

Rescue from Spice Intoxication: An Investigation in Rats of Agents to Reverse the Intoxicating and Dissociative Effects of Synthetic Cannabinoids



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Trends & Trajectories of Prescription Opioids in the Military Health System

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1.0 SUMMARY

Synthetic cannabinoid (SCB) use has increased recently, particularly among armed services personnel, due to a combination of increased availability and inadequate methods of detecting use. Intoxication with these substances has resulted in adverse events that require medical attention. Acute SCB intoxication can produce effects similar to those reported for cannabis and its primary psychoactive constituent, Δ^9 -tetrahydrocannabinol (THC), including relaxation, euphoria, disinhibition, and elevated heart rate. However, in some cases, SCBs can produce nausea and vomiting, which are only rarely associated with cannabis use, and even then, only in extremely frequent users. Even more troubling, SCBs have been reported to produce elevated blood pressure, which is not associated with cannabis intoxication. The most apparent and disturbing adverse effect of SCB intoxication is acute psychosis. While this can occur after cannabis use as well, acute psychosis produced by SCBs may be longer in duration and more severe. Most distressing are reports of seizures, renal failure, and death following SCB intoxication, which again are not associated with cannabis use. Reversing acute SCB intoxication is thus a clinical challenge that could improve outcomes for civilians and military personnel alike. Current clinical practice in cases of SCB intoxication is to administer antipsychotics and/or benzodiazepines, however the efficacy of these treatments remains unclear. Thus, the objective of this study was to identify clinically available medications that might be beneficial to reverse acute synthetic cannabinoid intoxication across several measures that reflect cannabinoid activity: decreased locomotion, catalepsy, body temperature, and antinociception (tail dip into 50°C water bath), as well as ataxia. In an attempt to capture reported hypertensive and psychotomimetic effects, we also assessed blood pressure and heart rate as well as pre-pulse inhibition of the startle response. We found rimonabant (a specific CB1 receptor antagonist) is able to reverse synthetic cannabinoid effects, however, current medications used to treat SCB intoxication (benzodiazepine, haloperidol) do not alter the course of SCB intoxication, and can exacerbate some SCB effects. No other treatment tested affected synthetic cannabinoid effects. Synthetic cannabinoids modestly reduced heart rate but did not affect blood pressure. Synthetic cannabinoids blunt the startle response, but do not affect prepulse inhibition of the startle response, suggesting that synthetic cannabinoid effects are more profound on sensory response rather than on sensory-motor gating. These results indicate that cannabinoids can diminish reflex arcs, but may not produce psychosis-like effects on their own. Rimonabant, and not diazepam or haloperidol, can reverse this impariment. The implication of this work is that the use of rimonabant (or a similar medication that blocks cannabinoid activity at CB1 receptors) for acute reversal of synthetic cannabinoid intoxiucation should be pursued. This use case would mitigate problems that arose when rimonabant was administered chronically for obesity.

2.0 INTRODUCTION

Rising use has produced a need for medications to reverse the acute effects of synthetic cannabinoids (SCB). Until recently, reversal of acute cannabinoid intoxication received little clinical attention ^{1,2}. This is due, in part, to the fact that until recently, virtually all cannabinoid use occurred by cannabis consumption. The primary psychoactive constituent in cannabis, Δ^9 -tetrahydrocannabinol (THC), exerts is effects via partial agonism of the cannabinoid CB1 receptor ³. While there is some evidence that chronic use of cannabis by vulnerable adolescents may exacerbate psychosis ⁴, acute toxicity is rarely of clinical concern.

However, a relatively new class of synthetic cannabinoids (SCB) has changed this calculation. These drugs, first synthesized by Huffman et al. ⁵, are easy to produce and many remain legal and readily accessible in gas stations or "head" shops. Further, these drugs allow users to evade detection by common urine screens ⁶. These factors have led to increased use among civilians and military personnel ⁷. This increase in use has, in turn, led to an alarming increase in emergency room admissions due to acute intoxication by SCBs ^{7.8}.

