to preliminary studies on their detection windows. *Anal Bioanal Chem* **405**:4691-4706.

Lefever TW, Marusich JA, Thomas BF, Barrus DG, Peiper NC, Kevin RC, Wiley JL (2017) Vaping synthetic cannabinoids: a novel preclinical model of e-cigarette use in mice. *Subst Abuse* **11**:1178221817701739.

Makriyannis A and Deng H (2000) Cannabimimetic Indole Derivatives, p 16, USA.

McGregor IS, Issakidis CN and Prior G (1996) Aversive effects of the synthetic cannabinoid CP 55,940 in rats. *Pharmacol Bioch Behav* **53**:657-664.

Musshoff F, Madea B, Kernbach-Wighton G, Bicker W, Kneisel S, Hutter M and Auwarter V (2013) Driving under the influence of synthetic cannabinoids ("Spice"): a case series. *Int J Legal Med* **128**:56-64.

Ovadia H, Wohlman A, Mechoulam R and Weidenfeld J (1995) Characterization of the hypothermic effect of the synthetic cannabinoid HU-210 in the rat. Relation to the adrenergic system and endogenous pyrogens. *Neuropharmacol* **34**:175-180.

Patton AL, Chimalakonda KC, Moran CL, McCain KR, Radominska-Pandya A, James LP, Kokes C and Moran JH (2013) K2 Toxicity: Fatal Case of Psychiatric Complications Following AM2201 Exposure. *J Forensic Sci* **58**:1676-1680.

JPET Fast Forward. Published on September 28, 2018 as DOI: 10.1124/jpet.118.250530
This article has not been copyedited and formatted. The final version may differ from this version.

Rawls SM, Cabassa J, Geller EB and Adler MW (2002) CB1 receptors in the preoptic anterior hypothalamus regulate WIN 55212-2 [(4,5-dihydro-2-methyl-4(4-morpholinylmethyl)-1-(1-naphthalenyl-carbonyl)-6H-pyrr olo[3,2,1ij]quinolin-6-one]-induced hypothermia. *J Pharmacol Exp Ther* **301**:963-968.

Schindler CW, Gramling BR, Justinova Z, Thorndike EB, Baumann MH (2017) Synthetic cannabinoids found in "spice" products alter body temperature and cardiovascular parameters in conscious male rats. *Drug Alcohol Depend* 179:387-394.

Seely KA, Brents LK, Radominska-Pandya A, Endres GW, Keyes GS, Moran JH and Prather PL (2012) A major glucuronidated metabolite of JWH-018 is a neutral antagonist at CB1 receptors. *Chem Res Toxicol* **25**:825-827.

Smith PB, Compton DR, Welch SP, Razdan RK, Mechoulam R and Martin BR (1994) The pharmacological activity of anandamide, a putative endogenous cannabinoid, in mice. *J Pharmacol Exp Ther* **270**:219-227.

Sobolevsky T, Prasolov I and Rodchenkov G (2012) Detection of urinary metabolites of AM-2201 and UR-144, two novel synthetic cannabinoids. *Drug Test Anal* **4**:745-753.

Stein EA, Fuller SA, Edgemond WS and Campbell WB (1996) Physiological and behavioural effects of the endogenous cannabinoid, arachidonylethanolamide (anandamide), in the rat. *Br J Pharmacol* **119**:107-114.

Tuv S, Krabseth H, Karinen R, Olsen K, Oiestad E and Vindenes V (2014) Prevalence of synthetic cannabinoids in blood samples from Norwegian drivers suspected of impaired driving during a seven weeks period. *Accid Anal Prev* **62**:26-31.

WHO (2014) AM-2201 Critical Review Report, Agenda item 4.7, pp 1-30, World Health Organization.

Wikstrom M, Thelander G, Dahlgren M and Kronstrand R (2013) An accidental fatal intoxication with methoxetamine. *J Anal Toxicol* **37**:43-46.

JPET Fast Forward. Published on September 28, 2018 as DOI: 10.1124/jpet.118.250530
This article has not been copyedited and formatted. The final version may differ from this version.

Yeakel JK and Logan BK (2013) Blood synthetic cannabinoid concentrations in cases of suspected impaired driving. *J Anal Toxicol* **37**:547-551.

JPET Fast Forward. Published on September 28, 2018 as DOI: 10.1124/jpet.118.250530
This article has not been copyedited and formatted. The final version may differ from this version.

FOOTNOTES

a) This study was supported by the Intramural Research Program of the National Institute on Drug Abuse (NIDA), National Institutes of Health (NIH) grant DA00523.

b) Partial results of this study were presented during the 51st meeting of the European Societies of Toxicology (Eurotox) in 2015 in Porto, Portugal.

