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Synthetic cannabinoids found in "spice" products alter body temperature and cardiovascular parameters in conscious male rats

Charles W. Schindler[a,b], **Benjamin R. Gramling**[a], **Zuzana Justinova**[b], **Eric B. Thorndike**[b], and **Michael H. Baumann**[a]

[a]Designer Drug Research Unit, Intramural Research Program, National Institute on Drug Abuse, Baltimore, MD

[b]Preclinical Pharmacology Section, Intramural Research Program, National Institute on Drug Abuse, Baltimore, MD

Abstract

Background—The misuse of synthetic cannabinoids is a persistent public health concern. Because these drugs target the same cannabinoid receptors as the active ingredient of marijuana, Δ^9-tetrahydrocannabinol (THC), we compared the effects of synthetic cannabinoids and THC on body temperature and cardiovascular parameters.

Methods—Biotelemetry transmitters for the measurement of body temperature or blood pressure (BP) were surgically implanted into separate groups of male rats. THC and the synthetic cannabinoids CP55,940, JWH-018, AM2201 and XLR-11 were injected s.c., and rats were placed into isolation cubicles for 3 h.

Results—THC and synthetic cannabinoids produced dose-related decreases in body temperature that were most prominent in the final 2 h of the session. The rank order of potency was CP55,940 > AM2201 = JWH-018 > THC = XLR-11. The cannabinoid inverse agonist rimonabant antagonized the hypothermic effect of all compounds. Synthetic cannabinoids elevated BP in comparison to vehicle treatment during the first h of the session, while heart rate was unaffected. The rank order of potency for BP increases was similar to that seen for hypothermia. Hypertensive effects of CP55,940 and JWH-018 were not antagonized by rimonabant or the neutral antagonist AM4113. However, the BP responses to both drugs were antagonized by pretreatment with either the ganglionic blocker hexamethonium or the α_1 adrenergic antagonist prazosin.

Correspondence: Charles W. Schindler, Designer Drug Research Unit, NIH/NIDA Intramural Research Program, 251 Bayview Blvd, Suite 200, Baltimore, MD 21224, (443) 740-2520, (443) 740-2933 (fax), cschind@helix.nih.gov.

Contributors

CWS and MHB were responsible for the conception and the design of the study. CWS and BRG wrote the first draft of the paper. CWS and EBT analyzed the data and CWS, ZJ and MHB interpreted the data. ZJ and EBT participated in the training of the animals. CWS, BRG, ZJ, EBT and MHB gave final approval of the article.

Conflicts of Interest

No conflict declared.

Conclusions—Our results show that synthetic cannabinoids produce hypothermia in rats by a mechanism involving cannabinoid receptors, while they increase BP by a mechanism independent of these sites. The hypertensive effect appears to involve central sympathetic outflow.

Keywords

Cannabinoids; THC; body temperature; blood pressure; rat

1. Introduction

Synthetic cannabinoids are new psychoactive substances that have become popular drugs of abuse (Castaneto et al., 2014; Seely et al., 2012). Individuals use these drugs due to curiosity about their effects, the intensity of the subjective experience, and the decreased likelihood of testing positive on urine toxicology screens (Deluca et al., 2012). The compounds exert their effects by acting at cannabinoid receptors, the same sites of action targeted by the active ingredient of marijuana, Δ^9-tetrahydrocannabinol (THC) (Banister et al., 2015; Wiley et al., 2013). The biological actions of most synthetic cannabinoids have not been fully characterized, and the only available information on the effects in human users is either anecdotal, or based on information from poison control centers and emergency room case reports (Spaderna, 2013). Adverse effects reported in human users include anxiety, agitation, hallucinations, tachycardia, hypertension, hyperglycemia, hyperemesis, seizures, dyspnea, and may also include kidney injury, stroke, encephalopathy, and in rare cases death (Cooper, 2016; Schifano et al., 2015). The extent to which the effect profiles of these compounds overlap with THC is a question of great importance to public health.

Data from the Drug Enforcement Administration show the incidence of synthetic cannabinoids in confiscated drug products has increased markedly since 2010 (U.S. Drug Enforcement Administration, 2014). Furthermore, there is a rapid appearance of new synthetic cannabinoids to replace those that are rendered illegal by drug control legislation. By way of example, the naphthoyl indole JWH-018 was the most abundant synthetic cannabinoid in 2011, but this drug was replaced by its fluorinated analog AM2201 after JWH-018 was banned by legislation in 2012. This trend has continued, as the tetramethylcyclopropyl indole XLR-11 became prominent after AM2201 was made illegal in 2013, and XLR-11 remained the most commonly encountered synthetic cannabinoid through 2015 (U.S. Drug Enforcement Administration, 2016).

One of the primary effects of THC in laboratory animals is a decrease in body temperature (Rawls and Benamar, 2011). The hypothermia produced by THC is mediated by cannabinoid-1 (CB_1) receptors (Ledent et al., 1999; Nava et al., 2000) in the central nervous system (CNS) (Schmeling and Hosko, 1977). JWH-018 and related synthetic cannabinoids produce temperature decreases in mice akin to those produced by THC (Brents et al., 2013; Vigolo et al., 2015; Wiley et al., 2102). While the effects of cannabinoids on body temperature are relatively straightforward, their effects on cardiovascular function are more complex (Malinowska et al., 2012; Randal et al., 2004). In anesthetized animals, the endocannabinoid anandamide produces a triphasic effect that consists of an initial bradycardia accompanied by a decrease in blood pressure (BP), which is followed by a

transient period of hypertension. Finally, there is a long lasting third phase consisting of decreased BP. In conscious animals, the delayed hypotensive phase is replaced by a hypertensive phase (Stein et al., 1996). The depressor response seen in anesthetized animals is blocked by pretreatment with cannabinoid antagonists (Lake et al., 1997; Malinowska et al., 2012) while the precise nature of the pressor response seen in conscious animals is not clear (Lake et al., 1997; Gardiner et al., 2002, 2009). Few studies have examined the effects of synthetic cannabinoids on cardiovascular function, but the effects are reportedly similar to THC or the endocannabinoids (Gardiner et al., 2002; Padley et al., 2003). Banister et al. demonstrated that JWH-018, AM2201 and XLR-11 produce bradycardia in conscious rats (Banister et al., 2013; 2015), but no data on BP effects have been reported.