Acute intoxication with SCB can be severe. Acute SCB intoxication can produce effects similar to those reported for cannabis, including relaxation, euphoria, disinhibition, and elevated heart rate ⁹. However, in some cases, SCBs can produce nausea and vomiting which are only rarely associated with cannabis intoxication ⁷, most often among chronic cannabis users ¹⁰. Even more troubling, SCBs have been reported to produce elevated blood pressure, which is also not typical of cannabis intoxication ^{7,8}. The most apparent and disturbing adverse effect of SCB intoxication is acute psychosis. While this can occur after cannabis use as well, acute psychosis produced by SCBs may be longer in duration and more severe ⁷. Most distressing are reports of seizures, renal failure, and death following SCB intoxication ¹¹. Reversing acute SCB intoxication is thus a clinical challenge that could improve outcomes among civilians and military personnel alike.

It remains unclear why SCBs appear to produce more severe acute intoxication than THC. Although adulteration is always possible for illicit or non-regulated drugs, our recent research suggests that SCBs obtained through common sources are very pure, which suggests that the adverse effects are due to the pharmacological activity of the SCB rather than to an adulterant ¹². One likely reason for the greater severity of SCB versus cannabis intoxication is that many SCBs act as full agonists at CB1 receptors, in contrast with THC which acts as a partial agonist ^{5,6}. CB1 receptor activation results in several downstream consequences. One of particular interest is glutamatergic hypofunction, apparently due to reduced glutamate release and n-methyl-D-aspartate (NMDA) glutamate receptor inactivation ^{e.g. 13,14}. This is effectively similar to the effects of psychotomimetics such as phencyclidine or ketamine ¹⁵. Such effects could explain some of the most severe effects of intoxication reported by emergency medical personnel ¹⁶.

Antagonists for SCBs are not clinically available. In the case of opioid overdose, emergency medical personnel have a powerful tool available to them: naltrexone. Naltrexone acts as an antagonist at the mu-opioid receptor and can displace the opioid agonist and thus almost instantaneously reverse the life-threatening effects of the opioid ¹⁷. Unfortunately, there is no parallel antagonist available for acute SCB intoxication. Rimonabant, like other selective CB1 antagonists, can reverse many SCB effects ^{3,18,19}. However, this drug was withdrawn from human use due to potentially dangerous mood disturbances ²⁰. These adverse effects were a consequence of chronic, rather than acute administration, nevertheless, the drug is not available for humans.

Other agents may be able to reverse acute SCB effects by action at other pharmacological sites.

In order to reverse SCB intoxication, we must look for alternative agents. Recent studies of the endocannabinoid system provide some possibilities. CB1 agonists can result in glumatergic hypofunction by decreasing glutamate release ¹⁴. Further, CB1 agonists also appear to inactivate NMDA receptors ²¹, which appears to be mediated by sigma-1 receptors ²¹. These studies suggest that CB1 agonists cause glutamatergic hypofunction both by reducing glutamate release and by inactivating NMDA receptors via a sigma-1 mediated mechanism.

Cannabinoid-induced glutamatergic hypofunction may be responsible for the neuroprotective effects of CB1 agoinsts some have reported ²². This activity may also explain why CB1 agonists may exacerbate psychosis among vulnerable adolescents ⁴. Further, the difference in efficacy between THC (partial agonist) and SCBs (full agonists) at CB1 receptors could explain why SCBs appear more dangerous than THC. While THC may decrease glutamatergic function, the extent of this action is likely limited by THC's relatively modest efficacy. In contrast, the full agonist activity of SCB may suppress glutamatergic function to such an extent that severe psychosis and other related symptoms could emerge.

Understanding the relationship between CB1 activation and glutamatergic function helps us understand of the physiological consequences of SCB use. However, it also provides potential targets for reversing acute SCB intoxication. *Increasing glutamate transmission or releasing NMDA receptors from the inhibitory control of CB1 receptors (via sigma-1 agonist administration) could reverse some of the most dangerous consequences of acute SCB intoxication. Importantly, there are agents with these actions already available to clinical care providers.*

Positive modulators of glutamate function are clinically available. While direct-acting NMDA agonists are not clinically available, positive modulators are available. These agents facilitate glutamate activation of NMDA receptors, similar to the way benzodiazepines act to modulate GABA-A receptors. <u>Piracetam</u> acts as a positive NMDA modulator and can increase the density of NMDA receptors on cerebral cell membranes ²³. Piracetam has been in clinical use for more than 30 years of clinical use ²⁴. <u>D-cycloserine</u> is a parital agonist at NMDA receptors and is also clinically available ²⁵. Both of these drugs can enhance NMDA receptor function and might reverse SCB-induced glutamatergic hypofunction.

