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Acute Cardiovascular and Cardiorespiratory Effects of JWH-018 in Awake and Freely Moving Mice: Mechanism of Action and Possible Antidotal Interventions?

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Abstract: JWH-018 is the most known compound among synthetic cannabinoids (SCs) used for their psychoactive effects. SCs-based products are responsible for several intoxications in humans. Cardiac toxicity is among the main side effects observed in emergency departments: SCs intake induces harmful effects such as hypertension, tachycardia, chest pain, arrhythmias, myocardial infarction, breathing impairment, and dyspnea. This study aims to investigate how cardio-respiratory and vascular JWH-018 (6 mg/kg) responses can be modulated by antidotes already in clinical use. The tested antidotes are amiodarone (5 mg/kg), atropine (5 mg/kg), nifedipine (1 mg/kg), and propranolol (2 mg/kg). The detection of heart rate, breath rate, arterial oxygen saturation (SpO2), and pulse distention are provided by a non-invasive apparatus (Mouse Ox Plus) in awake and freely moving CD-1 male mice. Tachyarrhythmia events are also evaluated. Results show that while all tested antidotes reduce tachycardia and tachyarrhythmic events and improve breathing functions, only atropine completely reverts the heart rate and pulse distension. These data may suggest that cardiorespiratory mechanisms of JWH-018-induced tachyarrhythmia involve sympathetic, cholinergic, and ion channel modulation. Current findings also provide valuable impetus to identify potential antidotal intervention to support physicians in the treatment of intoxicated patients in emergency clinical settings.

Keywords: JWH-018; synthetic cannabinoid; cardiovascular; respiratory; atropine; amiodarone; propranolol; nifedipine

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1. Introduction

The novel psychoactive substances (NPS) phenomenon has taken hold in the market for European and extra-European drugs of abuse [1]. These new synthetic substances, which are mainly traditional drug derivatives, result in a challenge for authorities due to their high potency as well as the difficult identification with screening tests [2,3]. The number of NPS has increased during the last decade, especially in the synthetic cannabinoids (SCs)

category [4]. Currently, SCs compounds are among the larger and various groups of NPS monitored by the United Nations Office on Drugs and Crime (UNODC) and European Monitoring Centre for Drugs and Drug Addiction [4–6].

Smoking mixtures containing SCs have been sold in the drug market since 2008 when JWH-018 was found in "Spice" and "K2", the typical brand names of SCs herbal mixtures [6,7]. Digitalization and technological progress have made the products available on the web market as well, where they have become popular among adolescents and young adults due to their low price and easy availability [7–10].

SCs have been found in different cases of intoxication, which mainly report CNS adverse effects, but cardiovascular (CV) and respiratory system damages can also be worrying [11–13]. Patients have shown symptoms such as tachycardia, hypertension, arrhythmias, chest pain, palpitations, respiratory acidosis, and dyspnea after assumptions of various SCs brands, such as Spice or K2, containing JWH-018 or Δ9-THC compounds [14–19]. Even bradycardic and hypotensive responses have been reported after SCs assumption, especially among the third-generation SCs [12,14,20]. Preclinical in vivo studies mainly revealed that synthetic cannabinoid-induced effects are bradycardia, bradyarrhythmias with sudden tachyarrhythmias, hypertension, and bradypnea [19], as our recent JWH-018 study also demonstrated [21]. Moreover, previous studies in rodents confirmed these effects after JWH-018 administration [22,23] as well as after administration of other SCs (i.e., CP-55,920 or AKB48) [24,25]. Moreover, smoking K2 products led to myocardial infarction in one 16-year-old boy [26]. Death cases have been reported after JWH-018 assumption, including a cardiac arrest case after SCs abuse [27–29]. These reports highlight the significant public health concerns regarding use of these NPS and the severity of life-threatening adverse effects due to SCs, thus underling the urgent need to characterize and develop effective treatment strategies for risks associated with both acute intoxication and chronic use. Actually, treatments of intoxication and withdrawal are still supportive and symptomatic, as no specific antidotes are available.

It is well known that CB1 receptor binding is the main cause of the effects induced by JWH-018 in preclinical models. In fact, numerous previous studies have shown that pretreatment with AM-251 prevented these effects [21,30], thus confirming the involvement of CB1 receptors that are highly expressed in CNS of rodents [31]. Centrally, CB1 receptors can also modulate cardiac and vascular functions involving the medulla and dorsal periaqueductal gray (dPAG) [32,33]. The role of CB2 receptors in the mechanisms underlying the effects induced by SCs in mice has been shown [21]. In fact, CB2 receptor selective antagonist, AM 630, reverted CV and respiratory effects of JWH-018 in a different manner from AM 251 [21]. In line with these findings, preclinical and clinical data state that CB receptors are G protein-coupled receptors involved in cognitive, cardiovascular, and metabolic functions. Notably, CB1 is in abundance in the mammalian brain, where it is responsible for psychoactive effects induced by cannabinoids, while CB2 receptors are mainly expressed in immune cells. It is worth noting that, although to a lesser extent, CB1 receptors are also situated in human peripheral tissues, such as the heart and vasculature [34]. CB2 receptors may also be further involved in CV and respiratory disorders observed in humans [35,36].

Previous studies suggested the involvement of both sympathetic and parasympathetic systems to explain cannabinoid-induced CV effects [32,37]. This is confirmed by both previous SCs studies carried out on rodents [32] and studies conducted on volunteers, which showed that tachyarrhythmias evoked by cannabis smoking were prevented by propranolol administration [37].

As shown in most recent research, modulation of CB1 and CB2 receptors could lead to interaction with ion channels [21,38]. Every phase of the cardiac reaction is regulated by voltage- and ligand-gated ion channels, which can be implicated in many types of arrhythmias due to, for instance, autonomic nervous system overstimulation or electrolyte imbalance [39]. Ion channels can be altered via CB receptor-dependent pathways that, through adenyl-cyclase inhibition, lead to sodium channel, calcium channel, and potas-

sium channel function modulations [40]. These compounds can also bind directly to ion channels, such as Δ9-THC, a cannabinoid receptor partial agonist that inhibits T-type calcium channels, CaV3.1, CaV3.2, and CaV3.3 with pEC50 of 5.81 + 0.02, 5.88 + 0.03, and 5.37 + 0.02, respectively [41]. Delta-9-THC is also able to inhibit voltage-gated sodium channels, as shown in studies carried out on neuroblastoma cells, and more recently, in rat ventricular myocytes [42,43]. Moreover, the interaction between endocannabinoid and potassium channels was reported in an in vitro study, which demonstrated that the endogenous cannabinoid anandamide (AEA) and arachidonoylglycerol (2-AG) blocked cardiac voltage-gated potassium channel (hKv1.5) with IC50 in the micromolar range on mouse fibroblasts (Ltk2 cells) [44].

Cardiovascular adverse effects can be caused by the interaction with CB receptors, but these can also be mediated by interaction with other substrates. Given the previous studies, which indicate the interactions between Δ9-THC and other endocannabinoids with ion-channels or with adrenergic and cholinergic receptors, the purpose of this investigation is to assess how JWH-018-induced CV responses are modulated with drugs that directly act on cardiac substrates. The evaluation has been performed by administering JWH-018 alone and by co-administration with amiodarone (class III antiarrhythmic drug), nifedipine (calcium channel blocker), atropine (anticholinergic drug), and propranolol (beta-blocker), drugs that are and have been widely used in emergency departments. This study also provides a valuable indication to identify potential antidotal intervention to support physicians in the treatment of SCs intoxicated patient in emergency clinical settings.

2. Results

2.1. Vehicle

To limit the number of mice, the same vehicle was used for all experiments. In vehicle-treated mice, basal HR (663 ± 3.8 bpm; Figure 1A), PD (222 ± 17 μm; Figure 1B), BR (277 ± 7.1 brpm; Figure 1C), and SpO2 (99.1 ± 1.2% SpO2; Figure 1D) did not change compared to the control (untreated) animals over the six-hour observation period.

2.2. JWH-018

As previously reported, JWH-018 (6 mg/kg) [21] rapidly reduced HR, inducing deep bradycardia and bradyarrhythmia alternated by sudden episodes of tachyarrhythmia that persisted up to six hours. In addition, PD values significantly decreased compared to basal values immediately after JWH-018 injection and during the last hours of the experiment due to a vasoconstrictor effect, which is also demonstrated by the systolic blood pressure increase. Moreover, systemic administration of JWH-018 rapidly induced a deep bradypnea combined with a transient reduction of the SpO_2 during the first hour of JWH-018 treatment [21].

2.3. Amiodarone

Amiodarone (5 mg/kg) administration by itself slightly induced an oscillatory effect on HR after one and three hours from the injection. The JWH-018-induced long-lasting effect on HR did not revert by amiodarone, which further reduced HR one hour after administration (Figure 1A, significant effect of treatment ($F_{3,1872} = 1454$, $p < 0.0001$), time ($F_{71,1872} = 36.57$, $p < 0.0001$), and time × treatment interaction ($F_{213,1872} = 9.388$, $p < 0.0001$)). Otherwise, amiodarone was able to counteract the insurgence of tachyarrhythmias (Figure 2A–F), especially during the last three hours of the experiment (Figure 2D, significant effect of treatment ($F_{1,112} = 39.22$, $p < 0.0001$), bin ($F_{7,112} = 6.018$, $p < 0.0001$), and bin × treatment interaction ($F_{7,112} = 4.978$, $p < 0.0001$); Figure 2E, significant effect of treatment ($F_{1,112} = 80.89$, $p < 0.0001$), bin ($F_{7,112} = 10.14$, $p < 0.0001$), and bin × treatment interaction ($F_{7,112} = 9.521$, $p < 0.0001$); Figure 2F, significant effect of treatment ($F_{1,112} = 86.18$, $p < 0.0001$), bin ($F_{7,112} = 10.25$, $p < 0.0001$), and bin × treatment interaction ($F_{7,112} = 9.353$, $p < 0.0001$)).

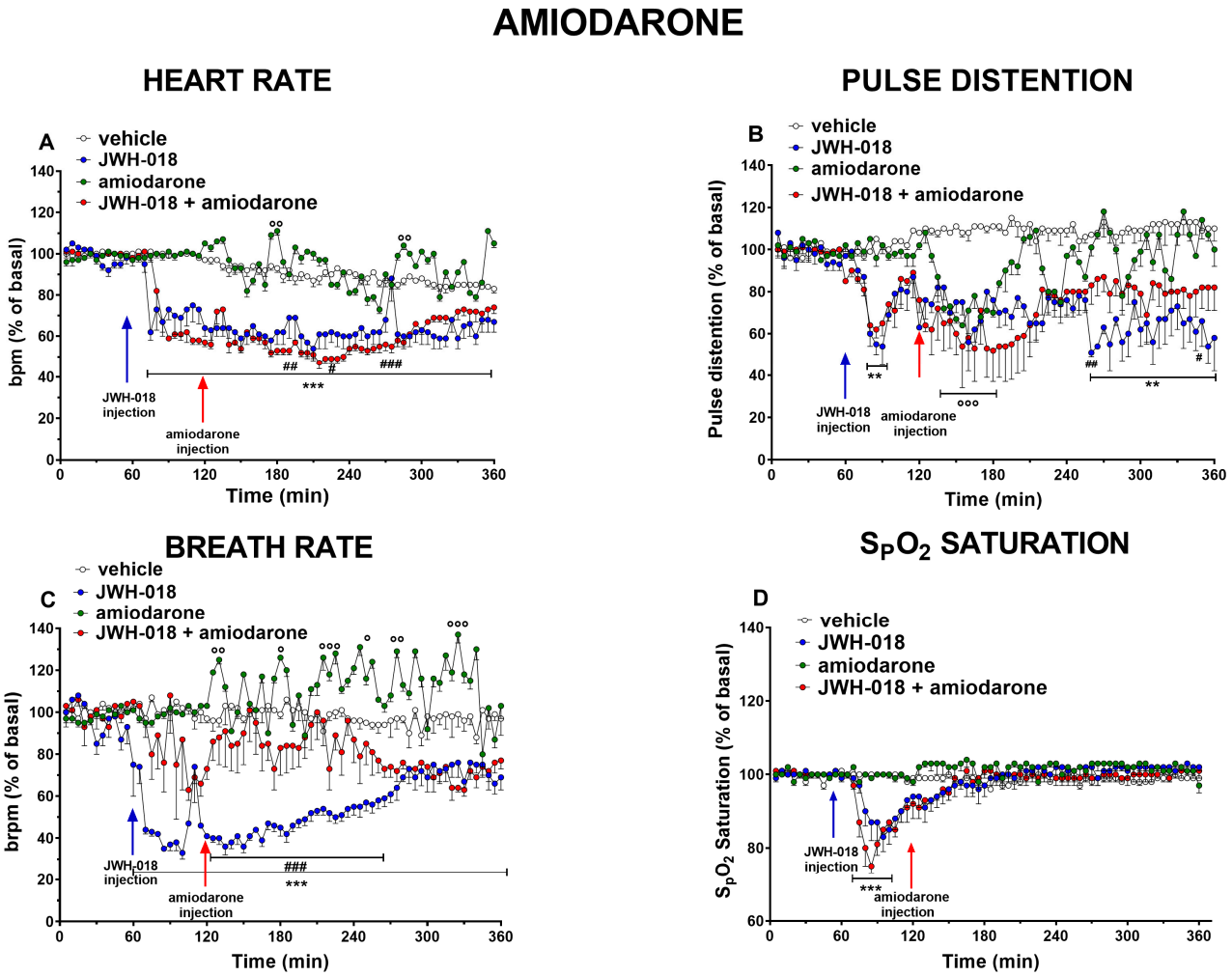

Figure 1. Effect of systemic administration of JWH-018 (6 mg/kg), amiodarone (5 mg/kg), and JWH-018 followed by amiodarone on heart rate (**A**), pulse distention (**B**), breath rate (**C**), and arterial oxygen saturation (**D**). Data are expressed as percentage of basal values in the form mean ± SEM of eight different evaluations for each group. Statistical analysis was performed by two-way ANOVA followed by Bonferroni's test for multiple comparisons. ** $p < 0.01$, *** $p < 0.001$, JWH-018 versus vehicle. # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$, JWH-018 versus JWH-018 + amiodarone. ° $p < 0.05$, °° $p < 0.01$, °°° $p < 0.001$, amiodarone versus vehicle.