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FIGURES LEGENDS

Figure 1. Chemical structures of AM-2201 and its major metabolites.

Figure 2. Dose-response effects of sc administered AM-2201 on body temperatures and catalepsy scores in male rats. Rats fitted with indwelling jugular catheters and temperature transponders received sc vehicle (1.0 mL/kg) or 0.1, 0.3, or 1.0 mg/kg AM-2201 at time zero. Temperatures were recorded and behaviors were scored just prior to blood sampling at 0.25, 0.5, 1, 2, 4, 8 and 24 h following injection. Values are expressed as mean±SEM for N=4-5 rats/group. Filled symbols indicate significant differences compared to vehicle at a given time point, $p<0.05$; unfilled symbols indicate no significant differences.

Figure 3. Time-concentration profiles for AM-2201, JWH-018 *N*-(5-hydroxypentyl), JWH-018 *N*-pentanoic acid, and AM-2201 N-(4-hydroxypentyl) in rats receiving sc administered AM-2201. Rats fitted with indwelling jugular catheters and temperature transponders received sc 0.1, 0.3, or 1.0 mg/kg AM-2201 at time zero. Blood samples were withdrawn via the catheters at 0.25, 0.5, 1, 2, 4, 8 and 24 h following injection and plasma specimens were assayed for analytes using LC-MS/MS. Data are for mean±SEM for N=4-5 rats/group. Filled symbols indicate significant differences compared to the low dose administered (0.1 mg/kg) at a given time point, $p<0.05$; unfilled symbols indicate no significant differences.

Figure 4. Correlations between plasma concentrations of AM-2201, JWH-018 *N*-(5-hydroxypentyl), and JWH-018 *N*-pentanoic acid versus body temperatures. Raw data from Figures 2 and 3 were used to construct the correlation matrices, such that analyte concentrations are plotted against temperature measures taken at the same time points. Pearson’s r and p values are shown.

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TABLES

Table 1. Pharmacokinetic constants for AM-2201 and its metabolites after sc administration of 0.1, 0.3, or 1.0 mg/kg AM-2201 in male rats

Analyte	AM-2201 Dose (mg/kg)	C_{max} (μg/L)	AUC $_{(0\text{-}24\ h)}$ (h.μg/L)	T_{max} (h)	$t_{1/2}$ (h)
AM-2201	0.1 (N = 5)	8.2 ± 1.7	51 ± 8	1.4 ± 0.2	4.3 ± 0.1
	0.3 (N = 5)	33 ± 8.9	149 ± 13	1.3 ± 0.3	4.6 ± 0.3
	1.0 (N = 4)	42 ± 9*	487 ± 74#	1.3 ± 0.4	6.3 ± 0.8*
JWH-018 *N*-(5-hydroxypentyl)	0.1 (N = 5)	0.17 ± 0.02	0.71 ± 0.11	1.8 ± 0.2	N.D.[a]
	0.3 (N = 5)	0.40 ± 0.04	2.1 ± 0.1	2.0 ± 0.0	N.D.[a]
	1.0 (N = 4)	0.91 ± 0.08#	11 ± 1#	3.5 ± 1.5	N.D.[a]
JWH-018 *N*-pentanoic acid	0.1 (N = 5)	0.12 ± 0.01	0.77 ± 0.09	4.8 ± 1.3	N.D.[a]
	0.3 (N = 5)	0.36 ± 0.02	5.4 ± 0.4*	6.8 ± 1.2	N.D.[a]
	1.0 (N = 4)	0.67 ± 0.10#	12.0 ± 1.5#	8.0 ± 0.8	N.D.[a]

Data are expressed as mean±SEM for N=4-5 rats/group.

[a] N.D. = not determined.

* indicates significant difference compared to 0.1 mg/kg, p<0.05.

# indicates significant difference compared to 0.1 and 0.3 mg/kg, p<0.05.

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Figure 1. Chemical structures of AM-2201 and its major metabolites.