There are clinically available sigma-1 receptor agonists. Sigma-1 receptor agonists can potentiate NMDA receptor-induced neuronal firing, and this action is blocked by haloperidol which acts as a sigma-1 antagonist ²⁶. Several clinically available drugs act as sigma-1 receptor agonists. Interestingly, these tend to be antidepressants. Imipramine, fluvoxamine, and fluoxetine have each been characterized as sigma-1 agonists, in addition to their more commonly considered activity as serotonergic agonists ²⁷. These agents might reverse aspects of SCB intoxication due to CB1-induced glutamatergic hypofunction by enhancing NMDA receptor activity. While the use of antidepressants to reverse cannabinoid intoxication may seem counter intuitive, there is evidence consistent with this notion. Imipramine tends to decrease the potency of the discriminative stimulus effects of THC in monkeys ²⁸. Fluoxetine has been shown to reverse the hypothermic effects of THC in mice ²⁹. and fluvoxamine has been shown to reverse cognitive deficits produced by the NMDA antagonist phencyclidine in mice ³⁰. Thus, it is plausible that these agents could reverse aspects of SCB intoxication.

Interestingly, sigma-1 receptor antagonists may exacerbate effects of SCBs. There are several clinically available sigma-1 antagonists. These agents include the antipsychotic haloperidol and the

antidepressant sertraline ^{27,31}. These agents might be expected to exacerbate some effects of SCBs by antagonizing sigma-1 receptors, further suppressing or preventing re-activation of NMDA receptors that are under the control of CB1 receptors ¹³.

Haloperidol has been shown to reverse THC-induced prepulse inhibition of startle, a widely used preclinical assay for antipsychotic activity ³². However, haloperidol had little effect on THC-induced locomotor suppression, catalepsy, or hypothermia ³³, and similarly had no effect on cognitive deficits produced by THC ³⁴. Haloperidol tended to potentiate a THC discrimination in monkeys ²⁸, and in humans, haloperidol had no effect or exacerbated behavioral effects of THC ³⁵. Thus, the existing evidence does not support the use of haloperidol (and by extension other antipsychotics with sigma-1 antagonist properties) to reverse acute SCB intoxication.

Medications that enhance glutamatergic function might reverse aspects of acute SCB intoxication. Together this evidence suggests that medications that enhance NMDA receptor signaling, either directly (positive modulators or partial agonists) or indirectly (sigma-1 agonists) might be able to reverse acute SCB intoxication. In contrast, medications that decrease glutamatergic function such as sigma-1 receptor antagonists are unlikely to reverse these effects, and may even exacerbate them. Extending examinations to other sigma-1 agonists and antagonists (including more specific agents that are not yet clinically available) could help guide current clinical practice as well as future drug development.

3.0 METHODS, ASSUMPTIONS, AND PROCEDURES

Tetrad Effects: Cannabinoids have been shown to produce consistent effects across four particular behavioral tests ³⁶. These tests include assays of hypothermia, antinociception, catalepsy, and locomotion. Effects of the SCB alone and in combination with each of the test drugs (and of the test drugs alone as well) will be assessed across these four assays. Previously, we have used this battery of tests to compare THC effects between C57/BL6 and DBA/2 mouse strains.

Locomotion: Locomotion is assessed using a procedure we have previously described. Briefly, we use four $30 \text{ cm} \times 15 \text{ cm} \times 15 \text{ cm}$ customized acrylic boxes with floors of a parallel grid of 2.3 mm stainless steel rods mounted 6.4 mm apart. Each box is enclosed in a commercially-available sound-and light-attenuating chamber. Four infrared light beams are evenly spaced 6 cm apart and located 2 cm from the floor of each box. Light beam disruptions are counted using commercially-available computer software. The floors and inside of the boxes are wiped with a damp sponge and the litter paper beneath the floors was changed between tests with different animals. Spontaneous locomotor activity is assessed over a 10-min period. The number of activity counts for each subject is summed over the 10-min period and dose-effect curves are constructed from the averages for the SCB in combination with each test agent.