Treatment with amiodarone alone transiently reduced PD during the first hour of the experiment and it slightly reverted the JWH-018-induced vasoconstriction during the fourth hour (Figure 1B, significant effect treatment ($F_{3,1872} = 336.6$, $p < 0.0001$), time ($F_{71,1872} = 3.769$, $p < 0.0001$), and time × treatment interaction ($F_{213,1872} = 2.654$, $p < 0.0001$)). Systemic amiodarone administration induced a slightly oscillatory effect on BR, but it restored the effects caused by JWH-018 during the first and second hours of the experiment (Figure 1C, a significant effect of treatment ($F_{3,1872} = 680.6$, $p < 0.0001$), time ($F_{71,1872} = 3.785$, $p < 0.0001$), and time × treatment interaction ($F_{213,1872} = 4.496$, $p < 0.0001$)). Finally, concerning oxygen saturation, amiodarone did not change the vehicle-treated mice or the transient JWH-018-induced effect in pretreated mice (Figure 1D, effect of treatment ($F_{3,1872} = 73.00$, $p < 0.0001$), time ($F_{71,1872} = 9.094$, $p < 0.0001$), and time × treatment interaction ($F_{213,1872} = 3.636$, $p < 0.0001$)).

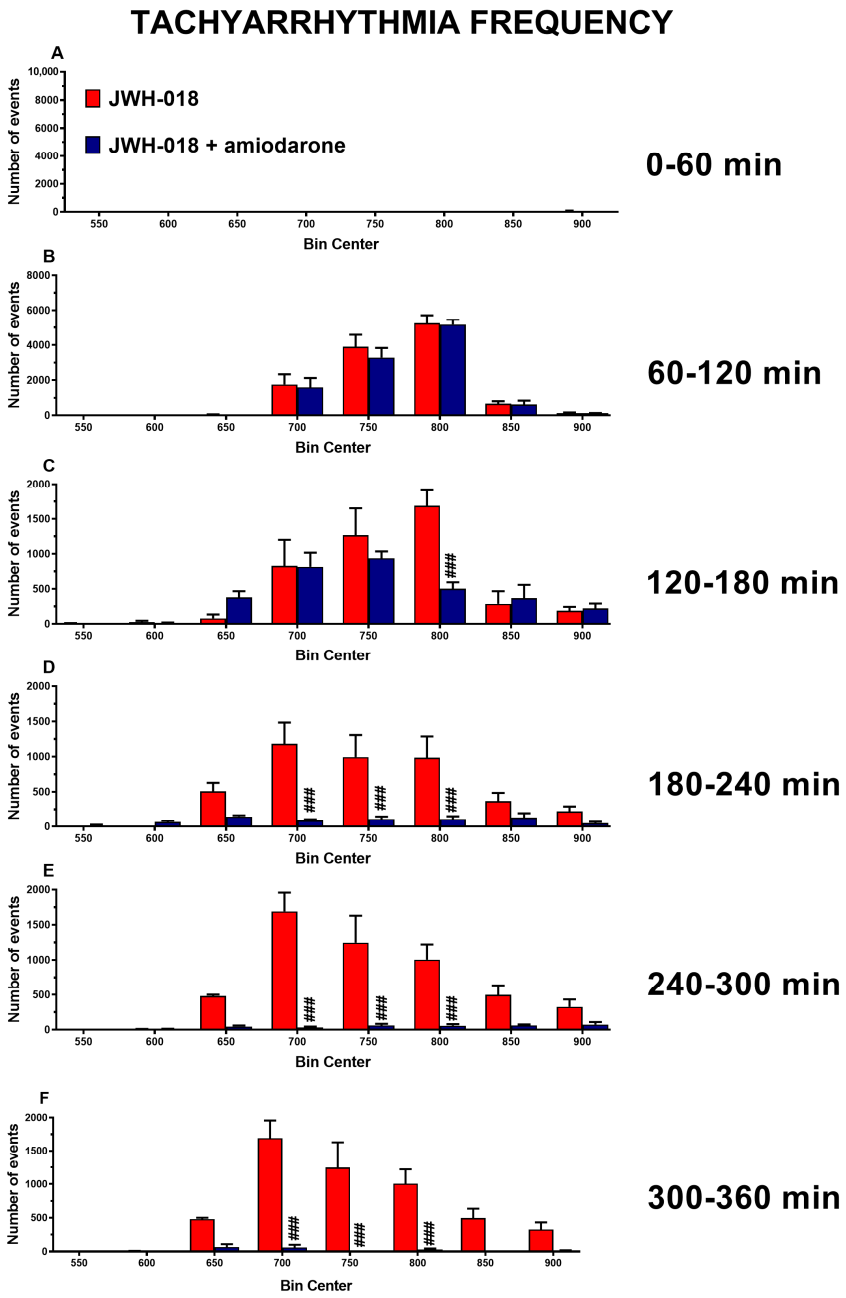

Figure 2. Frequency of tachyarrhythmia episodes between 0–60 (**A**), 60–120 (**B**), 120–180 (**C**), 180–240 (**D**), 240–300 (**E**), and 300–360 (**F**) minutes after administration of JWH-018 (6 mg/kg) or JWH-018 followed by amiodarone (5 mg/kg), expressed as number of events per mean heart rate value. Mean ± SEM of eight different evaluations for each group. Statistical analysis was performed by two-way ANOVA followed by Bonferroni's test for multiple comparisons. ### $p < 0.001$ versus JWH-018 + amiodarone.

2.4. Atropine

Administration of atropine 5 mg/kg did not alter basal parameters on HR, but it reverted the bradycardic effect induced by JWH-018 during the last three hours of the experiment (Figure 3A, significant effect of treatment ($F_{3,1872} = 747.2$, $p < 0.0001$), time ($F_{71,1872} = 14.18$, $p < 0.0001$), and time × treatment interaction ($F_{213,1872} = 6.370$, $p < 0.0001$)) and it reduced tachyarrhythmia episodes, in particular during the third, the fifth and the last hour of experiment (Figure 4C, significant effect of treatment ($F_{1,112} = 4.483$, $p = 0.0006$), bin ($F_{7,112} = 12.95$, $p < 0.0001$), and bin × treatment interaction (F7,112 = 4.034, $p = 0.0364$);

Figure 4E, significant effect of treatment ($F_{1,112}$ = 58.89, $p < 0.0001$), bin ($F_{7,112}$ = 10.74, $p < 0.0001$), and bin × treatment interaction ($F_{7,112}$ = 8.571, $p < 0.0001$); Figure 4F, significant effect of treatment ($F_{1,112}$ = 87.34, $p < 0.0001$), bin ($F_{7,112}$ = 9.646, $p < 0.0001$), and bin × treatment interaction ($F_{7,112}$ = 9.995, $p < 0.0001$)).

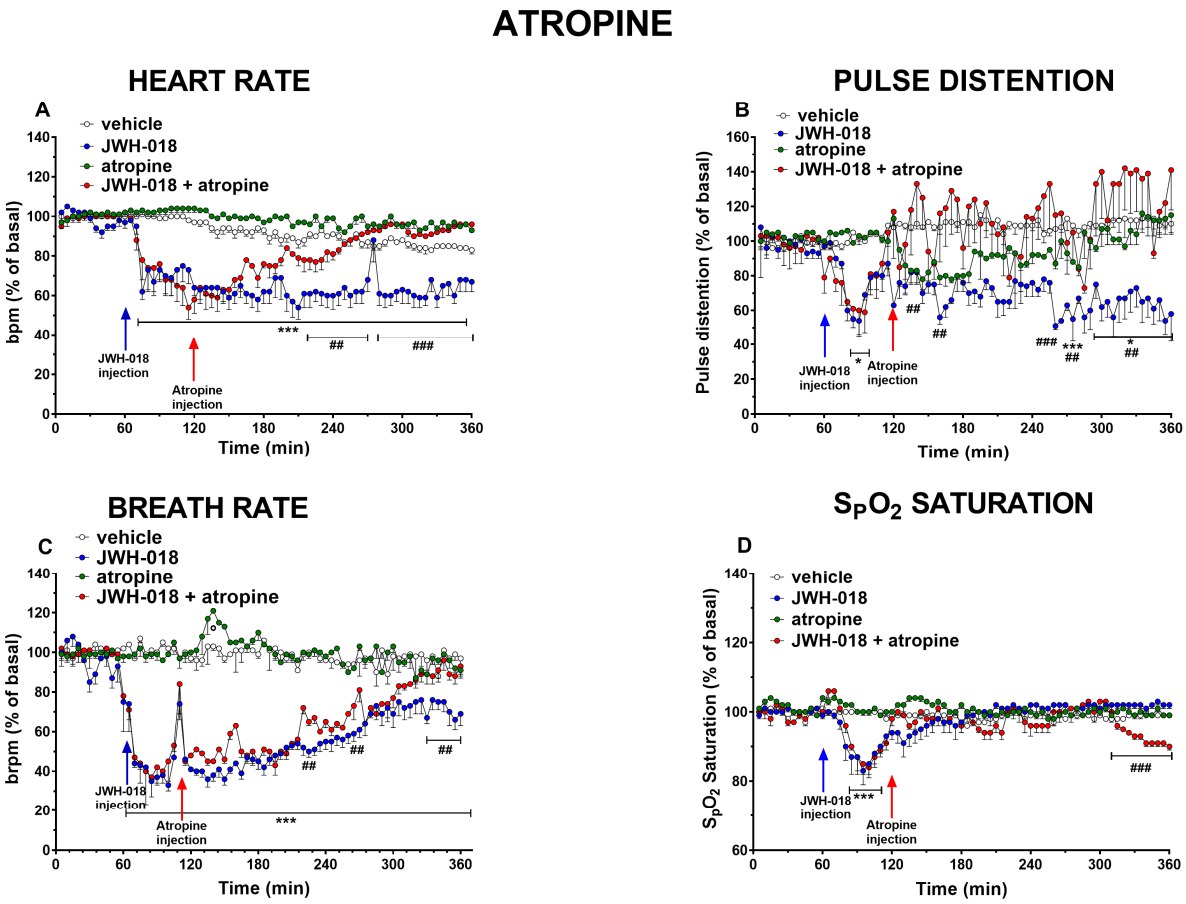

Figure 3. Effect of systemic administration of JWH-018 (6 mg/kg), atropine (5 mg/kg), or JWH-018 followed by atropine on heart rate (**A**), pulse distention (**B**), breath rate (**C**), and arterial oxygen saturation (**D**). Data are expressed as percentage of basal values in the form mean ± SEM of eight different evaluations for each group. Statistical analysis was performed by two-way ANOVA followed by Bonferroni's test for multiple comparisons. * $p < 0.05$, *** $p < 0.001$, JWH-018 versus vehicle. ## $p < 0.01$, ### $p < 0.001$ versus JWH-018 + atropine. ° $p < 0.05$, atropine versus vehicle.