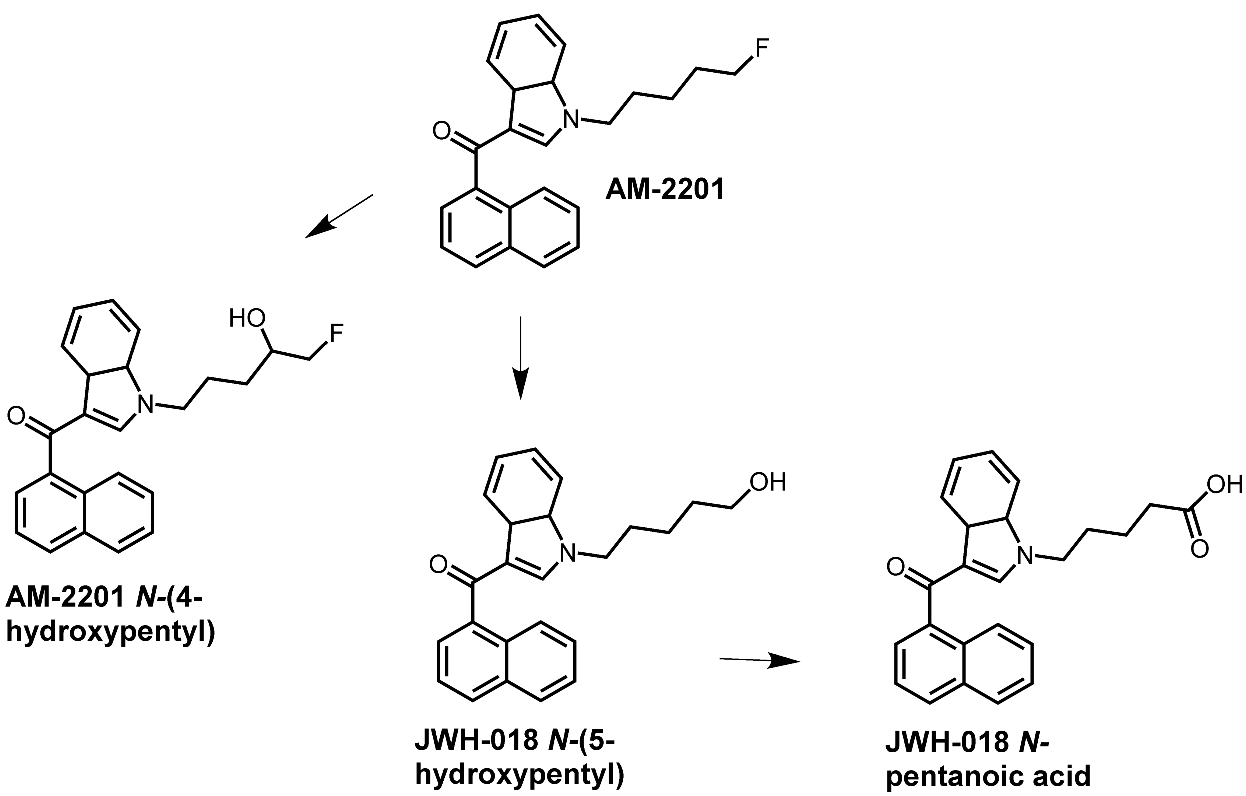

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Figure 2. Dose-response effects of sc administered AM-2201 on body temperatures and catalepsy scores in male rats.

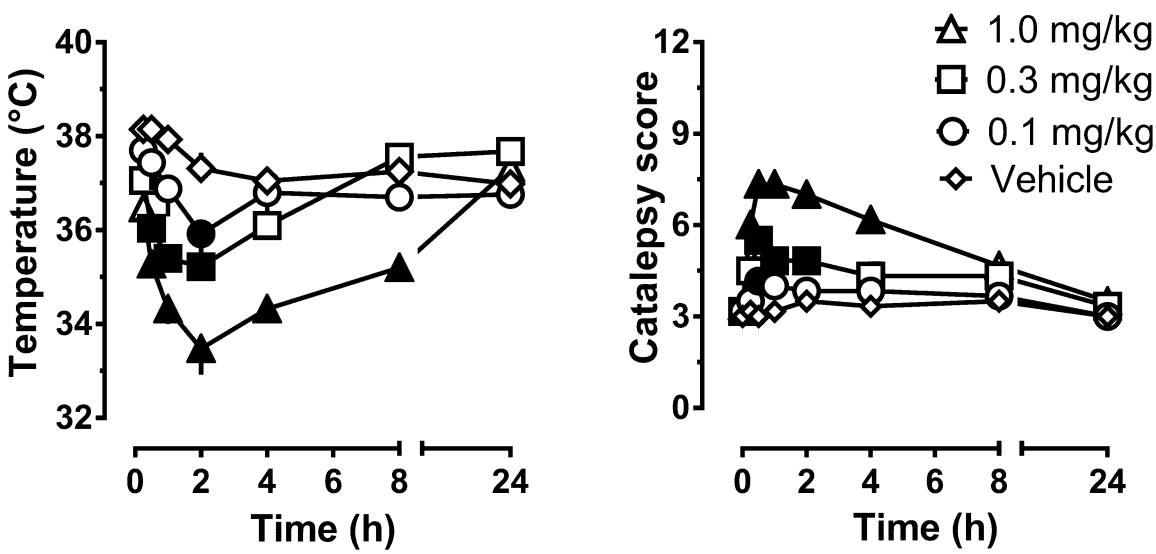

Figure 3. Time-concentration profiles for AM-2201, JWH-018 *N*-(5-hydroxypentyl), JWH-018 *N*-pentanoic acid, and AM-2201 N-(4-hydroxypentyl) in rats receiving sc administered AM-2201.