Given the recent rise in toxic exposures to synthetic cannabinoids (Clark et al., 2015; Hermanns-Clausen et al., 2013; Ibrahim et al., 2014; Mir et al., 2011; Shah et al., 2016; Young et al., 2012), it is important to further investigate the physiological effects of these compounds. Here, we used biotelemetry in male Sprague-Dawley rats to examine the effects of THC and the synthetic cannabinoids JWH-018, AM2201, XLR-11, and CP55,940 (a well-established reference cannabinoid agonist used in preclinical experiments) on body temperature and cardiovascular parameters. We further examined whether the observed effects were mediated at cannabinoid receptors by pretreating with the CB_1 inverse agonist rimonabant or the CB_1-selective neutral antagonist AM4113. As the effects of synthetic cannabinoids on BP did not appear to be mediated by cannabinoid receptors, we also employed pretreatment with the ganglionic blocker hexamethonium to determine whether the effects were mediated in the CNS, and the α_1 adrenergic antagonist prazosin to determine the involvement of the sympathetic nervous system.

2. Methods

2.1 Subjects

Nineteen adult male Sprague-Dawley rats (Charles River, Kingston, NY, USA) weighing 300–500 g were used for two separate experiments. They were individually housed in ventilated racks in a temperature and humidity controlled room with a 12 h reverse light/dark cycle (lights off at 7:00 am). All animals used in this study were maintained in facilities fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC). All procedures were approved by the Institutional Care and Use Committee of the NIDA/IRP and were conducted in accordance with the Guide for the Care and Use of Laboratory Animals.

2.2 Surgical Procedures

Model TA-F40 biotelemetry transmitters for the measurement of temperature (Data Sciences International, St. Paul, MN, USA) were surgically implanted into rats (N=6 rats) anesthetized with a combination of 50 mg/kg ketamine and 10 mg/kg xylazine. Following an incision of the abdominal wall, the transmitter was inserted into the abdominal cavity, the muscle was closed with sutures and the skin closed with wound clips. One to 2 weeks later the wound clips were removed. Model PA-C40 (N=6 rats, Data Sciences) or HD-S10 biotelemetry transmitters (N=7 rats, Data Sciences) for the measurement of BP were

implanted by surgeons at Data Sciences in St. Paul, MN. Briefly, the rats were anaesthetized with isoflurane and a midline incision was made in the abdominal wall. The intestines were retracted and the descending aorta isolated. The catheter of the transmitter was then inserted into the descending aorta and glued in place. The transmitter was secured to the abdominal wall and the muscle and skin sutured closed. Following recovery, the rats were shipped to the NIDA/IRP in Baltimore, MD where they underwent a 7-day quarantine.

2.3 Telemetric Measurements

Following recovery from surgery or release from quarantine, rats were adapted to the training procedures. Daily (Monday to Friday) rats were transported to the procedure room where food and water were removed from the home cage and the entire home cage was placed on top of a telemetry receiver (Data Sciences model RPC-1) that was located inside a small acoustical chamber (BRS/LVE, Laurel, MD, USA). Transmitters were only turned on during the experimental sessions. Data was collected for 10 sec every 1 min (DataQuest A.R.T. Gold, Data Sciences) for a total of 3 h. For the BP transmitters, heart rate was derived from the BP signal. Following initial adaptation, the rats were injected with saline (s.c.) 5 min prior to the session on Tuesdays and Fridays until their response following injections habituated and was indistinguishable from non-injection days.

Drug testing began following habituation to the injection procedure. Rats fitted with transmitters for temperature were injected (s.c.) with various doses of THC (0.3 – 3.0 mg/kg), JWH-018 (0.18 – 0.56 mg/kg), AM2201 (0.1 – 0.3 mg/kg), CP55,940 (0.01 – 0.1 mg/kg), XLR-11 (0.1 – 3.0 mg/kg) or their vehicles 5-min prior to being placed in the acoustical chamber. Following dose-effect testing, one dose of each drug was chosen for pretreatment studies with rimonabant. For these studies, rats were pretreated with rimonabant or vehicle 45 min prior to the cannabinoid injection or vehicle, and 5 min later placed in the acoustical chamber. All rats in the temperature studies received all treatments. The rats were approximately 3 to 10 months of age during testing.

Following injection adaptation, all rats fitted with the PA-C40 (N = 6) transmitters for measuring BP were injected (s.c.) with various doses of THC (1.0 – 3.0 mg/kg), JWH-018 (0.3 – 3.0 mg/kg), AM2201 (0.03 – 0.3 mg/kg), CP55,940 (0.03 – 0.3 mg/kg), XLR-11 (0.3 – 3.0 mg/kg) or their vehicles 5-min prior to being placed in the acoustical chamber. Following dose-effect testing, CP55,940 (0.3 mg/kg, 5 min pre-session) was chosen for pretreatment testing with rimonabant (1.0 mg/kg, 45 min prior to CP55,940) and AM4113 (3.0 mg/kg, 30 min prior to CP55,940). A lower dose of CP55,940 was chosen for pretreatment with hexamethonium (10.0 mg/kg, 20 min prior to CP55,940) and prazosin (0.3 mg/kg, 5 min prior to CP55,940). These rats were approximately 3 to 10 months of age during testing. All rats implanted with the HD-S10 (n = 7) transmitters were tested with JWH-018 (1.0 mg/kg, 5 min pre-session) and pretreated with rimonabant, AM4113, hexamethonium and prazosin in a manner identical to pretreatments prior to CP55,940. These rats had been in a prior study investigating the effects of methylenedioxypyrovalerone and its metabolites (Schindler et al., 2016). The effects of JWH-018 in these rats were similar to those rats used in the prior dose-effect experiments for JWH-018. These rats were approximately 7 to 9 months of age during testing.

For all studies, drug testing typically occurred on Tuesdays and Fridays with vehicle testing occurring on Thursdays. The order of drug treatment and dose within each group was non-systematic, although all animals within a group were typically tested with the same drug and dose on any given test day. Effects seen in dose-effect testing were nearly identical those seen in the pretreatment studies for the vehicle + agonist treatment, indicating that no change in the effects of the agonists occurred simply due to the passage of time or repeated testing.