Regarding PD (Figure 3B), atropine did not significantly alter the basal values, but it increased pulse distension in pretreated JWH-018 mice during the first and the last two hours of observation (effect of treatment ($F_{3,1872}$ = 128.6, $p < 0.0001$), time ($F_{71,1872}$ = 1.082, p = 0.3015), and time × treatment interaction ($F_{213,1872}$ = 1.415, p = 0.0002)). On BR (Figure 3C), atropine did not vary basal parameters in vehicle-treated mice, and it partially reverted the bradypnea effect induced by JWH-018 (effect of treatment ($F_{3,1872}$ = 1538, $p < 0.0001$), time ($F_{71,1872}$ = 19.02, $p < 0.0001$), and time × treatment interaction ($F_{213,1872}$ = 9.445, $p < 0.0001$)). Even about oxygen saturation (Figure 3D), atropine by itself did not modify vehicle values and it also did not revert the transitory effect induced by JWH-018 in pretreated mice, but a further decrease of ~10% was seen in the last hour of the experiment (effect of treatment ($F_{3,1872}$ = 78.60, $p < 0.0001$), time ($F_{3,1872}$ = 7.647, $p < 0.0001$), and time × treatment interaction ($F_{3,1872}$ = 4.516, $p < 0.0001$)).

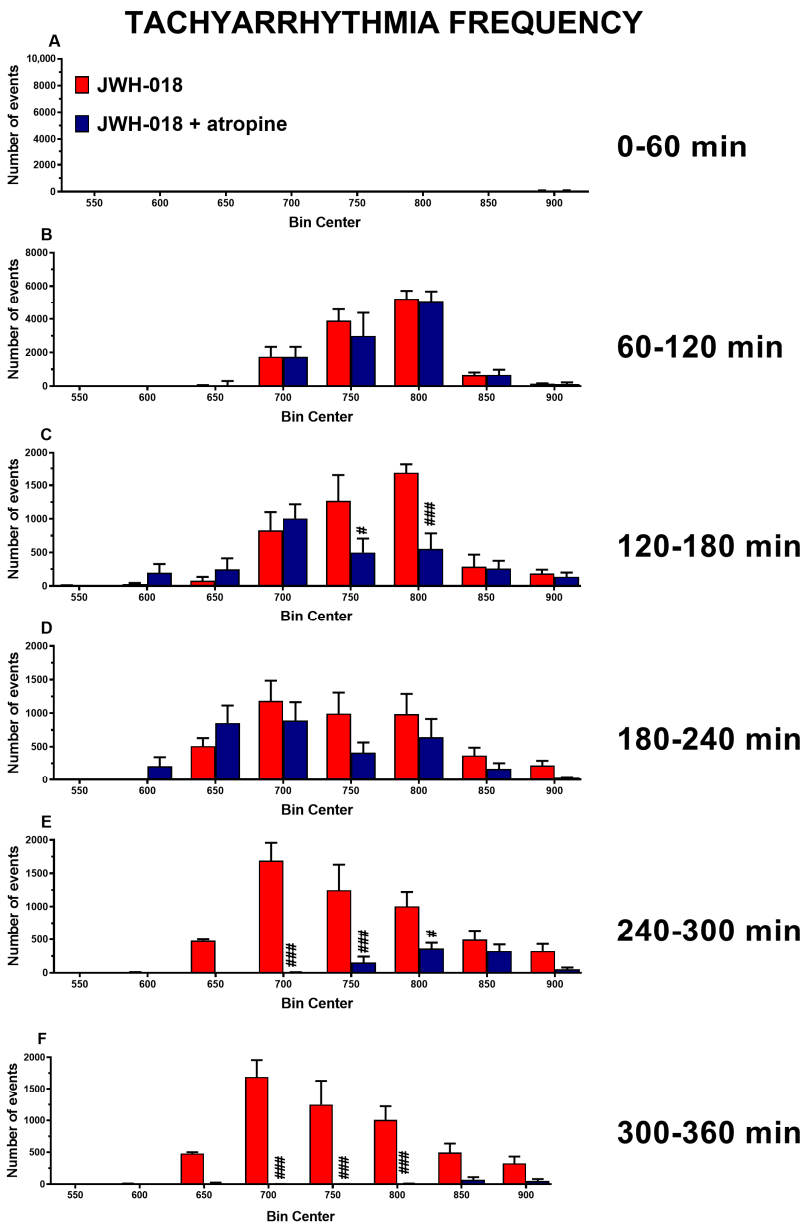

Figure 4. Frequency of tachyarrhythmia episodes between 0–60 (**A**), 60–120 (**B**), 120–180 (**C**), 180–240 (**D**), 240–300 (**E**), and 300–360 (**F**) minutes after administration of JWH-018 (6 mg/kg) or JWH-018 followed by atropine (5 mg/kg), expressed as number of events per mean heart rate value. Mean ± SEM of eight different evaluations for each group. Statistical analysis was performed by two-way ANOVA followed by Bonferroni's test for multiple comparisons. # $p < 0.05$, ### $p < 0.001$ versus JWH-018 + atropine.

2.5. Nifedipine

Nifedipine (1 mg/kg) did not affect the basal HR in vehicle-treated mice, and it did not modify the effect provoked by JWH-018 injection (Figure 5A, effect of treatment ($F_{3,1872} = 961.3$, $p < 0.0001$), time ($F_{71,1872} = 32.85$, $p < 0.0001$), and time × treatment interaction ($F_{213,1872} = 4.773$, $p < 0.0001$)).

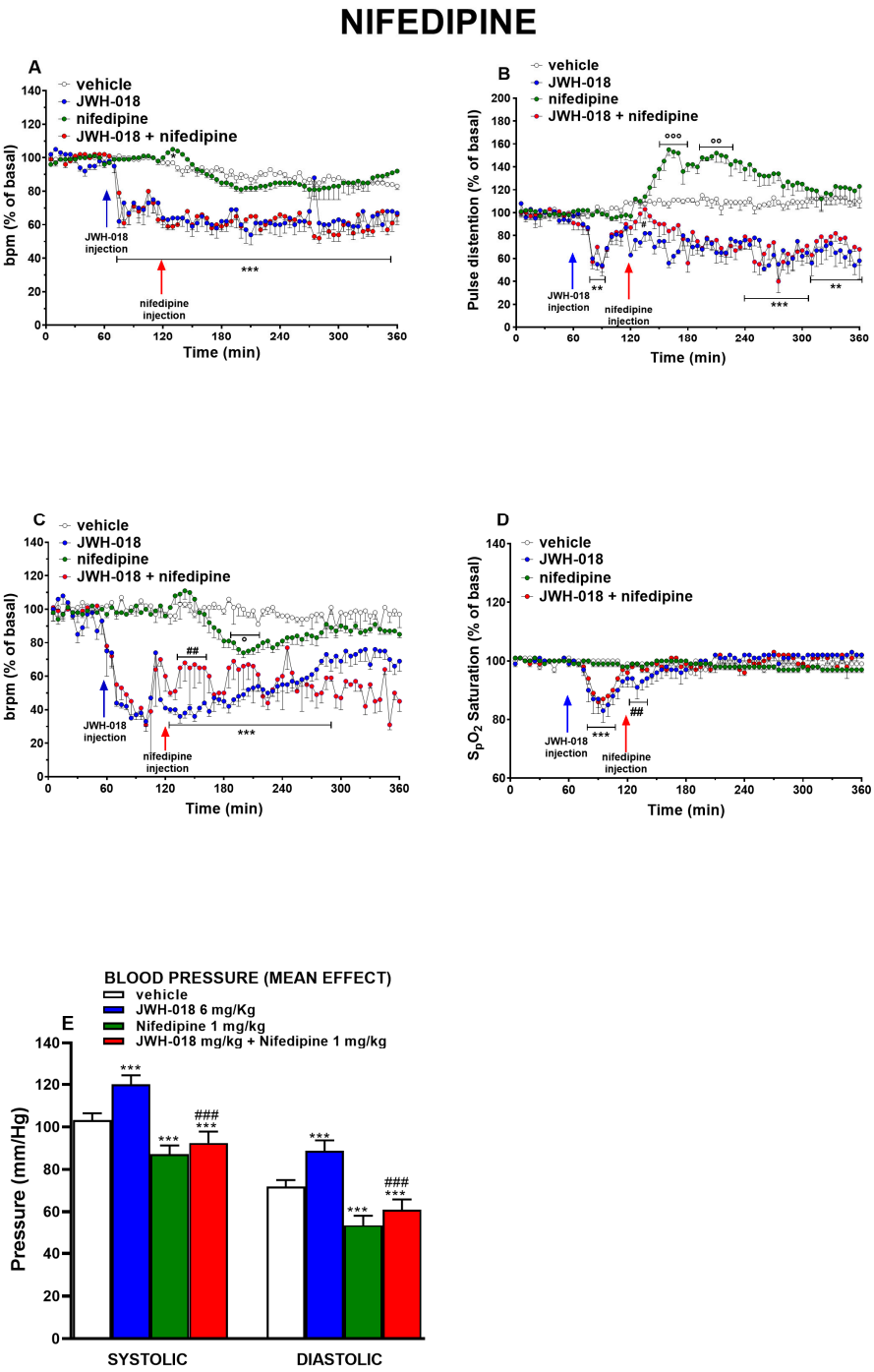

Figure 5. Effect of systemic administration of JWH-018 (6 mg/kg), nifedipine (1 mg/kg), or JWH-018 followed by nifedipine on heart rate (**A**), pulse distention (**B**), breath rate (**C**), and arterial saturation (**D**). Data are expressed as percentage of basal values in the form Mean ± SEM of eight different evaluations for each group. Statistical analysis was performed by two-way ANOVA followed by Bonferroni's test for multiple comparisons. ** $p < 0.01$, *** $p < 0.001$, JWH-018 versus vehicle. ## $p < 0.01$, JWH-018 versus JWH-018 + nifedipine. ° $p < 0.05$, °° $p < 0.01$, °°° $p < 0.001$, JWH-018 nifedipine versus vehicle. Changes in systolic and diastolic blood pressure (**E**). Data are expressed as absolute values (mmHg) of average effect. Statistical analysis was performed by one-way ANOVA followed by Bonferroni's test for multiple comparisons. *** $p < 0.001$ versus vehicle. ## $p < 0.01$ JWH-018, ### $p < 0.001$ versus JWH-018 + nifedipine.

The JWH-018-induced tachyarrhythmias were slightly reduced after nifedipine injection in the last two hours of experiment (Figure 6E, effect of treatment ($F_{1,112}$ = 6.417, p = 0.0127), bin ($F_{7,112}$ = 13.66, p < 0.0001), and bin × treatment interaction ($F_{7,112}$ = 1.945, p = 0.0689); Figure 6F, effect of treatment ($F_{1,112}$ = 7.537, p = 0.0070), bin ($F_{7,112}$ = 11.41, p < 0.0001), and bin × treatment interaction ($F_{7,112}$ = 4.934, p < 0.0001)).

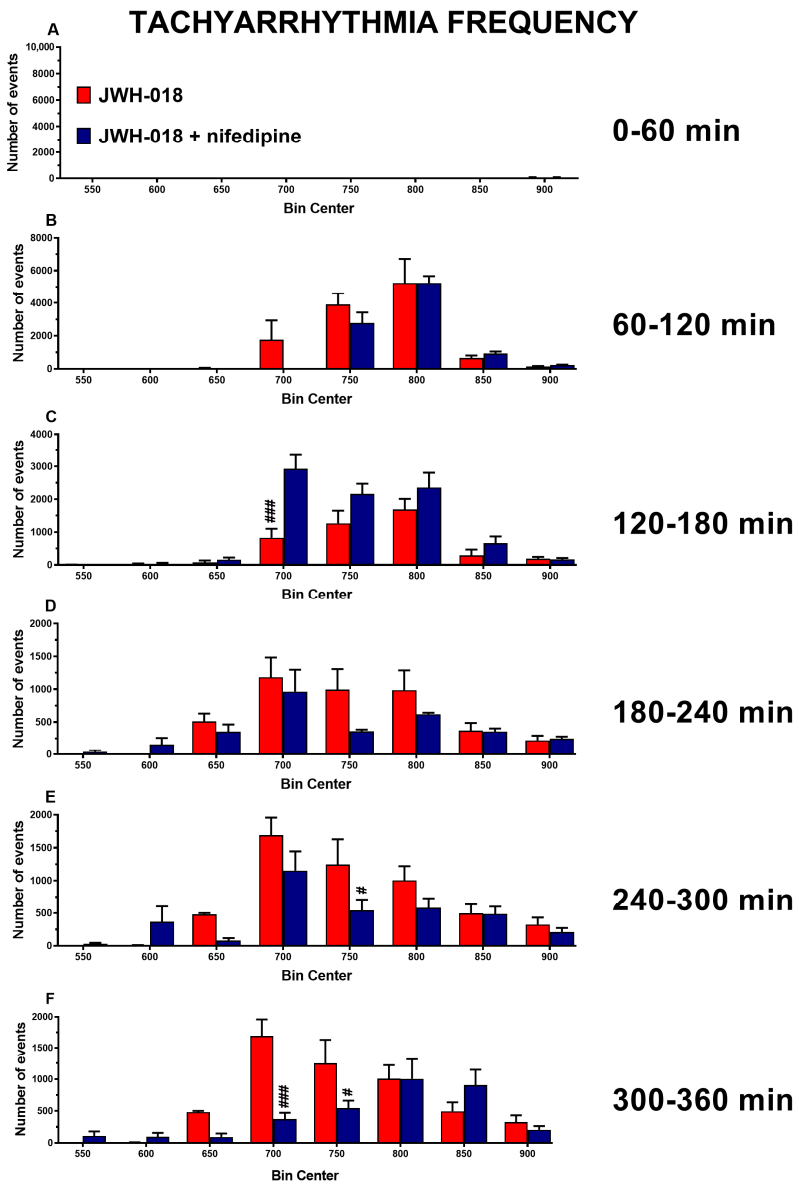

Figure 6. Frequency of tachyarrhythmia episodes between 0–60 (**A**), 60–120 (**B**), 120–180 (**C**), 180–240 (**D**), 240–300 (**E**), and 300–360 (**F**) minutes after administration of JWH-018 (6 mg/kg) or JWH-018 followed by nifedipine (5 mg/kg), expressed as number of events per mean heart rate value. Mean ± SEM of eight different evaluations for each group. Statistical analysis was performed by two-way ANOVA followed by Bonferroni's test for multiple comparisons. # p < 0.05, ### p < 0.001 versus JWH-018 + nifedipine.