Hypothermia: We measure the rectal temperature of mice by inserting a probe 2 cm into the rectum and obtaining a digital recording was obtained from a commercially available thermometer. Average temperatures for subjects in each treatment conditions are used to construct dose-effect curves for the SCB in combination with each test agent.

Antinociception: This procedure involves exposing the tail to an ambient heat source (i.e., water held at 50° C) and recording latency (in s) for the mouse to move the tail out of the water. A 10-s maximal latency is used to avoid damage to the mouse's tail. The latency for mice to move their tail is averaged for each treatment condition and then dose-effect curves for the SCB in combination with each test agent are constructed.

Catalepsy: Catalepsy is assessed with a procedure we have previously used. Briefly, catalepsy is examined using a horizontal cylindrical metal bar (diameter, 1 cm) supported 4 cm above the floor by two 8×8 -cm square pieces of Plexiglas. Mice are tested for catalepsy by placing their forepaws on the bar while the hind paws remain on the floor and then recording the time that both forepaws remained on the bar up to a maximum of 30 s. The latency to remove both paws from the bar is used as the measure for each treatment condition. Results are averaged and used to construct dose-effect curves for the SCB in combination with each test agent.

<u>Ataxia and cardiovascular effects</u>: In addition to the tetrad effects above, we also assess ataxia, heart rate, and blood pressure. We assess ataxia because this assay can detect glutamatergic hypofunction ³⁷. We assess heart rate and blood pressure because these represent other effects of concern to emergency medical personnel in cases of acute SCB intoxication³⁸.

Ataxia: We use a procedure we have previously described to measure ataxia ³⁷. Briefly, we use an inverted screen apparatus which consists of a 13 cm \times 13 cm wire mesh screens located 23 cm above the floor of four Plexiglas containers. Screens are connected to a rod and handle, which can be rotated 180° to invert them. Subjects failing to remain on the inverted wire mesh for at least 60-s were scored as having ataxia. The percentage of subjects rated as having ataxia is the measure for each treatment condition. These percentages are used to construct dose-effect curves for the SCB in combination with each test agent.

Heart rate and blood pressure: We assessed heart rate and blood pressure simultaneously using a commercially available apparatus (CODA monitor, Kent Scientific, Torrington, CT). We recorded heart rate and blood pressure in each mouse after each synthetic cannabinoid dose. Because we did not detect a significant effect on blood pressure, we did not assess effects of synthetic cannabinoids in combination with other agents.

Effects of JWH-018 alone: Each weekday, three flights of mice (4/flight) were tested. After measurement of temperature and baseline tail flick latency, SCB was administered and returned to their home cage for 10-min. Mice were then treated with vehicle, and again returned to their home cages for another 10-min. At this point, mice were removed from their home cage and tail-flick latency was measured. Mice were kept on the benchtop for the remainder of 5-min to ensure each test occurs at the same post-injection time. Mice were then placed into individual activity chambers for spontaneous locomotor activity assessment. Subsequently, rectal temperature was measured. Mice were then be tested for catalepsy, ataxia, and then heart rate and blood pressure. At least one week separated each test in each mouse to prevent tolerance to SCB effects.

Interactions between JWH-018 and test agents: Synthetic cannabinoid was administered and mice were placed in a holding cage alone for 10-min. Mice were then treated with a dose of one of the test agents, and again returned to the holding cage for another 10-min. At this point, mice were removed from their home cage and tail-flick latency, ataxia, and rectal temperature were measured. Mice were placed back into the holding cage for 10-min, and the tests were repeated. This occurred every 10-min for the next 50-min.