2.4 Drugs

JWH-018 (1-pentyl-3-(1-naphthoyl)indole), AM2201 (1-(5-fluoropentyl)-3-(1-naphthoyl)indole), and XLR-11 (1-(5-fluoropentyl)-1H-indol-3-yl)(2,2,3,3-tetramethylcyclopropyl)methanone) were obtained from the NIDA Drug Supply Program (Rockville, MD, USA). JWH-108, AM2201 and XLR-11were prepared in a vehicle of 5% ethanol, 5% Tween 80 and saline. CP55,940 (-[(1R,2R,5R)-5-hydroxy-2-(3-hydroxypropyl) cyclohexyl]-5-(2-methyloctan-2-yl)phenol) (Tocris Bioscience, Bristol, UK) was prepared in a vehicle of 3% ethanol, 2% Tween 80 and sterile water. THC (NIDA Drug Supply) was prepared in a vehicle of 20% cyclodextrin and saline. Rimonabant (5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-*N*-(piperidin-1-yl)-1*H*-pyrazole-3-carboxamide) (NIDA Drug Supply) was prepared in a vehicle of 2% ethanol, 2% Tween 80 and sterile water. AM4113 (N-piperidin-1-yl-2,4-dichlorophenyl-1H-pyrazole-3-carboxamide analog) was synthesized in the laboratory of Dr. Alexandros Makriyannis (Center for Drug Discovery, Northeastern University, Boston, MA, USA) and prepared in a vehicle of 6% DMSO, 6% Tween 80 and saline. Prazosin (Sigma Chemical, St. Louis, MO, USA) was prepared in sterile water and hexamethonium (Sigma Chemical) was prepared in saline. All drugs were administered s.c. in a volume of 1 ml/kg of body weight.

2.5 Data Analysis

For body temperature, time course data showed there was a delayed onset, with the effects of cannabinoids most prominent in the last 2 h of the session (see results). Therefore, for statistical analysis of temperature, the 1-min time points were averaged over the last 2 h of the session. For BP, time course data showed that hypertensive effects were most robust in the first h of the session following treatment with the cannabinoids. Therefore, for statistical analysis of BP, the 1-min time points were averaged over the first h of the session. The averaged data for temperature and BP were subjected to analysis-of-variance (ANOVA) followed by Tukey's multiple comparison test to determine significant differences between treatment groups (Prism ver 5.0f, Graphpad Software). There were no significant effects of the cannabinoids on heart rate (all p's > 0.07), so those data are not presented.

3. Results

3.1 Time Course of Temperature and Blood Pressure Effects of Synthetic Cannabinoids

Figure 1 shows the effects of the representative synthetic cannabinoids JWH-018 and XLR-11 on body temperature and BP in 10-min means over the full 3 h sessions. The other cannabinoids showed a similar time course. One striking difference between the effects of the drugs on temperature versus BP was the difference in time to peak effect. For body temperature, the various vehicles produced little change in temperature, while the

cannabinoids produced hypothermia that peaked well after the first h following drug administration. Specifically, temperature began to drop 30 min after drug administration and continued to drop for up to 2 h post-injection. By contrast, the most prominent effects of drugs on BP occurred early in the session. BP following vehicle administration dropped in the first h, while BP following the cannabinoids remained elevated. After 1 h to 90 min, BP following vehicle and the cannabinoids was similar. For subsequent analysis of dose-response effects of drugs on body temperature, the temperature responses were averaged over the final 2 h of the session. For analysis of dose-response effects of drugs on BP, the responses were averaged over the first h of the session.

3.2 Dose-effect Functions of Synthetic Cannabinoids on Body Temperature and Blood Pressure

The top panel of Figure 2 depicts the dose-effect function for cannabinoid agonists on body temperature averaged over the last 2 h of the sessions. Each of the drugs produced similar dose-dependent decreases in body temperature that were significantly below levels following vehicle injections (filled symbols, all p's < 0.05, JWH-018 [$F_{4,29} = 10.69$], AM2201 [$F_{3,23} = 8.80$], XLR-11 [$F_{3,23} = 15.3$], CP55,940 [$F_{3,23} = 24.0$], THC [$F_{3,23} = 9.26$]). However, there were clear differences in potency across the various cannabinoids tested. THC and XLR-11 were the least potent of the cannabinoids, while CP55,940 was the most potent. The structurally-related compounds JWH-018 and AM2201 had comparable potency and were intermediate between CP55,940 and THC.

The bottom panel of Figure 2 depicts the dose-effect function for cannabinoid agonists on BP averaged over the first h of the sessions. The synthetic cannabinoids produced similar dose-dependent increases in BP that were significantly higher than that observed following vehicle injections (filled red symbols, all p's < 0.05, JWH-018 [$F_{4,29} = 7.47$], AM2201 [$F_{3,23} = 4.65$], XLR-11 [$F_{3,23} = 10.14$], CP55,940 [$F_{3,23} = 14.64$]). There was a similar trend for THC. Although ANOVA revealed a significant effect of THC on BP ($F_{2,17} = 5.38$), post-hoc tests failed to demonstrate significant effects between doses. The rank order of potency for the various cannabinoids to produce hypertension mimicked that observed for effects on body temperature. THC and XLR-11 were the least potent of the cannabinoids, while CP55,940 was the most potent. JWH-018 and AM2201 had comparable potency at increasing BP and were intermediate between CP55,940 and THC.

3.3 Pretreatment Experiments

As shown in Figure 3, the CB_1 inverse agonist/antagonist rimonabant (1 mg/kg) antagonized the effects of each of the cannabinoid agonists on body temperature. ANOVA results revealed that each of the cannabinoid agonists significantly altered body temperature (all p's < 0.05, JWH-018 [$F_{3,23} = 3.17$], AM2210 [$F_{3,23} = 15.22$], XLR-11 [$F_{3,23} = 46.71$], CP55,940 [$F_{3,23} = 12.95$], THC [$F_{3,23} = 25.35$]). Specifically, for each drug tested, body temperature for the vehicle + agonist group was significantly lower when compared to all other groups. No other drug treatments were significant, except that the rimonabant + THC group was slightly lower than rimonabant + vehicle group ($p < 0.05$), suggesting that rimonabant may have only partially reversed the effect of THC on body temperature.