Nifedipine administration itself induced an increase of PD (Figure 5B) of ~50% with respect to basal values. Despite this, the administration of nifedipine after JWH-018 injection slightly reverted the vasoconstrictor effect caused by the latter, only during the first 30 min of the experiment (Figure 5B, a significant effect of treatment ($F_{3,1872}$ = 867.6, p < 0.0001), time ($F_{71,1872}$ = 3.378, p < 0.0001), and time × treatment interaction ($F_{213,1872}$ = 5.162, p < 0.0001)). This effect was confirmed by the analysis on BP-2000 that registered the

blood pressure changes in one hour. In particular, both systolic (Figure 5E, significant effect of treatment $F_{3,28} = 92.29$, $p < 0.0001$) and diastolic (Figure 5E, significant effect of treatment $F_{3,28} = 92.29$, $p < 0.0001$) blood pressure of JWH-018 were reduced by nifedipine administration. Nifedipine slightly reduced basal BR one hour following the treatment, and in JWH-018-pretreated mice, it abolished the reduction of breath rate during the first hour of the experiment (Figure 5C, a significant effect of treatment ($F_{3,1872} = 654.9$, $p < 0.0001$), time ($F_{71,1872} = 8.586$, $p < 0.0001$), and time × treatment interaction ($F_{213,1872} = 3.623$, $p < 0.0001$)). The effect on oxygen saturation subsequent to nifedipine administration did not reveal a difference from the vehicle. However, nifedipine was able to slightly increase oxygen saturation immediately after injection when it was administered after JWH-018 (Figure 5D, the effect of treatment ($F_{3,1872} = 4.110$, $p = 0.0064$), time ($F_{71,1872} = 7.553$, $p < 0.0001$), and time × treatment interaction ($F_{213,1872} = 3.959$, $p < 0.0001$)).

2.6. β1 β2 Blocker

Administering propranolol, a non-selective β1 β2 blocker (2 mg/kg), by itself did not significantly change the HR compared to the basal rate. Nevertheless, propranolol systemic administration (2 mg/kg) after JWH-018 injection firstly reduced and subsequently increased the bradycardic effect induced by JWH-018 (Figure 7A, significant effect of treatment ($F_{3,1872} = 476.3$, $p < 0.0001$), time ($F_{71,1872} = 23.55$, $p < 0.0001$), and time × treatment interaction ($F_{213,1872} = 5.412$, $p < 0.0001$)).

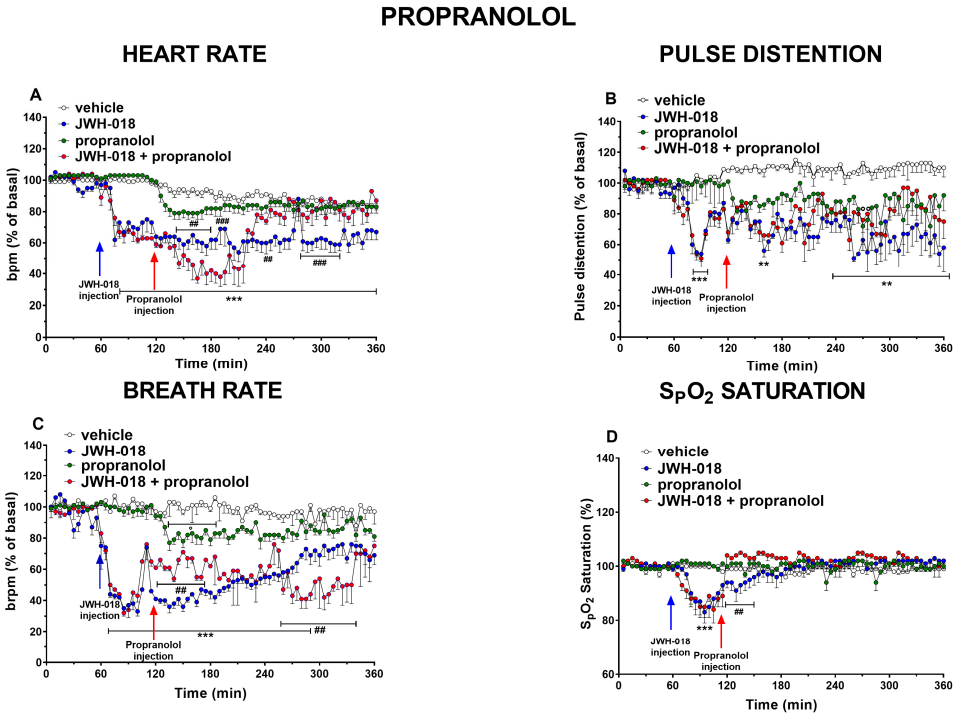

Figure 7. Effect of systemic administration of JWH-018 (6 mg/kg), propranolol (2 mg/kg), or JWH-018 followed by propranolol on heart rate (**A**), pulse distention (**B**), breath rate (**C**), and oxygen blood saturation (**D**). Data are expressed as percentage of basal values in the form mean ± SEM of four different evaluations for each group. Statistical analysis was performed by two-way ANOVA followed by Bonferroni's test for multiple comparisons. ** $p < 0.01$, *** $p < 0.001$, JWH-018 versus vehicle. ## $p < 0.01$, ### $p < 0.001$, JWH-018 versus JWH-018 + propranolol. ° $p < 0.05$, propranolol versus vehicle.

Moreover, propranolol drastically reduced the JWH-018-induced tachyarrhythmic events immediately after injection up to the end of the experiment (Figure 8C, significant effect of treatment ($F_{1,112} = 36.99$, $p < 0.0001$), bin ($F_{7,112} = 7.018$, $p < 0.0001$), and bin × treat-

ment interaction ($F_{7,112}$ = 6.650, $p < 0.0001$); Figure 8D, significant effect of treatment ($F_{1,112}$ = 33.68, $p < 0.0001$), bin ($F_{7,112}$ = 6.926, $p < 0.0001$), and bin × treatment interaction ($F_{7,112}$ = 3.425, $p = 0.0023$); Figure 8E, significant effect of treatment ($F_{1,112}$ = 20.80, $p < 0.0001$), bin ($F_{7,112}$ = 8.628, $p < 0.0001$), and bin × treatment interaction ($F_{7,112}$ = 4.869, $p < 0.0001$); Figure 8F, significant effect of treatment ($F_{1,112}$ = 40.74, $p < 0.0001$), bin ($F_{7,112}$ = 8.954, $p < 0.0001$), and bin × treatment interaction ($F_{7,112}$ = 5.122, $p < 0.0001$)).

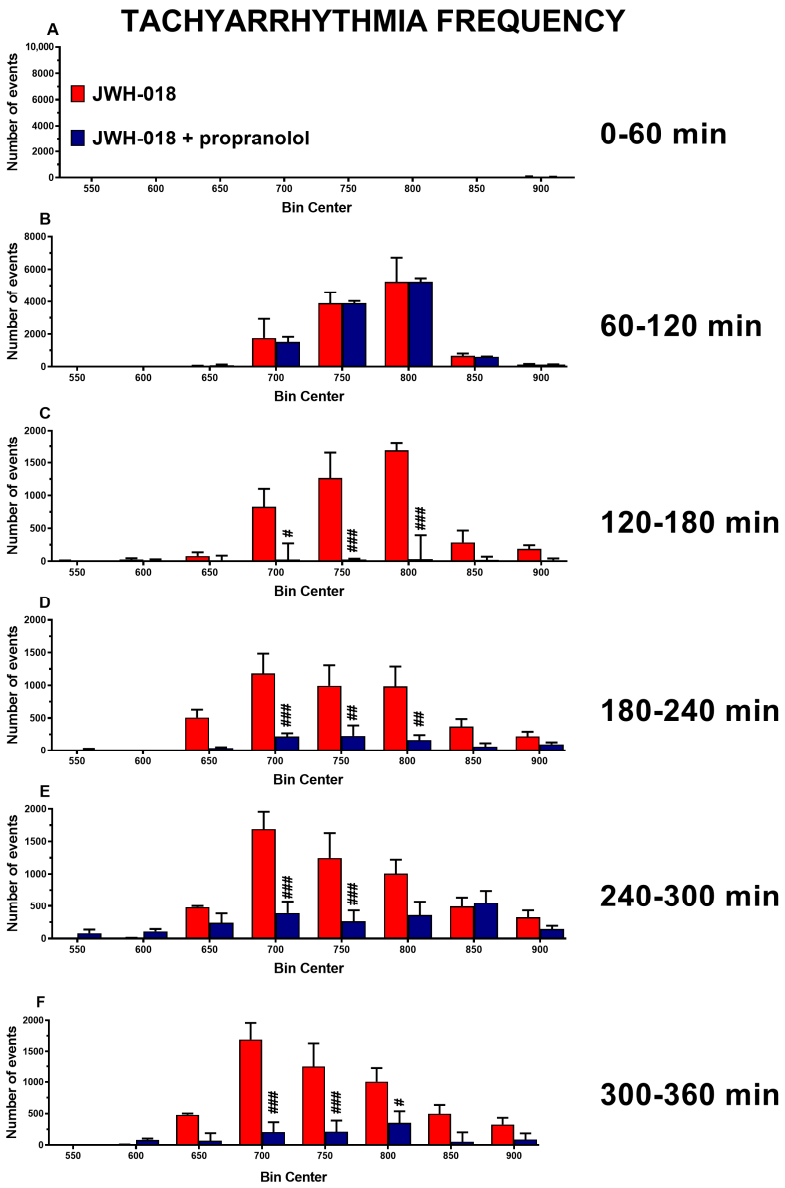

Figure 8. Frequency of tachyarrhythmia episodes between 0–60 (**A**), 60–120 (**B**), 120–180 (**C**), 180–240 (**D**), 240–300 (**E**), and 300–360 (**F**) minutes after administration of JWH-018 (6 mg/kg) or JWH-018 followed by propranolol (2 mg/kg), expressed as number of events per mean heart rate value. Mean ± SEM of eight different evaluations for each group. Statistical analysis was performed by two-way ANOVA followed by Bonferroni's test for multiple comparisons. # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ versus JWH-018 + propranolol.

The treatment with propranolol alone slightly decreased the pulse distension, especially after 150 min from the injection, but it did not alter pulse distension reduction induced by JWH-018 (Figure 7B, effect of treatment ($F_{3,1872}$ = 257.5, $p < 0.0001$), time ($F_{71,1872}$ = 2.773, $p < 0.0001$), and time × treatment interaction ($F_{213,1872}$ = 1.738, $p < 0.0001$)). After propra-

nolol alone administration, basal parameters of BR (Figure 7C) were slightly reduced of ~20%. Propranolol was able to increase BR in JWH-018 pretreated mice, immediately after injection, while during last two hours it further reduced the effect caused by JWH-018 (Figure 7C, significant effect of treatment ($F_{3,1872}$ = 826.0, $p < 0.0001$), time ($F_{71,1872}$ = 12.69, $p < 0.0001$), and time × treatment interaction ($F_{213,1872}$ = 4.369, $p < 0.0001$)). Finally, the effect on SpO2 after propranolol administration by itself did not change respect to vehicle-treated mice; however, immediately after propranolol injection, the effects of JWH-018 were abolished (Figure 7D, significant effect of treatment ($F_{3,1872}$ = 21.05, $p < 0.0001$), time ($F_{71,1872}$ = 7.185, $p < 0.0001$), and time × treatment interaction ($F_{213,1872}$ = 4.211, $p < 0.0001$)).