Prepulse Inhibition of Startle: Fifteen minutes prior to each test session, each mouse was administered a dose (i.p.) of either JWH-018, JWH-073, or vehicle and placed in a separate cage for ten minutes. Mice were then administered a dose of haloperidol, diazepam, cannabidiol, or rimonabant (per group assignment) or vehicle. Mice were immediately placed in the startle response chamber. This was repeated with at least one week separating each dose combination in each mouse. **Experiment 1: Dose effects on PPI** White noise at 70dB served as background noise throughout the session. After being placed in the chamber, mice were exposed to background noise for 5 min, then trials commenced. Startle trials consisted of a 20ms presentation of background noise, followed 100ms later by a 40ms presentation of whitenoise at 70, 80, 100, or 120 dB. Inhibition trials consisted of a presentation of a 20ms white noise prepulse of 70, 74, 82 or 86 dB, followed 100ms later by a 40ms white noise pulse of 120 dB. Each session consisted of presentation of a 70 dB prepulse and 120dB pulse, and were not used in the analysis. The intertrial interval ranged between ten and twenty seconds, averaging 15 sec. Trials were pseudorandomly arranged with fifteen of each trial type presented per session. Data was recorded at moment of startle tone emission for both trial types.

Experiment 2: Timing effects on PPI Each session began with a five-minute acclimation period of background noise of 70dB. Trials consisted of a 20ms prepulse of either 70 or 86 dB followed by a 40ms 120dB startle pulse, with the timing of the prepulse varying across trials. Depending on the trial, The prepulse preceded the pulse by 3, 10, 30, 100, 300, or 3000 ms.

Analyses: Data for JWH-018 alone or for JWH-018 in combination with each test agent were analyzed using a linear mixed effect model with dose of cannabinoid, dose of test agent, and time as betweensubjects factors. This model was analyzed using ANOVA with kenward-roger contrasts and significance set at alpha=0.05. Ataxia data were converted into binary values (pass/fail) and analyzed using a general linear probit regression and then analyzed using ANOVA with kenward-roger contrasts and significance set at alpha=0.05. Contrasts were applied to determine differences between vehicle alone and other treatment conditions at each time point.

JWH-018 produced the expected effects on rectal temperature, catalepsy, antinociception, and locomotor activity, as well as increasing ataxia on an inverted screen.



Figure 1: Effects of JWH-018 on locomotion (top left), antinociception (bottom left), rectal temperature (bottom right), and catalepsy and ataxia (top right). Each point represents the mean \pm S.E.M. for n=13 mice. Doses of 1 and 3 mg/kg were effective in each assay. Analysis of variance indicated that dose was significant for each measure (F[6,73]=34.3 (Temperature); 25.7 (Antinociception), 14.4 (catalepsy) and 5.2 (locomotion), p<0.05), and that for each measure, doses of 1 and 3 mg/kg produced effects that were significantly different than vehicle after correction for multiple comparisons (p<0.05).

JWH-018 has modest effects on heart rate, and no reliable effect on blood pressure

Effects on systolic, diastolic, and heart rate were not significantly affected across the dose range tested. While this is surprising in light of one report in rats and numerous clinical case reports describing patients presenting with extreme hypertension after self-reported synthetic cannabinoid use ^{38,39}, it is generally consistent with preclinical literature and clinical literature with THC, both of which show a highly transient, if any, effect of cannabinoid agonsts on blood pressure ^{40,41}. This suggests that observation of hypertension among synthetic cannabinoid users might reflect other constituents of the products used recreationally (e.g. stimulants) or a response to the medical emergency itself and the situation experienced in the emergency department.



Figure 2: Cardiovascular effects of JWH-018. Mice were restrained for approximately 15-min while blood pressure and heart rate were repeatedly sampled. The average for each measure during each session was determined and averaged for all mice. Points represent the mean \pm S.E.M for 8-15 mice at each dose.

Rimonabant reverses JWH-018 effects on temperature, analgesia, and ataxia.

JWH-018 or vehicle was administered immediately after the 'Baseline' observations shown in Fig. 3, below. Rimonabant (3.0 mg/kg) or vehicle was administered immediately after the first 10-min observation, as indicated in the figure. JWH-018 (3 mg/kg, open triangles) produces hypothermia, increases tail withdrawal latency from warm water, and ataxia on an inverted screen. Administration of 3 mg/kg rimonabant alone (filled circles) does not affect latency for a mouse to remove its tail from 50°C water (top left), body temperature (top right), or ataxia (falling off of an inverted screen within 30-sec, bottom). Rimonabant reverses the hypothermic, antinociceptive, and ataxic effect of JWH-018 within 10-min.