The effects of CP55,940 and JWH-018 on BP were tested in pretreatment experiments. Figure 4 shows the effects of the various pretreatment drugs on the BP increases following CP55,940 administration. CP55,940 significantly increased BP compared to vehicle + vehicle control groups under all pretreatment conditions (e.g., $F_{3,23} > 10.0$, $p < 0.01$). However, neither the CB_1 inverse agonist/antagonist rimonabant (1.0 mg/kg) nor the CB_1 neutral antagonist AM4113 (3.0 mg/kg) was able to antagonize the effect of CP55,940 on BP. More specifically, CP55,940 (0.3 mg/kg) significantly increased BP following both vehicle and antagonist pretreatment, and neither of those groups differed from each other. It is noteworthy that rimonabant + vehicle also significantly increased BP, complicating interpretation of the results. Unlike the cannabinoid antagonists, the α_1 adrenergic antagonist prazosin (0.3 mg/kg) and the ganglionic blocker hexamethonium (10 mg/kg) reversed the effects of 0.1 mg/kg CP55,940.

Figure 5 shows the effects of the various pretreatment drugs on the BP increases following JWH-018 administration. JWH-018 (1.0 mg/kg) significantly increased BP under all pretreatment conditions when compared to vehicle + vehicle groups (e.g., $F_{3,27} > 19.8$, $p < 0.01$). Similar to the results with CP55,940, neither rimonabant nor AM4113 was able to reverse the hypertension produced by JWH-018. Here, AM4113 + vehicle significantly increased blood pressure, similar to the effects of rimonabant + vehicle in the CP55,490 experiments described above, complicating interpretation. Prazosin antagonized the BP increase produced by JWH-018, though the prazosin + vehicle group demonstrated a slightly decreased BP response. Similarly, hexamethonium reversed the hypertension produced by JWH-018, but hexamethonium + vehicle group had reduced BP compared to the vehicle + vehicle group.

4. Discussion

Based on the rising misuse of synthetic cannabinoids in humans and the associated risk of toxic exposure (Clark et al., 2015; Hermanns-Clausen et al., 2013; Ibrahim et al., 2014; Mir et al., 2011; Shah et al., 2016; Young et al., 2012), there is clearly a need for basic research investigating the physiological effects of these compounds. Although many synthetic cannabinoids induce effects that are similar to THC, their greater potency may lead to an increased propensity for adverse effects when these compounds are misused as substitutes for THC. The findings reported here on body temperature and BP in rats confirm that synthetic cannabinoids abused by humans have effects that are similar to THC, but the synthetic compounds can be much more potent than THC.

There were clear differences in potency across the drugs tested in our study, but THC and synthetic cannabinoids reduced body temperature in a dose-related manner and to the same extent. At room temperature, the observed decreases in body temperature were evident by around 30 min post-injection, but did not reach their nadir until greater than 90 min following injection. The cannabinoid CB_1 inverse agonist rimonabant antagonized the hypothermic responses to all drugs tested. Our body temperature results agree with previous work using THC (e.g., Fennessy and Taylor, 1977; Pertwee and Tavendale, 1977; Taffe et al., 2015) and other synthetic cannabinoids (De Fry et al., 2004; Fan et al., 1994; Ovadia et al., 1995; Rawls et al., 2002; Tai et al., 2015). In particular, the present temperature data in

rats are similar to those reported by Banister et al. (2015) who showed that JWH-018, AM2201 and XLR-11 produce robust hypothermia in conscious rats fitted with biotelemetry transmitters (Banister et al., 2015). Furthermore, the relative potency data that we report here agrees well with the findings of Banister and coworkers. While not directly tested in this study, the decrease in body temperature produced by cannabinoids appears to be mediated in the CNS (Schmeling and Hosko, 1977), most likely in the preoptic anterior hypothalamus (Ovadia et al., 1995; Rawls et al., 2002, but see Schmeling and Hosko, 1976). Importantly, the hypothermic response to cannabinoids is associated with decreases in central oxygen consumption (Fitton and Pertwee, 1982; Pertwee and Tavendale, 1977).

Unlike the delayed and sustained effects on body temperature, the synthetic cannabinoids had rapid and transient effects on BP. More specifically, rats receiving synthetic cannabinoids displayed elevated BP immediately following injection when compared to rats receiving vehicle injections. There were clear differences in potency to increase BP across the drugs tested, but all synthetic cannabinoids produced a similar effect. It is noteworthy that BP responses following THC administration were not significantly elevated when compared to vehicle control, though there was trend in that direction at higher doses. The hypertension we observed following JWH-018, AM2201 and XLR-11 is in agreement with previous research in conscious animals treated with cannabinoid agonists (Gardiner et al., 2001, 2002), as well as endocannabinoids (Gardiner et al.; 2009; Lake et al., 1997; Stein et al., 1996). Banister et al. (2015) showed that JWH-018, AM2201 and XLR-11 produce substantial decreases in HR in conscious rats fitted with biotelemetric transmitters (Banister et al., 2015). We found no evidence that cannabinoids produce bradycardia in our experiments. While we have no ready explanation for the differences between our work and that of Banister and colleagues, the resting HR measures in our rats decreased substantially after vehicle injections (data not shown) whereas those in the Banister study remained elevated throughout 6 h test sessions. Thus, reduced HR responses for the vehicle control rats in our study may have precluded detection of bradycardia after cannabinoid drug administration. A number of differences between the two studies may have contributed to these differences in resting HR. Banister et al. (2015) used a different strain of rat, injections were given i.p. rather than s.c., and injections were also given 1 hr following the start of the experimental session, rather than prior to the session as in the present study.

Perhaps the most intriguing finding from our experiments is that the BP effects of synthetic cannabinoids were not antagonized by the CB_1 inverse agonist rimonabant nor by the CB_1 neutral antagonist AM4113. These results suggest that effects of synthetic cannabinoids on BP might not involve CB_1 receptors. The pressor effect observed following anandamide treatment in conscious rats is not antagonized by CB_1 antagonists (Lake et al., 1997), and the brief pressor response following anandamide treatment in anesthetized animals is also not antagonized by cannabinoid antagonists (Grzeda et al., 2015; Kwolek et al., 2005; Malinowska et al., 2010). In contrast to the present results, Gardiner et al. (2002) reported increases in BP in conscious rats following treatment with the synthetic cannabinoids WIN55,210 and HU-210, and these responses were antagonized by the cannabinoid antagonist AM251. Similarly, Gardiner et al. (2009) reported that anandamide-induced BP increases in conscious rats were also antagonized by AM251. The most obvious difference between our findings and those of Gardiner et al. is the use of the cannabinoid antagonist

AM251 in prior work. Rimonabant and AM251 are structurally similar and both function as CB_1 inverse agonists. AM251 has a slightly greater binding affinity and is more selective for CB_1 receptors when compared to rimonabant (Lan et al., 1999). Nevertheless, at the doses used in this study, both rimonabant and AM4113 should have produced antagonism at CB_1 receptors akin to that afforded by AM251, and rimonabant clearly antagonized the hypothermic effects of THC and the synthetic cannabinoids. Therefore, the reason for the discrepancies in the effects of various cannabinoid antagonists across studies requires further investigation.