3. Discussion

The use of both natural and synthetic cannabinoids has dramatically increased in the past decades due to legalization, to the diffusion as mass culture and, more recently, to the diffuse increase in substance abuse during the COVID-19 pandemic and restrictive measures [45,46]. The recent change in the attitude and the increase in consumption have shed light on the increased CV risk in cannabinoid consumers, as demonstrated by the recently published clinical statements by the American Heart Association and the European Association of Preventive Cardiology [47,48], which highlighted the increased risk of myocardial infarction, arrhythmias, sudden cardiac death, and stroke. Therefore, it is of utmost importance to evaluate the possible pharmacological antidotes, their efficacy, and possible harmful effects.

Currently, no antidote is available for SCs poisoning and the cardiotoxic effects due to NPS. In addition, specific practice guidelines still need to be developed for intoxicated patients. Supportive care and symptom management are the mainstay of the treatment; applying intravenous fluids to treat electrolyte and fluid disturbances is particularly critical to cardiac function. Since patients rarely fit precisely into a particular toxidrome, but rather present overlapping signs and symptoms from manifold syndrome groups, the development of a prompt differential diagnosis is difficult and challenging, requiring relevant cardiology and neurology evaluation. Rapid and consensual treatment of agitation is crucial, also for the healing of cardiovascular toxic effects [3]. In particular, in the pharmacological management of SCs intoxication, the initial treatments of severe hypertension include nitrates, benzodiazepines (BDZs), α-adrenergic antagonists (e.g., prazosin and phentolamine), and β-blockers (e.g., labetalol), these latter used with caution, since potential paradoxical hypertension may occur. Atypical antipsychotics, e.g., haloperidol, ziprasidone, quetiapine, and olanzapine, results are more beneficial than BDZs as fist-line medicaments, even seldom the use of these medications have the potential to worsen SCs-induced QTc prolongation, possibly causing additional cardiac complications, including Torsades de Pointes [49,50].

Moreover, naltrexone, nabilone and naloxone have been proposed lately as potential pharmacotherapy for treating SCs withdrawal [51,52]. Recently, many studies have indicated that prophylactic treatment with CB1 receptor antagonists can block cannabimimetic effects both in animals and humans. Therefore, single-use CB1 receptor inverse agonists could perhaps provide an acceptable temporary single-dose antidote [53,54]. Nonetheless, a never-ending effort is devoted to evaluating the effectiveness of drugs usually employed in Emergency Departments (EDs) for the treatment of NPS-induced adverse effects, with the goal of identifying novel, effective, antidotal therapeutic strategies to be adopted in the critical management of SCs intoxicated patients.

Considering the CV and respiratory effects in mice reported in our preclinical study [21], this work evaluated the interaction between JWH-018 effects and different CV drugs that can be used as symptomatic "antidotes" such as amiodarone (class III anti-arrhythmic drug), nifedipine (calcium channel blocker), atropine (anticholinergic drug), and propranolol (beta-blocker drug), with the aim of counteracting JWH-018-induced effects, by administering cardio-active drugs. Specifically, concerning the choice of currently employed selected antiarrhythmics and antihypertensive medicines, it has to be highlighted that amiodarone

is the most commonly prescribed antiarrhythmic drug (AAD) and the most used drug in EDs and Critical Care Units. The other tested AADa, i.e., atropine, nifedipine, and propranolol, are well known to be recommended by clinical practice guidelines for their use in combination with the abovementioned amiodarone or as an alternative effective treatment in the acute management of adverse cardiovascular outcomes (e.g., arrhythmias, refractory ventricular fibrillation/tachycardia) commonly used in EDs [55–57] when narrowing an early differential diagnosis enabling the identification of a NPS-induced toxidrome and achieving patient-centered decision making are even more challenging.

Our results showed that only atropine has completely reverted JWH-018-induced bradycardia, and it was the only tested antidote that reverted the JWH-018-induced vasoconstriction. All antidotes reduced the tachyarrhythmia onset induced by JWH-018. All drugs tested, in particular amiodarone administration, reverted respiratory rate. The effect of these substances will be further examined below.

3.1. Amiodarone

The first substance examined was amiodarone, which is a class III anti-arrhythmic drug and one of the most used antiarrhythmic drugs in the emergency department [58], acting both on ion channels (K^+, Na^+, and Ca^{2+}) and adrenergic receptors [59] in the sinus node and in the atrio-ventricular (A-V) node. Amiodarone, administered as a dose of 5 mg/kg, slightly worsened JWH-018-provoked (6 mg/kg) bradycardia, in the central hours of the experiment (Figure 1A). On the contrary, it prevented the onset of tachyarrhythmic events, especially during the last three hours of the experiment, dropping the number of events to zero (Figure 2D–F). The JWH-018-induced effect on PD was not significantly changed during the first two hours after amiodarone administration, but it slightly reverted in the fourth hour of the experiment (Figure 1B). Moreover, amiodarone reverted the reduction of BR caused by JWH-018, but it did not change the JWH-018-induced SpO2 reduction (Figure 1C,D).

In vivo cardiac effects caused by JWH-018 (6 mg/kg), as bradycardia interspersed by sudden tachyarrhythmia events, could be caused by CB1, which can lead to autonomic nervous system modulations and ion channel disbalances [60]. An electrophysiological study showed that amiodarone slows upstroke velocity of action potential and decreases excitability and conductibility of cardiac cells inhibiting K^+, Na^+, and Ca^{2+} currents [61]. Beyond these mechanisms, amiodarone is able to diminish sympathetic tone with α and β receptor blockage [56,62]. Action on ion channel and sympathetic tone decrease might be able to further increase JWH-018-induced bradycardia, but also reducing tachyarrhythmic spikes. Although amiodarone seems to worse the bradycardic effect on HR in JWH-018 pretreated mice, this effect could be advantageous in humans. Differently from mice, the most common cardiac symptom reported following SCs use is tachycardia, due to different sympathovagal responses probably caused by the lower dosage taken [16,63]. Indeed, two clinical cases reported that treatment with amiodarone after cannabis or SCs assumption was effective to regularize cardiac rhythm and resolve tachycardia [64,65] due to its direct effect on sinus-atrial node frequency. Likewise, amiodarone regularized all HR changes (arrhythmias and tachycardia) induced by JWH-018 on mice [21]. The inhibition of adrenergic receptors could also explain respiratory response trend following amiodarone administration [66–68]. Amiodarone induced tachypnea administered by itself, but also reverted JWH-018-induced bradypnea. This is consistent with clinical cases, which reported tachypnea after amiodarone assumption [69,70].

3.2. Atropine

Another attempt to revert the effect of JWH-018 was made by administering 5 mg/kg of atropine, a competitive reversible antagonist of the muscarinic acetylcholine receptors [71]. Atropine was widely used in clinical cases to counteract cardiac abnormalities such as brady-asystolic cardiac arrest [72] or acute myocardial infarction [73] or A-V block [74]. The data obtained seemed to indicate that atropine is able to shorten the du-

ration of the effect of JWH-018 (6 mg/kg) on heart rate (Figure 3A), and also to reduce vasoconstriction (Figure 3B). JWH-018-induced BR reduction was reverted after atropine administration (Figure 3C), while SpO2 did not show significant changes (Figure 3A). Moreover, atropine seemed to decrease the number of tachyarrhythmic events registered after its injection, mainly during the last two hours (Figure 4C,E,F).

The choice of trying to revert JWH-018-induced CV effects with atropine was made in line with the evidence that reported a possible involvement of the vagus nerve, due to cannabinoid receptor activation, resulting in bradycardia [60]. As expected, the increase in HR recorded after atropine treatment confirmed the involvement of vagal activity in the bradycardic effect after administration of JWH-018 (6 mg/kg). Acting as an antagonist for muscarinic receptors, atropine blocks the stimulation of the parasympathetic system with its vagolytic action on sinus-atrial (S-A) and A-V nodes, increasing heart rate [75–78].

Beyond the expected effect on HR, atropine was also able to decrease JWH-018-induced tachyarrhythmia, particularly during the last two hours of the experiment. By increasing the heart rate, atropine was effective in reducing ventricular ectopic beats, thus improving cardiac dysrhythmia [71,79]. Moreover, atropine decreases the cardiac automaticity in the S-A node [80], improves A-V nodal conduction, avoiding A-V block, which has often been found after SCs administration [81,82]. This evidence indicates that the tachyarrhythmia reduction after atropine administration could be due to a cardiac conduction improvement. According to this, a clinical report showed that atropine was also used to treat a "spice"-intoxicated patient with cardiac dysfunction [16].

Regarding PD, results showed an increase after atropine administration, differently from what was expected based on the evidence. Indeed, atropine should block the peripheral vasodilation, preventing the acetylcholine action [83], and this was confirmed by in vivo studies on dogs [84,85]. Nevertheless, the pulse distension increase is consistent with Abraham and colleagues' study [86], which showed the hypotensive effect of atropine in hypertensive rats. Actually, these findings demonstrated that atropine can modulate noradrenergic system response and this action underlies its hypotensive effect [86]. This has probably been a physiological reflex of mice due to an already existent high sympathetic tone [86]. An excessive sympathetic activity to the cardiovascular system may paradoxically activate cardiac sensory nerves in the vagus nerve, causing reflex inhibition of sympathetic activity to blood vessels, leading to vasodilation [87].

Vagal innervation could be involved in the JWH-018-induced depressant breathing effect [88,89]. The central effect of atropine could solve this effect. Moreover, the atropine could also interfere with the oxygen blood saturation, which further decreases the JWH-018-induced effect, during the last hour after atropine administration [90].

3.3. Nifedipine

Nifedipine is a Ca^{2+} channel blocker that is commonly used to treat hypertensive emergencies and as antianginal medication [91]. Despite this, administration of 1 mg/kg of nifedipine was unexpectedly inefficient in reverting the effect of JWH-018 in pulse distension values (Figure 5B). Nifedipine was also ineffective on bradycardia induced by JWH-018 even if it prevented the sudden increase of HR (Figure 6A) and the tachyarrhythmic events during the last hours (Figure 6E,F). Moreover, nifedipine was able to restore the JWH-018-induced breath rate and SpO2 reduction, immediately after administration (Figure 5C,D).

Nifedipine was used to try to manage a common effect described in many case reports of cannabinoid intoxication: hypertension. Despite nifedipine increasing PD by itself, it did not revert the effect induced by JWH-018 in mice during all experiments, except during the first hour, and the effect was confirmed by BP-2000 blood pressure analysis. This evidence could suggest that hypertension induced by JWH-018 was probably caused by a peripheral action that involves calcium channel [92] during the first hour, while during the following hours of the experiment the central action on dorsal periaqueductal gray (dPAG) could prevail [93]. However, the possible variability factors related to the technique

applied (such as heat, restraint of the mouse, and inflation of the cuff on the tail) should be considered [94].

In line with the action mechanism of nifedipine on the calcium channel [91], the obtained results may suggest a possible involvement of these latter for the onset of arrhythmias [95,96], as previously hypothesized [21].

Breathing parameters of JWH-018 were slightly reverted immediately after nifedipine injection. This is in accordance with previous in vitro and in vivo studies, which showed the nifedipine through Ca^{2+} channel block within airway smooth cells, diminished airway resistance, leading to increase of breathing frequency [97–100]. Moreover, in line with our results on the SpO2 parameter, a study carried out by Watanabe and colleagues showed that nifedipine was also able to increase oxygen saturation level in hypoxic rats [101].

3.4. Propranolol

As a last attempt to solve both the JWH-018-induced cardiac effects and the possible onset of hypertension, it was decided to administer propranolol, a β-blocker commonly used as both an antihypertensive and antiarrhythmic drug [102]. The results showed that injection of 2 mg/kg of propranolol caused a further decrease in HR during first hours, but it reverted the HR at the end of the experiment, and it dropped tachyarrhythmic events immediately after injection (Figures 7A and 8C–F, respectively). On the contrary, JWH-018-induced breath rate reduction was partially increased immediately after propranolol administration and it was further reduced at the end of the experiment (Figure 7C). Moreover, propranolol increased the SpO_2 values (Figure 7D). The pulse distension was not affected by propranolol (Figure 7B).