Haloperidol (an antipsychotic used to treat suspected cases of synthetic cannabinoid intoxication in the Emergency Department), has no effect on JWH-018 induced hypothermia, antinociception, or ataxia

Baseline 10 30 40 50 20 Time since JWH-018 administration (min)

Rimonabant

JWH-018 (3 mg/kg, open triangles) produces hypothermia, increases tail withdrawal latency from warm water, and ataxia on an inverted screen. Administration of 3 mg/kg haloperidol alone (filled circles) does not affect latency for a mouse to remove its tail from 50°C water (top left), or body temperature (top right). There was evidence for a partial reversal of ataxia (falling off of an inverted screen within 30-sec, bottom). Haloperidol does not reverse the hypothermic, antinociceptive, or ataxic effect of JWH-018.

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This suggests that anti-psychotic administration is not beneficial in cases of synthetic cannabinoid-induced psychosis. Instead, recovery appears to continue as it would without haloperidol exposure. This is interesting because it implies a different mechanism than that which is responsible for schizophrenia. Still, the relationship repeatedly reported between cannabinoid use and schizophrenia in vulnerable individuals indicates these mechanism might be complementary.



Diazepam (a benzodiazepine used to treat suspected cases of synthetic cannabinoid intoxication in the Emergency Department), enhanced JWH-018 induced hypothermia, antinociception, and ataxia

This suggests that diazepam and other GABAergic sedatives could be problematic if used or administered in conjunction with synthetic cannabinoids. This appears to be a similar interaction as that between ethanol and cannabinoids, where effects are additive or possibly super-additive. Extreme caution should be used when these drugs are used in combination.



D-cycloserine (a NMDA glutamatergic partial agonist) does not affect JWH-018 behavioral effects.

D-cycloserine (a putative cognitive enhancer that acts as a partial agonist at NMDA receptors) has no effect on JWH-018 induced antinociception (top), hypothermia (middle), or ataxia (bottom). This indicates that reversing downstream inhibition of NMDA function as a consequence of synthetic cannabinoid action is insufficient to reverse acute cannabinoid intoxication.



Piracetam (a NMDA glutamatergic positive allosteric modulator) does not affect JWH-018 behavioral effects.

Figure 7: Piracetam (a putative cognitive enhancer that positively modulates NMDA receptors) has no effect on JWH-018 induced antinociception (top), hypothermia (middle), or ataxia (bottom). Once again, this indicates that reversing downstream inhibition of NMDA function as a consequence of synthetic cannabinoid action is insufficient to reverse acute cannabinoid intoxication.

In addition to these compounds, we have investigated the interactions with JWH-018 effects of the antidepressants fluvoxamine and imipramine, the benzodiazepine inverse agonist flunitrazepam, the sigma -1 antagonists NE-100 and SR1A, and intravenous administration of liposyn (which binds lipid soluble drugs in the blood). None of these agents affected JWH-018 effects on rectal temperature, antinociception, or ataxia.

Prepulse-Inhibition of Startle





Rimonabant reverses the effect of JWH-018 on the startle reflex in response to presentation of a 120dB tone.



Points represent the mean of 8-16 mice. Y-axis represents maximal startle response in mV, and the x-axis are different tone intensities. Effects on the startle response are dose-dependent. Rimonabant dose-dependently reversed the effect, though the reversal was incomplete even at 3 mg/kg.

Prepulse inhibition of startle: Effect of varying prepulse timing

We also examined effects of JWH-018 on inhibition of startle when a 120dB tone was preceded by a 70dB (background noise) or an 86dB tone at different prepulse times. We again found that JWH-018 decreased startle response, but did not affect prepulse inhibition.