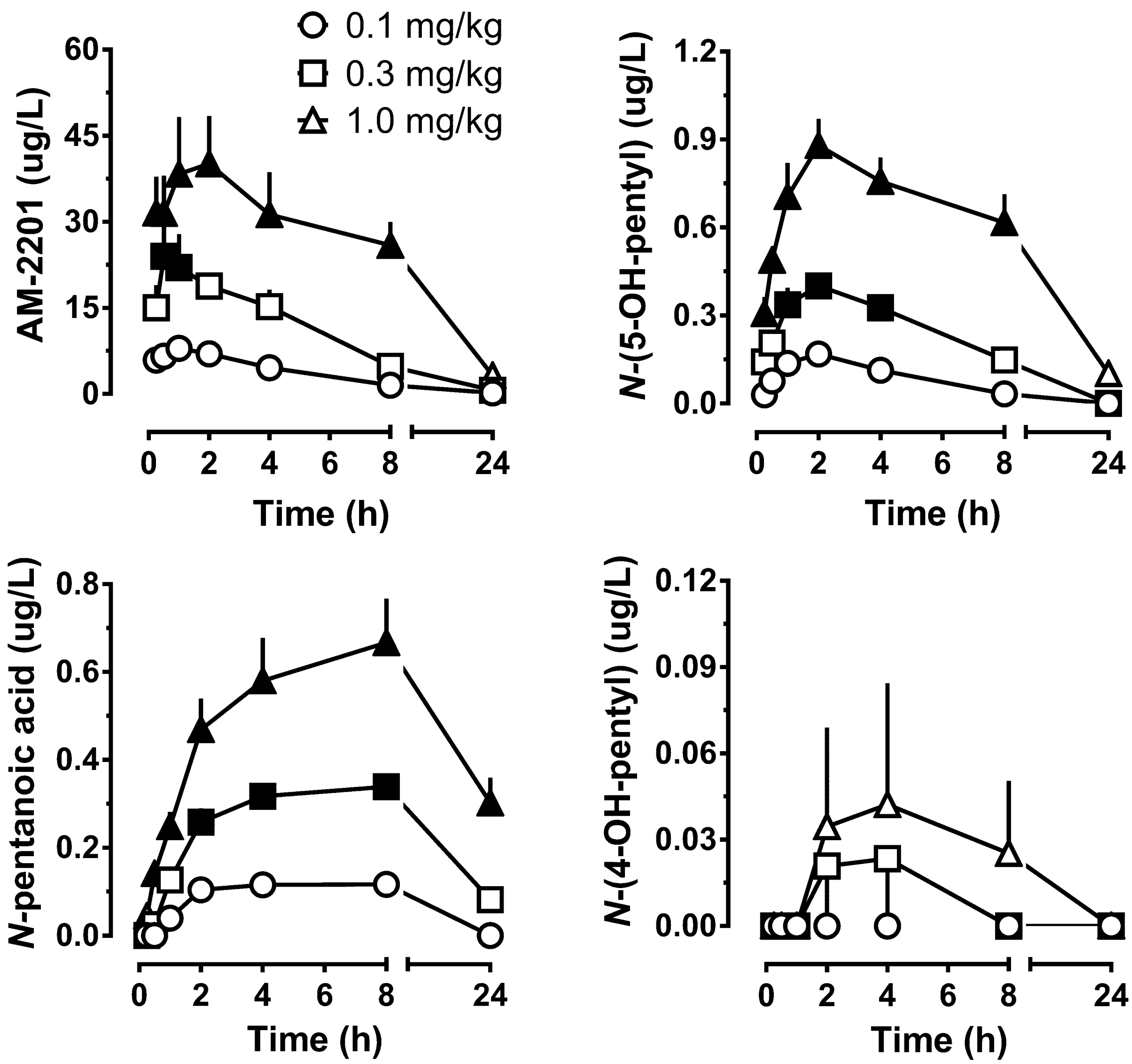

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Figure 4. Correlations between plasma concentrations of AM-2201, JWH-018 *N*-(5-hydroxypentyl), and JWH-018 *N*-pentanoic acid versus body temperatures.