The increase in BP seen is our experiments most likely results from an increase in central sympathetic outflow. Pretreatment with the ganglionic blocker hexamethonium antagonized the hypertensive effects for both CP55,940 and JWH-018. In addition, the α_1 adrenergic antagonist prazosin also antagonized the BP effect for both drugs. The finding that pressor effects produced by synthetic cannabinoids are centrally mediated confirms previous results with other cannabinoid compounds. Gardiner et al. (2001) reported that the ganglionic blocker pentolinium antagonizes the pressor effect seen following treatment with the synthetic cannabinoid WIN55,212-2. Padley et al. (2003) found that central injections of WIN55,212-2 or the synthetic cannabinoid HU-210 increase sympathetic nerve activity. Grzeda et al. (2015) and Malinowska et al. (2010) showed that central administration of CP55,940 or anandamide produces a pressor effect, even when given along with the cannabinoid CB_1 inverse agonist AM251. Collectively, the available findings suggest that synthetic cannabinoids produce a centrally-mediated sympathetic pressor effect that is independent of cannabinoid receptors. This effect appears to predominate when the drugs are administered systemically in conscious animals (Lake et al., 1997; Stein et al., 1996).

It is unclear why the body temperature effects of cannabinoids are antagonized by cannabinoid CB_1 antagonists but the BP effects are not. Both responses appear to be centrally-mediated, where CB_1 receptors are abundantly expressed (Pertwee, 2010). In addition, both effects are seen with synthetic cannabinoids that are structurally distinct. For example, CP55,940 and JWH-018 produce similar effects on body temperature and BP with analogous pharmacological control, despite their differences in structure. Finally, the rank order of potency for the cannabinoids tested here was similar for their effects on body temperature and BP. A recent study by Wiley et al. (2016) employed *in vitro* receptor screening methods and failed to identify non-cannabinoid sites of action for JWH-018 or other related synthetic cannabinoids, so it is difficult to speculate about what other receptor mechanisms might be involved in the hypertensive effects of synthetic cannabinoids examined here.

5. Conclusion

THC and synthetic cannabinoids produce cannabinoid receptor-mediated decreases in body temperature in conscious rats. Some of the synthetic cannabinoids showed much greater potency than THC, but hypothermic effects of all drugs were antagonized by the same dose of the cannabinoid inverse agonist rimonabant. In addition to their effects on body temperature, the synthetic cannabinoids produce increases in BP compared to vehicle-treated controls. This hypertensive effect does not appear to involve cannabinoid receptors, but is

mediated via central sympathetic control. Whether the physiological effects of the synthetic cannabinoids reported here in rats are relevant to the recent reports of toxicity following synthetic cannabinoid use in humans is unclear (Clark et al., 2015; Hermanns-Clausen et al., 2013; Ibrahim et al., 2014; Mir et al., 2011; Shah et al., 2016; Young et al., 2012), but our results do suggest the possibility that adverse effects and toxicity associated with certain synthetic cannabinoids may not be simply explained by potent activation of cannabinoid receptors.

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Highlights

- Physiological effects of abused synthetic cannabinoids were evaluated in rats
- All cannabinoids decreased body temperature
- Hypothermic effects of cannabinoids were blocked by a cannabinoid receptor antagonist
- Synthetic cannabinoids increased blood pressure through non-cannabinoid mechanisms
- Hypertensive effects are dependent upon central sympathetic outflow