In the heart, as expected, propranolol initially further decreased the low cardiac frequency of JWH-018-treated mice, causing a further increase of vagal activity due to β receptor block [102–104]. Concerning heart rate, treatment with propranolol induced an effect that could depend on the basal tone of JWH-018-injected mice, similar to the atropine effect on pulse distension. The increment of already high vagal tone in mice probably has led to a paradoxical effect. Indeed, following the initial and expected further decrease of heart rate, our results showed an increased HR during the last hours of the experiment, and this evidence was already shown in a clinical study [105]. Moreover, the reduction of tachyarrhythmia onset was in line with clinical studies with the well-known evidence that propranolol reduces arrhythmias, especially supraventricular tachyarrhythmias [106–109]. In particular, propranolol exerts its action on cardiac β1 receptors [109], leading to the reduction of ventricular contraction through catecholamines block on A-V node and hence could reduce and improve the chronotropic cardiac effect on mice [106]. The diminution of cardiac output, as well as the central and peripheral reduction of the sympathetic tone caused by propranolol [110], should suggest an increase in pulse distension. Despite the cardiac action, reduction of pulse distension induced by JWH-018 was not modulated by propranolol administration and this could suggest the hypothesis to exclude the involvement of sympathetic nerves from JWH-018-induced hypertension. Moreover, the breath rate trend could be related to heart failure. The effect could be closely linked to a probable JWH-018-induced ventricular dysfunction that could lead to respiratory damage [111,112]. Indeed, in heart failure patients, β blockers were able to improve pulmonary hemodynamics, diminishing the liquid content of the lung tissue and its relative effects on bronchial, alveolar, and interstitial tissue [113]. This could suggest why propranolol initially reverted JWH-018-induced bradypnea. Moreover, propranolol was able to abolish oxygen saturation reduction in accordance with Khambatta and colleagues' preclinical study [114]. The decrease of sympathetic tone could then prevail, further decreasing breath rate [115].

Our study is limited to the use of male mice and this choice was made based on increasing evidence of the greater risk of consumption among male adults than female and the higher susceptibility of males to SCs effects has been shown. In fact, previous reports have shown emergency room assistance has been required more frequently for males (78%) than

females (22%) patients following SCs intoxication in respect to cannabis intoxication [116]. Moreover, males have accounted for the 73.9% of patients who have contacted poison centers after SCs use and reported tachycardia among main adverse effects [14]. In line with these findings, Fogel and colleagues have more recently demonstrated that women can be less sensitive to the effects induced by high dosages of THC [117]. Despite this, both clinical [118] and preclinical [119,120] evidence suggest that females are more susceptible to cannabinoid-induced effects, even though cardiovascular responses are not mentioned among these studies.

Overall, our study sheds light on the CV effects of four pharmacological agents that are commonly used in Emergency Departments. In particular, the use of amiodarone and propranolol, and possibly other beta-blockers that must be tested, are particularly interesting because both of them can be used in the presence of coronary artery diseases and acute myocardial infarction. A systematic review [121] including 115 studies and other reports [19] has proven the increased risk of cardiovascular diseases, acute myocardial infarction, and ischemic stroke in healthy and young people consuming cannabis and synthetic cannabiminetics, so it will be pivotal to translate these findings to humans. In fact, the presence of tachycardia is the most reliable marker to study the effects of cannabinoids in humans [122] but it is also a predictor of an increased risk of CV morbidity and mortality because it leads to a reduction in the cardiac stroke volume and impairs the myocardial oxygen supply–demand. HR reduction is therefore a protective effect in the presence of acute myocardial infarction alone, and also in cannabinoid-induced tachycardia and their synergic effects could be particularly detrimental.

4. Materials and Methods

4.1. Animals

Male outbred ICR (CD-1®) mice (N = 112), 25–30 g (Centralized Preclinical Research Laboratory, University of Ferrara, Italy) were group-housed (five mice per cage; floor area per animal was 80 cm^2; minimum enclosure height was 12 cm) in a colony room under a constant temperature (23–24 °C) and humidity (45–55%). Food (Diet 4RF25 GLP; Mucedola, Settimo Milanese, Milan, Italy) and tap water were available ad libitum during the entire time the animals spent in their home cages. The daylight cycle was artificially maintained (dark between 7 p.m. and 7 a.m.). The experiments were performed during the light phase. The experimental protocol followed in the present study was in accordance with the new European Communities Council Directive of September 2010 (2010/63/EU), a revision of the Directive 86/609/EEC, and was approved by the Italian Ministry of Health (license no. 223/2021-PR and extension CBCC2.46.EXT.21) and the Ethics Committee of the University of Ferrara. According to the ARRIVE guidelines, all possible efforts were made to minimize the number of animals used, minimize the animals' pain and discomfort, and reduce the number of experimental subjects. In this study, the determination of the number of animals to be used (sample size) and the calculation of the appropriate power in the statistical data analysis (power analysis) was determined using the simulation software G*Power 3.1.9.2 (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany) [123]. Following the manual G*Power 3.1.9.2 we then carried out the Prior power analysis [124] that allows calculation of the number of animals to be used (N or sample size). Thus, in function of the actual power level of the analysis ($0.99 < \beta < 1.00$) of the level of significance ($\alpha = 0.05$) to be achieved and according to the magnitude of the effect reported in the present tests (effect size f) a sample size of eight animals per group was calculated. In the cardiorespiratory studies (vehicle, JWH-018 6 mg/kg, amiodarone 5 mg/kg, atropine 5 mg/kg, nifedipine 1 mg/kg, propranolol 2 mg/kg, JWH-018 + amiodarone, JWH-018 + atropine, JWH-018 + nifedipine, or JWH-018 + propranolol) eight mice were used per group (total mice used: 80). For systolic and diastolic pressure study (saline, JWH-018 6 mg/kg, nifedipine, and JWH + nifedipine) eight mice were used per group (total mice used: 32). For all experiments, only male mice were used, following international trends that identify men as the main cannabinoid consummators [125].

4.2. Drug Preparation and Dose Selection

JWH-018 was purchased from LGC Standards (LGC Standards S.r.L., Sesto San Giovanni, Milan, Italy) while amiodarone, atropine, nifedipine, and propranolol were from Tocris (Tocris, Bristol, UK). Drugs were initially dissolved in absolute ethanol (final concentration was 5%) and Tween 80 (2%) and brought to the final volume with saline (0.9% NaCl). The solution made with ethanol, Tween 80, and saline was also used as the vehicle. Amiodarone (5 mg/kg), atropine (5 mg/kg), nifedipine (1 mg/kg), and propranolol (2 mg/kg) were administered 60 min after JWH-018 injection. Drugs were administered by intraperitoneal injection (i.p.) in a volume of 4 µL/g.

The dose selection was evaluated based on a previous study by our group [21] that showed severe CV effects induced by 6 mg/kg JWH-018. In line with HED formula and with dosage scale reported by users [126,127], this dose represents a toxic dose of JWH-018 in humans. The dosage of amiodarone [128], atropine [129,130], nifedipine [131], and propranolol [132] were chosen from previous preclinical studies on rodents.

4.3. Evaluation of Cardiorespiratory Changes

As previously reported, to monitor the cardiorespiratory parameters in awake and freely moving animals without using invasive instruments and handling, a collar with a sensor was used to detect heart rate (HR), breath rate (BR), oxygen blood saturation (SpO2), and pulse distension (PD) with a frequency of 15 Hz [133–136]. During the experiment, the mouse was allowed to freely move around its cage (30 × 30 × 20 cm) while having no access to food or water while being monitored by the sensor collar through the software MouseOx Plus 1.6 (STARR Life Sciences® Corp., Oakmont, PA, USA). In the first hour of acclimation, a fake collar similar in design to the collar used in the test but without a sensor was used to minimize the potential stress of the mouse during the experiment. The collar with the sensor was then applied, while the baseline parameters were monitored for 60 min. Subsequently, drugs or the vehicle were administered. Amiodarone (5 mg/kg), atropine (5 mg/kg), nifedipine (1 mg/kg), and propranolol (2 mg/kg) were administered 60 min after JWH-018 injection. The data were recorded for 5 h.

Due to its primary effects on blood pressure, the co-administration with nifedipine (1 mg/kg) is also evaluated on BP-2000 system. As previously reported [133], systolic and diastolic blood pressure were measured by tail-cuff plethysmography using a BP-2000 blood pressure analysis system (Visitech Systems, Apex, NC, USA). For each session, mice were placed in a metal box restraint with its tail passing through the optical sensor and compression cuff before finally being taped to the platform. A traditional tail-cuff occluder was placed proximally on the animal's tail, which was then immobilized with tape in a V-shaped block between a light source above and a photoresistor below. Upon inflation, the occluder stopped blood flow through the tail, while upon deflation, the sensor detected the blood flow return. The restraint platform was maintained at 37 °C. Before experiments, mice were acclimated to restraint and tail-cuff inflation for 5–7 days. On the test day, 10 measurements were made to collect basal blood pressure. Upon the tenth analysis, the software was paused, and mice were injected with either drug treatments or the vehicle; animals were then repositioned in the restraints, and 60 measurements were acquired.

4.4. Data and Statistical Analysis

Data related to HR, PD, BR, and SpO2 changes are expressed as a percentage of basal value. While tachyarrhythmia analysis expressed in histograms represents the number of tachyarrhythmia events (divided in each hour, for 6 h). A tachyarrhythmic event was recorded when heart pulse was almost >200 pulses higher compared to mean basal HR, after vehicle or drug administration [21]. The statistical analysis of the dose–response curve of different substances and the analysis of tachyarrhythmia frequencies was performed by two-way ANOVA followed by Bonferroni's test for multiple comparisons.

The significance level was set at $p < 0.05$. Cardiovascular data are expressed as a percentage of the baseline value with mean ± standard error of the mean (SEM) of the

eight independent experiments. Tachyarrhythmia data are expressed as a number of events with mean ± SEM of the eight independent experiments. All statistical analyses were performed using GraphPad Prism 8.0.1 software (GraphPad Prism, San Diego, CA, USA). Changes in systolic and diastolic blood pressure were expressed as absolute values (mmHg) of average effect. The effects of different average effect of each substance were analyzed by a one-way ANOVA followed by Bonferroni's test for multiple comparisons where appropriate. Data were reported as mean standard error of the mean (SEM) of at least eight independent experiments.

5. Conclusions

In conclusion, our study helps to better clarify the mechanisms under cardio-respiratory dysfunctions induced by JWH-018 (6 mg/kg). In particular, our results corroborated a previous study hypothesis [21] in which heart rate decrease was linked to vagal tone increase via CB1 or CB2 receptors. Indeed, only muscarinic receptor block, through atropine administration, entirely enhanced JWH-018-induced bradycardia one hour from administration. Beyond this, our results showed that all examined CV drugs improved tachyarrhythmias induced by JWH-018, suggesting different mechanisms behind onset of tachyarrhythmias, including vagal and sympathetic tone disbalance, and ion channel involvement. Regarding pulse distension, only atropine slightly increased it, but as above explained, this could be a reflex mechanism. Finally, even breathing responses suggested different mechanisms involved. Again, all drugs tested improved respiratory rate, in particular amiodarone administration. These data, beyond suggesting and defining different mechanisms behind JWH-018-induced CV damage, are important to identify possible antidotes in case of JWH-018 or other SCs cardiac intoxication, highlighting the strong public health impact induced by JWH-018 diffuse use.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved Ministry of Health (protocol code 223/2021-PR and extension CBCC2.46.EXT.21). All applicable international, national and/or institutional guidelines for the care and use of animals were followed. All procedures performed in the studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted. Project activated in collaboration with the Presidency of the Council of Ministers-DPA Anti-Drug Policies (Italy).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the first author (Beatrice Marchetti) and corresponding author (Matteo Marti) for researchers of academic institutes who meet the criteria for access to the confidential data.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

AM 251	1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-N-(piperidin-1-yl)-1H-pyrazole-3-carboxamide
A-V	Atrio-Ventricular
BR	Breath Rate
CNS	Central Nervous System
CV	Cardiovascular
dPAG	Dorsal Periaqueductal Gray
HR	Heart Rate
JWH-018	1-pentyl-3-(1-naphthoyl)indole
NPS	Novel Psychoactive Substances
PD	Pulse Distention
S-A	Seno-Atrial
SCs	Synthetic Cannabinoids
SpO2	Oxygen blood saturation
Δ9-THC	(-)-Δ9-THC or Dronabinol®