Figure 10: Effect of varying prepulse stimulus time (relative to a 120dB startle tone). Symbols show the mean \pm S.E.M. for n=22 mice. Open circles show the effect of the startle tone with no prepulse (70dB tone was always present as background noise). Open triangles show response when the startle tone was preceded by an 86dB at the times indicated. Inhibition of startle was apparent at prepulse times up to 3000 msec. JWH-018 (3 mg/kg, filled symbols) decreased the startle response (circles), but did not affect prepulse inhibition (triangles, which have the same slope, but a lower intercept).

In addition to these studies, we also examined effects of another synthetic cannabinoid (JWH-073) on startle and prepulse inhibition, as well as antagonism of those effects by rimonabant. Results were substantially similar to those obtained with JWH-018. Further, we examined the ability of cannabidiol (1, 1.78, 3 mg/kg) to blunt JWH-018 effects on the startle response as cannabidiol has been reported to reverse some effects of cannabinoid agonists. However, we found no evidence that cannabidiol affected JWH-018 effects on the startle response, though it is possible that higher doses might have exhibited an effect.

5.0 CONCLUSIONS

As expected, JWH-018 produced dose-related effects on the tetrad (locomotor activity, catalepsy, antinociception, and rectal temperature) as well as on ataxia. This pattern is consistent with previously descibed effects of cannabinoid CB1 agonists. Cardiovascular effects were less profound, with modest decreases in heart rate apparent, but not dose-dependent, and no reliable effect on blood pressure. These results are consistent with previous reports of minimal cardiovascular effects of cannabinoids on cardiovascular function.

Rimonabant (a specific CB1 receptor antagonist) is able to reverse synthetic cannabinoid effects. This is not unexpected, as the observed effects of SCB are mediated via CB1 receptor activation. In contrast, current medications used to treat SCB intoxication (diazepam, haloperidol) do not alter the course of SCB intoxication, and can exacerbate some SCB effects. This was somewhat unexpected, as these medications are currently in clinical use in cases of suspected SCB intoxication. Additionally, no other treatment tested affected synthetic cannabinoid effects, despite the proposed rationale that these agents might act as functional antagonists of SCB.

We determined that synthetic cannabinoids blunt the startle response, but do not affect prepulse inhibition of the startle response. This suggests that synthetic cannabinoid effects are more profound on sensory response rather than on sensorymotor gating. These results indicate that cannabinoids can diminish reflex arcs, but may not produce psychosis-like effects on their own. Rimonabant, and not diazepam or haloperidol, can reverse this impariment, consistent with other effects of SCB we observed.

In contrast with expectations, none of the agents tested affected SCB effects on these measures, with the notable exception of rimonabant, the CB1 antagonist. This indicates that reversing downstream effects of CB1 agonist activity is unlikely to provide relief from SCB intoxication. Further, the lack of effect of medications currently in clinical use for SCB intoxication (diazepam, haloperidol) demonstrates the need for effective reversal agents, similar to the use of naltrexone in the case of opioid overdose. The one agent that was extremely effective at reversing SCB intoxication, rimonabant, is no longer approved for human use. Unfortunately, this particular medication was prohibited from human use due to mood disruption among those taking it chronically for obesity. A medication with similar pharmacological action might be useful as a reversal agent, and its use as an acute reversal agent might mitigate concerns that arose from chronic use of rimonabant for obesity.

6.0 **RECOMMENDATIONS**

The finding that among all agents tested, only Rimonabant was able to rescue mice from synthetic cannabinoid intoxication has implications in the treatment of acute synthetic cannabinoid intoxication. Rimonabant had been submitted to the Food and Drug Administration for approval in the United States in 2005. However, upon review by an internal advisory committee, the FDA determined the manufacturer had failed to adequately demonstrate its safety and ultimately the application was withdrawn ⁴². This was largely due to mood disruption reported by people in Europe (where the drug had already been approved) using it chronically, as directed, for obesity. There is little evidence that acute treatment with rimonabant would have the same liability, especially when used to reverse the severely disruptive effects of synthetic cannabinoids. Thus, to the extent that synthetic cannabinoid intoxication remains a public heath threat, the medical community should reconsider approval of rimonabant, or another, similar cannabinoid CB1 receptor antagonist, strictly for acute use in cases of suspected synthetic cannabinoid intoxication.

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