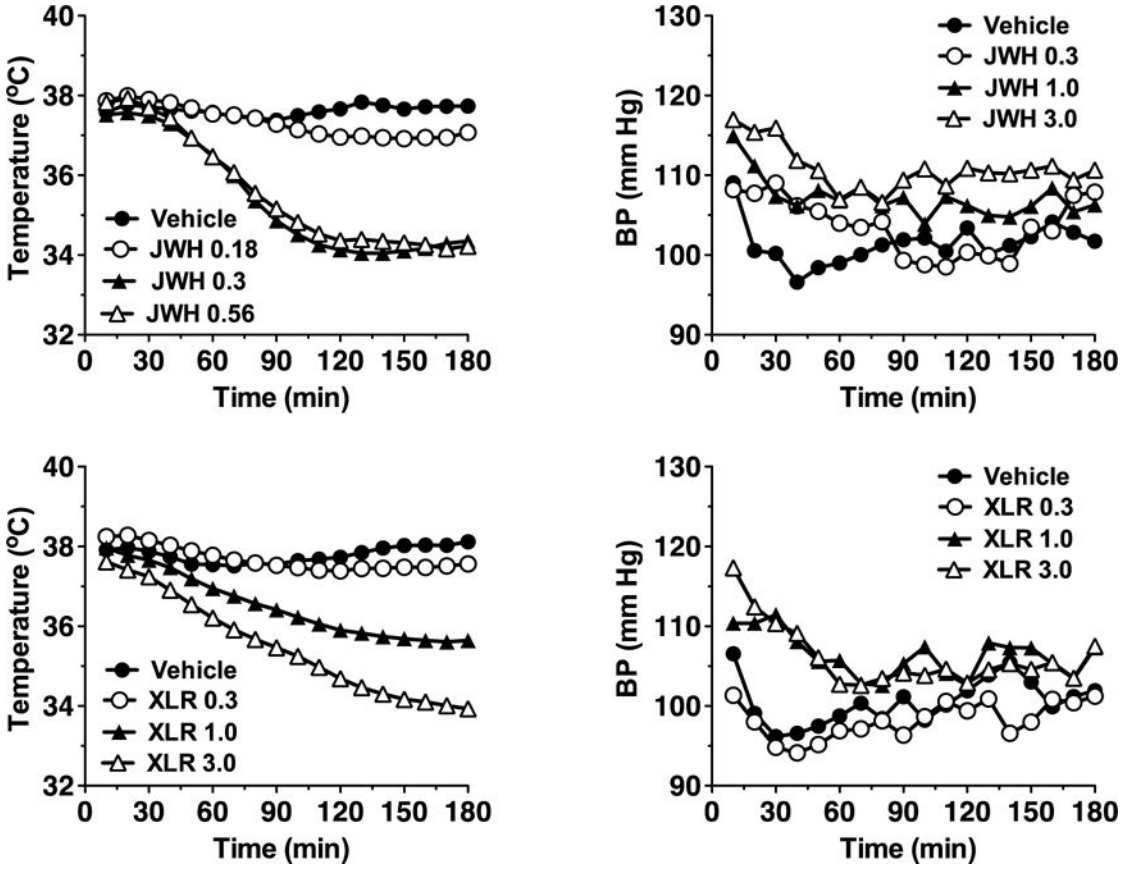

Fig. 1.

Time course for body temperature (left-hand panels) and blood pressure (BP) (right-hand panels) following various doses of the synthetic cannabinoids JWH-018 and XLR-11. Each point is a 10-min mean and each line represents the mean of 6 rats.

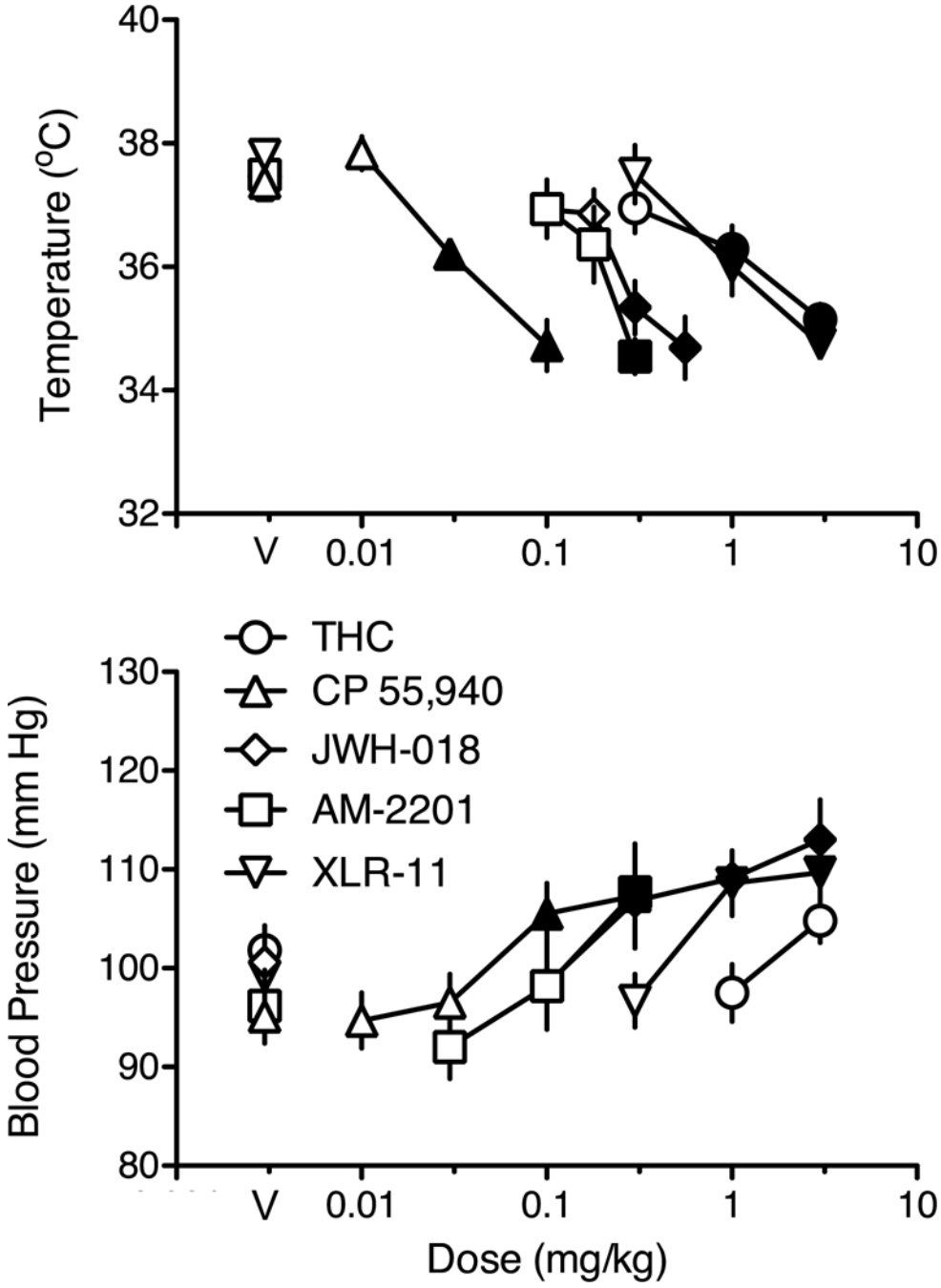

Fig. 2.

Dose-effect functions for the effects of THC and the synthetic cannabinoids on body temperature (top panel) in the last 2 h of the sessions and blood pressure (bottom panel) in the first 1 h of the sessions. Solid symbols indicate significant ($p < 0.05$) differences from the appropriate paired vehicle treatment (points above V). Each point is the mean of 6 rats ± SEM.