References

1. Pisarska, A.; Deluca, P.; Demetrovics, Z.; Moskalewicz, J.; ReDNet, G. Novel psychoactive substances (NPS)-knowledge and experiences of drug users from Hungary, Poland, the UK and the USA. *Neuropsychopharmacol. Hung.* **2019**, *21*, 152–163.
2. Kennedy, J.; Shanks, K.G.; Van Natta, K.; Prieto Conaway, M.C.; Wiseman, J.M.; Laughlin, B.; Kozak, M. Rapid screening and identification of novel psychoactive substances using PaperSpray interfaced to high resolution mass spectrometry. *Clin. Mass Spectrom.* **2016**, *1*, 3–10. [CrossRef]
3. Locatelli, C.A.; Lonati, D.; Petrolini, V.M. New drugs of abuse and cardiovascular function. In *Brain and Heart Dynamics*; Govoni, S., Politi, P., Vanoli, E., Eds.; Springer: Cham, Switerland, 2020; pp. 1–27. [CrossRef]
4. European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). *European Drug Report 2021: Trends and Developments*; Publications Office of the European Union: Luxembourg, 2021.
5. United Nations Office on Drugs and Crime (UNODC). Current NPS threats. Available online: https://www.unodc.org/documents/scientific/NPS_threats-IV.pdf (accessed on 12 January 2023).
6. Adamowicz, P. Blood concentrations of synthetic cannabinoids. *Clin. Toxicol.* **2021**, *59*, 246–251. [CrossRef]
7. Theunissen, E.L.; Reckweg, J.T.; Hutten, N.R.P.W.; Kuypers, K.P.C.; Toennes, S.W.; Neukamm, M.A.; Halter, S.; Ramaekers, J.G. Psychotomimetic symptoms after a moderate dose of a synthetic cannabinoid (JWH-018): Implications for psychosis. *Psychopharmacology* **2021**, *239*, 1251–1261. [CrossRef] [PubMed]
8. Bretteville-Jensen, A.L.; Tuv, S.S.; Bilgrei, O.R.; Fjeld, B.; Bachs, L. Synthetic cannabinoids and cathinones: Prevalence and markets. *Forensic Sci. Rev.* **2013**, *25*, 7–26. [PubMed]
9. Norman, C.; McKirdy, B.; Walker, G.; Dugard, P.; NicDaéid, N.; McKenzie, C. Large-scale evaluation of ion mobility spectrometry for the rapid detection of synthetic cannabinoid receptor agonists in infused papers in prisons. *Drug Test Anal.* **2021**, *13*, 644–663. [CrossRef] [PubMed]
10. Tai, H.; Swartz, M.D.; Marsden, D.; Perry, C.L. The Future of Substance Abuse Now: Relationships among Adolescent Use of Vaping Devices, Marijuana, and Synthetic Cannabinoids. *Subst. Use Misuse* **2021**, *56*, 192–204. [CrossRef]
11. Seely, K.A.; Lapoint, J.; Moran, J.H.; Fattore, L. Spice drugs are more than harmless herbal blends: A review of the pharmacology and toxicology of synthetic cannabinoids. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **2012**, *39*, 234–243. [CrossRef]
12. Hermanns-Clausen, M.; Kneisel, S.; Szabo, B.; Auwärter, V. Acute toxicity due to the confirmed consumption of synthetic cannabinoids: Clinical and laboratory findings. *Addiction* **2013**, *108*, 534–544. [CrossRef]
13. Gurney, S.M.; Scott, K.S.; Kacinko, S.L.; Presley, B.C.; Logan, B.K. Pharmacology, toxicology, and adverse effects of synthetic cannabinoid drugs. *Forensic Sci. Rev.* **2014**, *26*, 53–78.
14. Forrester, M.B.; Kleinschmidt, K.; Schwarz, E.; Young, A. Synthetic cannabinoid exposures reported to Texas poison centers. *J. Addict. Dis.* **2011**, *30*, 351–358. [CrossRef]
15. Harris, C.R.; Brown, A. Synthetic cannabinoid intoxication: A case series and review. *J. Emerg. Med.* **2013**, *44*, 360–366. [CrossRef]
16. Tait, R.J.; Caldicott, D.; Mountain, D.; Hill, S.L.; Lenton, S. A systematic review of adverse events arising from the use of synthetic cannabinoids and their associated treatment. *Clin. Toxicol.* **2016**, *54*, 1–13. [CrossRef]
17. Alon, M.H.; Saint-Fleur, M.O. Synthetic cannabinoid induced acute respiratory depression: Case series and literature review. *Respir. Med. Case Rep.* **2017**, *22*, 137–141. [CrossRef]
18. Alipour, A.; Patel, P.B.; Shabbir, Z.; Gabrielson, S. Review of the many faces of synthetic cannabinoid toxicities. *Ment. Health Clin.* **2019**, *9*, 93–99. [CrossRef]
19. Weresa, J.; Pędzińska-Betiuk, A.; Mińczuk, K.; Malinowska, B.; Schlicker, E. Why do marijuana and synthetic cannabimimetics induce acute myocardial infarction in healthy young people? *Cells* **2022**, *11*, 1142. [CrossRef]

20. Kane, E.M.; Hinson, J.S.; Jordan, C.D.; Paziana, K.; Sauber, N.J.; Rothman, R.E.; Stolbach, A.I. Bradycardia and hypotension after synthetic cannabinoid use: A case series. *Am. J. Emerg. Med.* **2016**, *34*, 2055.e1–2055.e2. [CrossRef]
21. Marchetti, B.; Bilel, S.; Tirri, M.; Arfè, R.; Corli, G.; Roda, E.; Locatelli, C.A.; Cavarretta, E.; De Giorgio, F.; Marti, M. The Old and the New: Cardiovascular and respiratory alterations induced by acute jwh-018 administration compared to δ9-thc-a preclinical study in mice. *Int. J. Mol. Sci.* **2023**, *24*, 1631. [CrossRef]
22. Banister, S.D.; Wilkinson, S.M.; Longworth, M.; Stuart, J.; Apetz, N.; English, K.; Brooker, L.; Goebel, C.; Hibbs, D.E.; Glass, M.; et al. The synthesis and pharmacological evaluation of adamantane-derived indoles: Cannabimimetic drugs of abuse. *ACS Chem. Neurosci.* **2013**, *4*, 1081–1092. [CrossRef]
23. Schindler, C.W.; Gramling, B.R.; Justinova, Z.; Thorndike, E.B.; Baumann, M.H. Synthetic cannabinoids found in "spice" products alter body temperature and cardiovascular parameters in conscious male rats. *Drug Alcohol. Depend.* **2017**, *179*, 387–394. [CrossRef]
24. Schmid, K.; Niederhoffer, N.; Szabo, B. Analysis of the respiratory effects of cannabinoids in rats. *Naunyn Schmiedebergs Arch. Pharmacol.* **2003**, *368*, 301–308. [CrossRef] [PubMed]
25. Bilel, S.; Tirri, M.; Arfè, R.; Stopponi, S.; Soverchia, L.; Ciccocioppo, R.; Frisoni, P.; Strano-Rossi, S.; Miliano, C.; De-Giorgio, F.; et al. Pharmacological and behavioral effects of the synthetic cannabinoid AKB48 in rats. *Front. Neurosci.* **2019**, *13*, 1163. [CrossRef] [PubMed]
26. Mir, A.; Obafemi, A.; Young, A.; Kane, C. Myocardial infarction associated with use of the synthetic cannabinoid K2. *Pediatrics* **2011**, *128*, e1622–e1627. [CrossRef] [PubMed]
27. Shanks, K.G.; Dahn, T.; Terrell, A.R. Detection of JWH-018 and JWH-073 by UPLC-MS-MS in postmortem whole blood casework. *J. Anal. Toxicol.* **2012**, *36*, 145–152. [CrossRef] [PubMed]
28. Trecki, J.; Gerona, R.R.; Schwartz, M.D. Synthetic cannabinoid-related illnesses and deaths. *N. Engl. J. Med.* **2015**, *373*, 103–107. [CrossRef] [PubMed]
29. Ibrahim, S.; Al-Saffar, F.; Wannenburg, T. A Unique case of cardiac arrest following K2 abuse. *Case Rep. Cardiol.* **2014**, *2014*, 120607. [CrossRef]
30. Ossato, A.; Vigolo, A.; Trapella, C.; Seri, C.; Rimondo, C.; Serpelloni, G.; Marti, M. JWH-018 impairs sensorimotor functions in mice. *Neuroscience* **2015**, *300*, 174–188. [CrossRef]
31. Tsou, K.; Brown, S.; Sañudo-Peña, M.C.; Mackie, K.; Walker, J.M. Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. *Neuroscience* **1998**, *83*, 393–411. [CrossRef]
32. Niederhoffer, N.; Szabo, B. Cannabinoids cause central sympathoexcitation and bradycardia in rabbits. *J. Pharmacol. Exp. Ther.* **2000**, *294*, 707–713.
33. Dean, C.; Hillard, C.J.; Seagard, J.L.; Hopp, F.A.; Hogan, Q.H. Components of the cannabinoid system in the dorsal periaqueductal gray are related to resting heart rate. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2016**, *311*, R254–R262. [CrossRef]
34. Pacher, P.; Steffens, S.; Haskó, G.; Schindler, T.H.; Kunos, G. Cardiovascular effects of marijuana and synthetic cannabinoids: The good, the bad, and the ugly. *Nat. Rev. Cardiol.* **2018**, *15*, 151–166. [CrossRef]
35. Steffens, S.; Pacher, P. Targeting cannabinoid receptor CB(2) in cardiovascular disorders: Promises and controversies. *Br. J. Pharmacol.* **2012**, *167*, 313–323. [CrossRef]
36. Tahamtan, A.; Samieipoor, Y.; Nayeri, F.S.; Rahbarimanesh, A.A.; Izadi, A.; Rashidi-Nezhad, A.; Tavakoli-Yaraki, M.; Farahmand, M.; Bont, L.; Shokri, F.; et al. Effects of canna-binoid receptor type 2 in respiratory syncytial virus infection in human subjects and mice. *Virulence* **2018**, *9*, 217–230. [CrossRef]
37. Beaconsfield, P.; Ginsburg, J.; Rainsbury, R. Marihuana smoking. Cardiovascular effects in man and possible mechanisms. *N. Engl. J. Med.* **1972**, *287*, 209–212. [CrossRef]
38. Richards, J.R. Mechanisms for the risk of acute coronary syndrome and arrhythmia associated with phytogenic and synthetic cannabinoid use. *J. Cardiovasc. Pharmacol. Ther.* **2020**, *25*, 508–522. [CrossRef]
39. Nerbonne, J.M.; Kass, R.S. Molecular physiology of cardiac repolarization. *Physiol. Rev.* **2005**, *85*, 1205–1253. [CrossRef]
40. Masini, E.; Sgambellone, S.; Lanzi, C. Psychostimulants and cardiovascular function. In *Brain and Heart Dynamics*; Govoni, S., Politi, P., Vanoli, E., Eds.; Springer: Cham, Switzerland, 2020; pp. 829–841. [CrossRef]
41. Ross, H.R.; Napier, I.; Connor, M. Inhibition of recombinant human T-type calcium channels by Delta9-tetrahydrocannabinol and cannabidiol. *J. Biol. Chem.* **2008**, *283*, 16124–16134. [CrossRef]
42. Turkanis, S.A.; Partlow, L.M.; Karler, R. Delta-9-tetrahydrocannabinol depresses inward sodium current in mouse neuroblastoma cells. *Neuropharmacology* **1991**, *30*, 73–77. [CrossRef]
43. Al Kury, L.T.; Voitychuk, O.I.; Yang, K.H.; Thayyullathil, F.T.; Doroshenko, P.; Ramez, A.M.; Shuba, Y.M.; Galadari, S.; Howarth, F.C.; Oz, M. Effects of the endogenous cannabinoid anandamide on voltage-dependent sodium and calcium channels in rat ventricular myocytes. *Br. J. Pharmacol.* **2014**, *171*, 3485–3498. [CrossRef]
44. Barana, A.; Amorós, I.; Caballero, R.; Gómez, R.; Osuna, L.; Lillo, M.P.; Blázquez, C.; Guzmán, M.; Delpón, E.; Tamargo, J. Endocannabinoids and cannabinoid analogues block cardiac hKv1.5 channels in a cannabinoid receptor-independent manner. *Cardiovasc. Res.* **2010**, *85*, 56–67. [CrossRef]
45. Motyka, M.A.; Al-Imam, A. Representations of psychoactive drugs' use in mass culture and their impact on audiences. *Int. J. Environ. Res. Public Health* **2021**, *18*, 6000. [CrossRef] [PubMed]
46. Borgonhi, E.M.; Volpatto, V.L.; Ornell, F.; Rabelo-da-Ponte, F.D.; Kessler, F.H.P. Multiple clinical risks for cannabis users during the COVID-19 pandemic. *Addict. Sci. Clin. Pract.* **2021**, *16*, 5. [CrossRef] [PubMed]