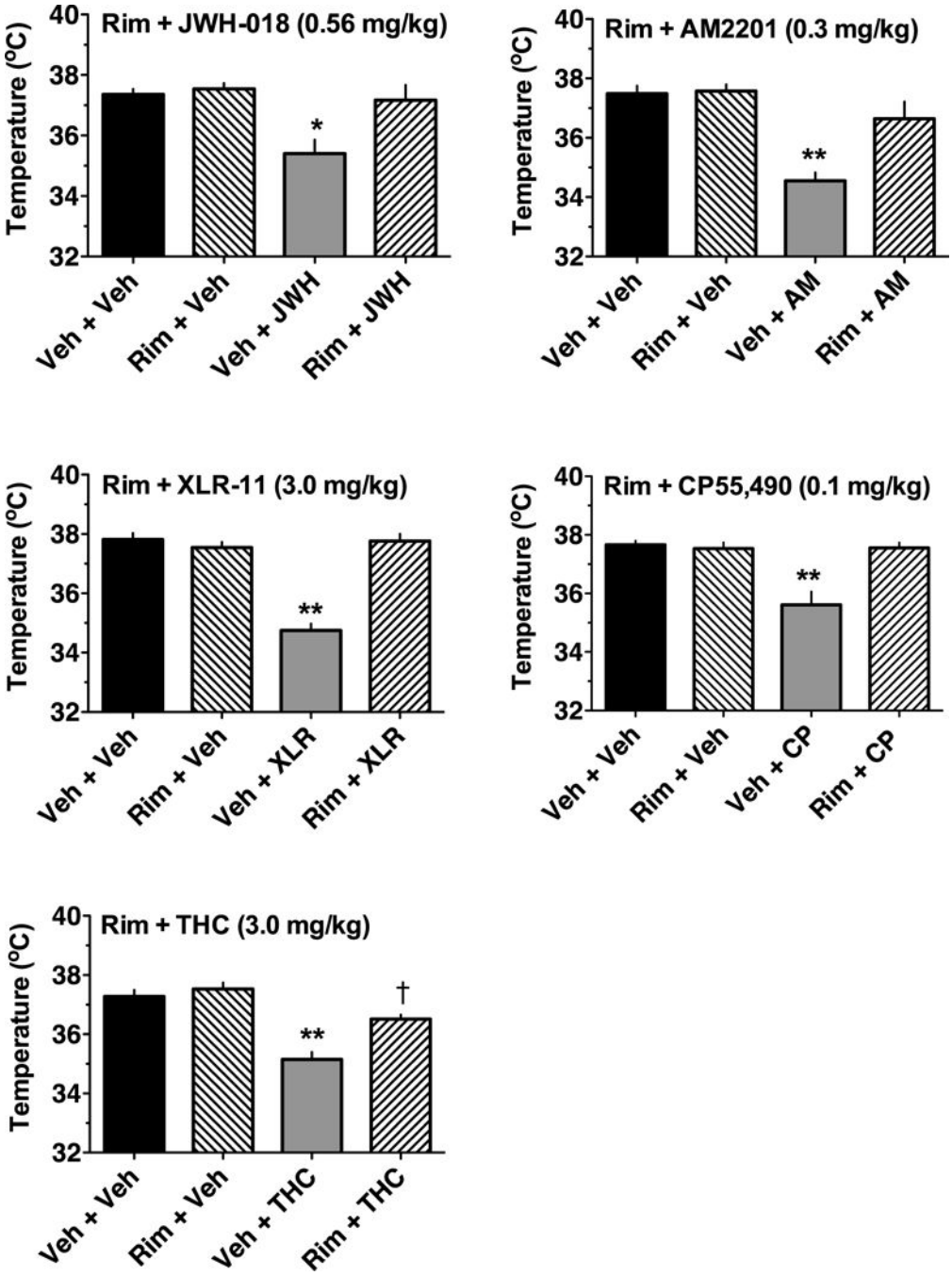

Fig. 3.

Effects of pretreatment with the cannabinoid inverse agonist rimonabant (Rim, 1 mg/kg) on the hypothermic effect of THC and the synthetic cannabinoids at the indicated doses. *Significantly different from all other groups (one symbol $p < 0.05$, two symbols $p < 0.01$). †Significantly different from the Rim + Veh group ($p < 0.05$). Veh = vehicle. Each bar is the mean of 6 rats ± SEM.

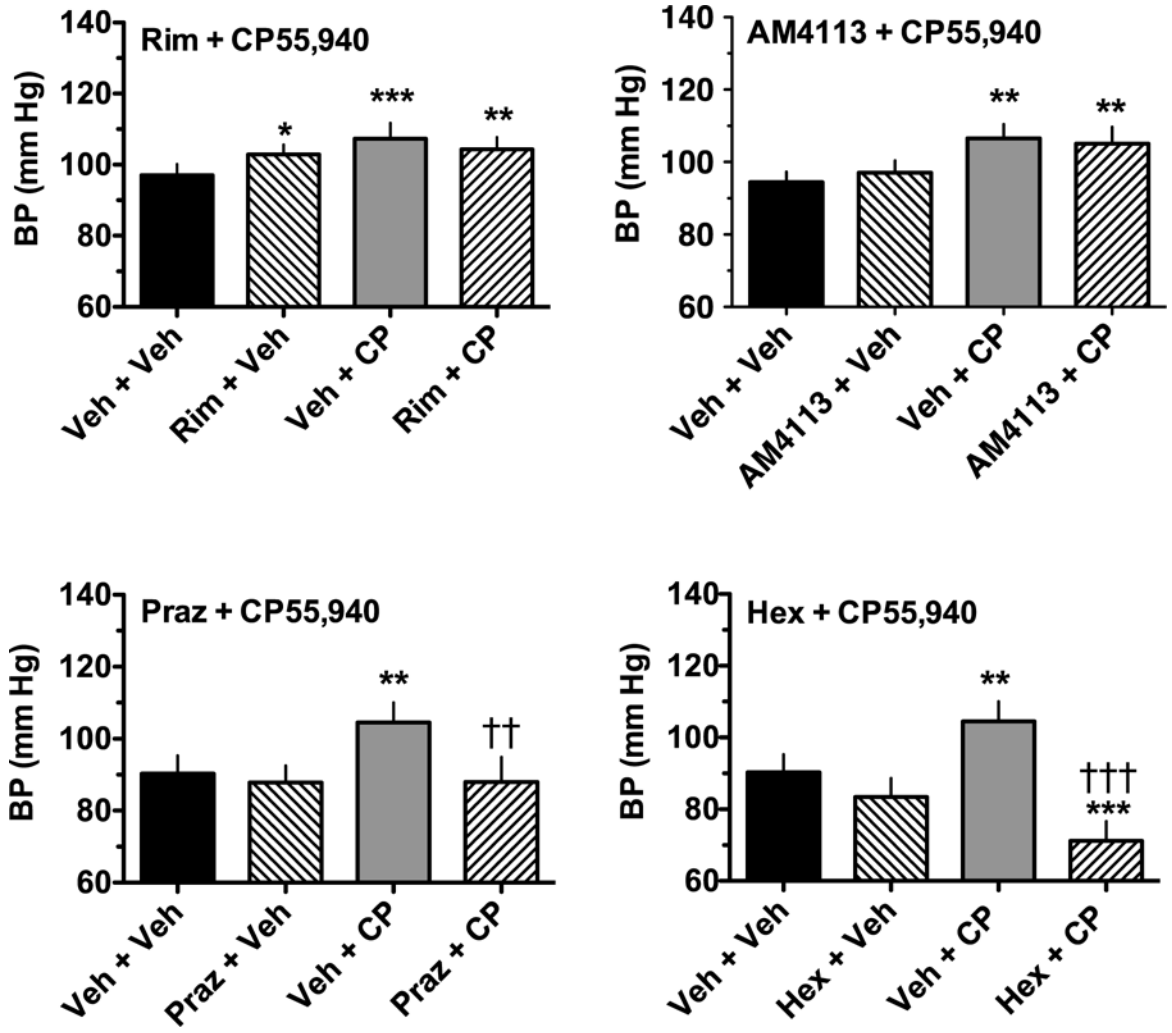

Fig. 4.

The top panels show the effects of pretreatment with rimonabant (Rim, 1 mg/kg) or AM4113 (3.0 mg/kg) on the blood pressure (BP) effect of CP55,940 (CP, 0.3 mg/kg). The bottom panels show the effects of prazosin (Praz, 0.3 mg/kg) and hexamethonium (Hex, 10 mg/kg) on the BP effect of CP55,940 (CP, 0.1 mg/kg). *Significant difference from Veh + Veh. †Significant difference from Veh + CP. One symbol $p < 0.05$, two symbols $p < 0.01$, three symbols $p < 0.001$. Each bar is the mean of 6 rats ± SEM.

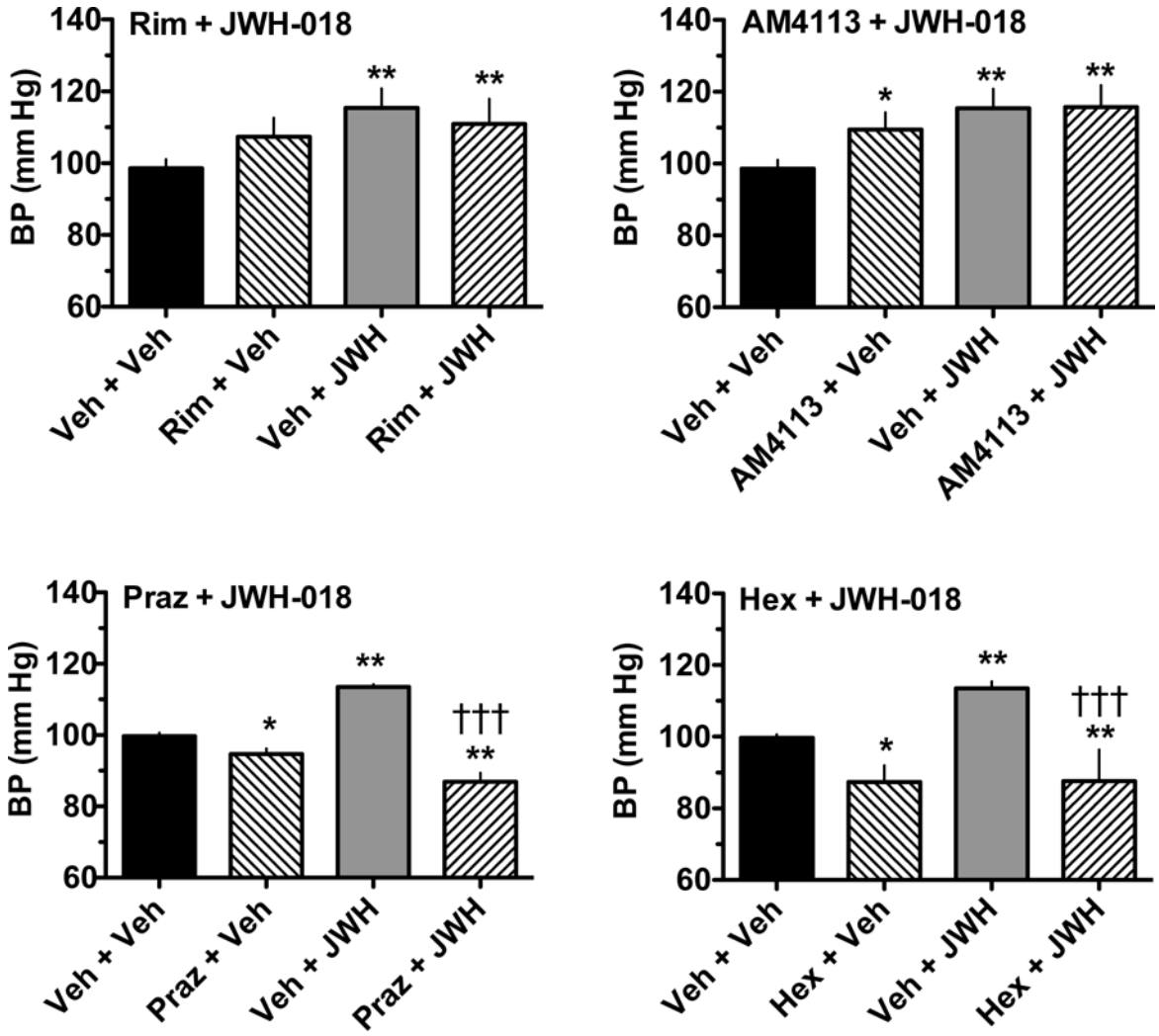

Fig. 5.

The top panels show the effects of pretreatment with rimonabant (Rim, 1 mg/kg) and AM4113 (3.0 mg/kg) on the blood pressure (BP) effect of JWH-018 (JWH, 1.0 mg/kg). The bottom panels show the effects of prazosin (Praz, 0.3 mg/kg) and hexamethonium (Hex, 10 mg/kg) on the BP effect of JWH-018 (JWH, 1.0 mg/kg). *Significant difference from Veh + Veh. †Significant difference from Veh + JWH. One symbol $p < 0.05$, two symbols $p < 0.01$, three symbols $p < 0.001$. Each bar is the mean of 7 rats ± SEM.