47. Page, R.L., 2nd; Allen, L.A.; Kloner, R.A.; Carriker, C.R.; Martel, C.; Morris, A.A.; Piano, M.R.; Rana, J.S.; Saucedo, J.F.; American Heart Association Clinical Pharmacology Committee and Heart Failure and Transplantation Committee of the Council on Clinical Cardiology; et al. Medical marijuana, recreational cannabis, and cardiovascular health: A scientific statement from the american heart association. *Circulation* **2020**, *142*, e131–e152. [CrossRef] [PubMed]
48. Adami, P.E.; Koutlianos, N.; Baggish, A.; Bermon, S.; Cavarretta, E.; Deligiannis, A.; Furlanello, F.; Kouidi, E.; Marques-Vidal, P.; Niebauer, J.; et al. Cardiovascular effects of doping substances, commonly prescribed medications and ergogenic aids in relation to sports: A position statement of the sport cardiology and exercise nucleus of the European Association of Preventive Cardiology. *Eur. J. Prev. Cardiol.* **2022**, *29*, 559–575. [CrossRef]
49. Von Der Haar, J.; Talebi, S.; Ghobadi, F.; Singh, S.; Chirurgi, R.; Rajeswari, P.; Kalantari, H.; Hassen, G.W. Synthetic cannabinoids and their effects on the cardiovascular system. *J. Emerg. Med.* **2016**, *50*, 258–262. [CrossRef] [PubMed]
50. Hancox, J.C.; Kalk, N.J.; Henderson, G. Synthetic cannabinoids and potential cardiac arrhythmia risk: An important message for drug users. *Ther. Adv. Drug Saf.* **2020**, *11*, 2042098620913416. [CrossRef]
51. Cooper, Z.D. Adverse effects of synthetic cannabinoids: Management of acute toxicity and withdrawal. *Curr. Psychiatry Rep.* **2016**, *18*, 52. [CrossRef]
52. Jones, J.D.; Nolan, M.L.; Daver, R.; Comer, S.D.; Paone, D. Can naloxone be used to treat synthetic cannabinoid overdose? *Biol. Psychiatry* **2017**, *81*, e51–e52. [CrossRef]
53. Pryce, G.; Baker, D. Antidote to cannabinoid intoxication: The CB1 receptor inverse agonist, AM251, reverses hypothermic effects of the CB1 receptor agonist, CB-13, in mice. *Br. J. Pharmacol.* **2017**, *174*, 3790–3794. [CrossRef]
54. Meredith, G.; DeLollis, M.; Shad, M.U. Potential treatment for overdose with synthetic cannabinoids. *Med. Cannabis Cannabinoids* **2020**, *3*, 74–75. [CrossRef]
55. Trappe, H.J. Concept of the five 'A's for treating emergency arrhythmias. *J. Emerg. Trauma Shock* **2010**, *3*, 129–136. [CrossRef] [PubMed]
56. Dan, G.A.; Martinez-Rubio, A.; Agewall, S.; Boriani, G.; Borggrefe, M.; Gaita, F.; van Gelder, I.; Gorenek, B.; Kaski, J.C.; Kjeldsen, K.; et al. ESC Scientific Document Group. Antiarrhythmic drugs-clinical use and clinical decision making: A consensus document from the European Heart Rhythm Association (EHRA) and European Society of Cardiology (ESC) Working Group on Cardiovascular Pharmacology, endorsed by the Heart Rhythm Society (HRS), Asia-Pacific Heart Rhythm Society (APHRS) and International Society of Cardiovascular Pharmacotherapy (ISCP). *Europace* **2018**, *20*, 731–732an. [CrossRef] [PubMed]
57. Dominic, P.; Ahmad, J.; Awwab, H.; Bhuiyan, M.S.; Kevil, C.G.; Goeders, N.E.; Murnane, K.S.; Patterson, J.C.; Sandau, K.E.; Gopinathannair, R.; et al. Stimulant drugs of abuse and cardiac arrhythmias. *Circ. Arrhythm. Electrophysiol.* **2022**, *15*, e010273. [CrossRef] [PubMed]
58. Testa, A.; Ojetti, V.; Migneco, A.; Serra, M.; Ancona, C.; De Lorenzo, A.; Gentiloni Silveri, N. Use of amiodarone in emergency. *Eur. Rev. Med. Pharmacol. Sci.* **2005**, *9*, 183–190. [PubMed]
59. Mujović, N.; Dobrev, D.; Marinković, M.; Russo, V.; Potpara, T.S. The role of amiodarone in contemporary management of complex cardiac arrhythmias. *Pharmacol. Res.* **2020**, *151*, 104521. [CrossRef]
60. Niederhoffer, N.; Szabo, B. Effect of the cannabinoid receptor agonist WIN55212-2 on sympathetic cardiovascular regulation. *Br. J. Pharmacol.* **1999**, *126*, 457–466. [CrossRef]
61. Kodama, I.; Kamiya, K.; Toyama, J. Cellular electropharmacology of amiodarone. *Cardiovasc. Res.* **1997**, *35*, 13–29. [CrossRef]
62. Campbell, N.; Agarwal, K.; Alidoost, M.; Miskoff, J.A.; Hossain, M. Acute fulminant hepatic failure and renal failure induced by oral amiodarone: A case report and literature review. *Cureus* **2020**, *12*, e8311. [CrossRef]
63. Richards, J.R.; Bing, M.L.; Moulin, A.K.; Elder, J.W.; Rominski, R.T.; Summers, P.J.; Laurin, E.G. Cannabis use and acute coronary syndrome. *Clin. Toxicol.* **2019**, *57*, 831–841. [CrossRef]
64. Yamanoglu, A.; Celebi Yamanoglu, N.G.; Evran, T.; Sogut, O. How much can synthetic cannabinoid damage the heart? A case of cardiogenic shock following resistant ventricular fibrillation after synthetic cannabinoid use. *J. Clin. Ultrasound.* **2018**, *46*, 605–609. [CrossRef]
65. Sampat, P.J.; Riaz, S.; Bisen, M.; Carhart, R. An unusual case of ventricular tachycardia in a young patient associated with cannabis use. *Case Rep. Cardiol.* **2020**, *2020*, 8813930. [CrossRef] [PubMed]
66. Billington, C.K.; Penn, R.B.; Hall, I.P. β2 agonists. *Handb. Exp. Pharmacol.* **2017**, *237*, 23–40. [CrossRef] [PubMed]
67. Vaillancourt, M.; Chia, P.; Sarji, S.; Nguyen, J.; Hoftman, N.; Ruffenach, G.; Eghbali, M.; Mahajan, A.; Umar, S. Autonomic nervous system involvement in pulmonary arterial hypertension. *Respir. Res.* **2017**, *18*, 201. [CrossRef] [PubMed]
68. Norman, K.; Nappe, T.M. Alpha receptor agonist toxicity. In *StatPearls*; StatPearls Publishing: Treasure Island, FL, USA, 2022.
69. Manresa, F.; Dorca, J.; Rodriguez Sanchon, B.; Romero Colomer, P. Recurrent form of amiodarone-induced pneumonitis. *Chest* **1984**, *86*, 944. [CrossRef]
70. Deşer, S.B.; Yücel, S.M. Management of hyperacute amiodarone-induced pulmonary toxicity. *J. Cardio-Vasc.-Thorac. Anaesth. Intensive Care Soc.* **2018**, *24*, 187–189. [CrossRef]
71. Lemaire-Hurtel, A.S. Drugs involved in drug-facilitated crime—Pharmacological aspects. In *Toxicological Aspects of Drug-Facilitated Crimes*; Klintz, P., Ed.; Academic Press: Cambridge, MA, USA, 2014; pp. 47–49. [CrossRef]
72. Coon, G.A.; Clinton, J.E.; Ruiz, E. Use of atropine for brady-asystolic prehospital cardiac arrest. *Ann. Emerg. Med.* **1981**, *10*, 462–467. [CrossRef]

73. Swart, G.; Brady, W.J., Jr.; DeBehnke, D.J.; Ma, O.J.; Aufderheide, T.P. Acute myocardial infarction complicated by hemodynamically unstable bradyarrhythmia: Prehospital and ED treatment with atropine. *Am. J. Emerg. Med.* **1999**, *17*, 647–652. [CrossRef]
74. Brady, W.J., Jr.; Harrigan, R.A. Diagnosis and management of bradycardia and atrioventricular block associated with acute coronary ischemia. *Emerg. Med. Clin. N. Am.* **2001**, *19*, 371–384. [CrossRef]
75. Carleton, R.A.; Miller, P.H.; Graettinger, J.S. Effects of ouabain, atropine, and ouabain and atropine on A-V nodal conduction in man. *Circ. Res.* **1967**, *20*, 283–288. [CrossRef]
76. Gravenstein, J.S.; Ariet, M.; Thornby, J.I. Atropine on the electrocardiogram. *Clin. Pharmacol. Ther.* **1969**, *10*, 660–666. [CrossRef]
77. Lowenstein, J.; Steele, J.M., Jr. Appraisal and reappraisal of cardiac therapy: Prazosin. *Am. Heart J.* **1978**, *95*, 262–265. [CrossRef] [PubMed]
78. Ishii, M.; Kurachi, Y. Muscarinic acetylcholine receptors. *Curr. Pharm. Des.* **2006**, *12*, 3573–3581. [CrossRef] [PubMed]
79. Neeld, J.B., Jr.; Allen, A.T.; Coleman, E.; Frederickson, E.L.; Goldberg, L.I. Cardiac rate and rhythm changes with atropine and methscopolamine. *Clin. Pharmacol. Ther.* **1975**, *17*, 290–295. [CrossRef] [PubMed]
80. Rosen, K.M.; Loeb, H.S.; Sinno, M.Z.; Rahimtoola, S.H.; Gunnar, R.M. Cardiac conduction in patients with symptomatic sinus node disease. *Circulation* **1971**, *43*, 836–844. [CrossRef]
81. Schweitzer, P.; Mark, H. The effect of atropine on cardiac arrhythmias and conduction. Part 1. *Am. Heart J.* **1980**, *100*, 119–127. [CrossRef] [PubMed]
82. Anzillotti, L.; Marezza, F.; Calò, L.; Banchini, A.; Cecchi, R. A case report positive for synthetic cannabinoids: Are cardiovascular effects related to their protracted use? *Leg. Med.* **2019**, *41*, 101637. [CrossRef]
83. Coffman, J.D.; Cohen, R.A. Cholinergic vasodilator mechanism in human fingers. *Am. J. Physiol.* **1987**, *252 Pt 2*, H594–H597. [CrossRef]
84. Laitinen, L.A.; Laitinen, M.V.; Widdicombe, J.G. Parasympathetic nervous control of tracheal vascular resistance in the dog. *J. Physiol.* **1987**, *385*, 135–146. [CrossRef]
85. Cabrera, E.; Levenson, J.; Armentano, R.; Barra, J.; Pichel, R.; Simon, A. Constricting and stiffening action of atropine on aortic response to angiotensin in dogs. *Hypertension* **1988**, *11 Pt 2*, I103–I107. [CrossRef]
86. Abraham, S.; Cantor, E.H.; Spector, S. Studies on the hypotensive response to atropine in hypertensive rats. *J. Pharmacol. Exp. Ther.* **1981**, *218*, 662–668.
87. Larson, R.A.; Chapleau, M.W. Increased cardiac sympathetic activity: Cause or compensation in vasovagal syncope? *Clin. Auton. Res.* **2018**, *28*, 265–266. [CrossRef]
88. Doherty, P.A.; McCarthy, L.E.; Borison, H.L. Respiratory and cardiovascular depressant effects of nabilone, N-methyllevonantradol and delta 9-tetrahydrocannabinol in anesthetized cats. *J. Pharmacol. Exp. Ther.* **1983**, *227*, 508–516.
89. Richardson, C.A.; Herbert, D.A.; Mitchell, R.A. Modulation of pulmonary stretch receptors and airway resistance by parasympathetic efferents. *J. Appl Physiol. Respir. Environ. Exerc. Physiol.* **1984**, *57*, 1842–1849. [CrossRef]
90. Stewart, H.J. The effect of increased heart rate due to the injection of atropine on the oxygen saturation of the arterial and venous blood of patients with heart disease. *J. Clin. Investig.* **1926**, *3*, 241–251. [CrossRef]
91. Khan, K.M.; Patel, J.; Schaefer, T.J. Nifedipine. In *StatPearls*; StatPearls Publishing: Treasure Island, FL, USA, 2022.
92. Ralevic, V.; Kendall, D.A. Cannabinoid modulation of perivascular sympathetic and sensory neurotransmission. *Curr. Vasc. Pharmacol.* **2009**, *7*, 15–25. [CrossRef] [PubMed]
93. Dean, C. Cannabinoid and GABA modulation of sympathetic nerve activity and blood pressure in the dorsal periaqueductal gray of the rat. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2011**, *301*, R1765–R1772. [CrossRef] [PubMed]
94. Fink, G.D. Does Tail-cuff plethysmography provide a reliable estimate of central blood pressure in mice? *J. Am. Heart Assoc.* **2017**, *6*, e006554. [CrossRef]
95. Pogwizd, S.M.; Schlotthauer, K.; Li, L.; Yuan, W.; Bers, D.M. Arrhythmogenesis and contractile dysfunction in heart failure: Roles of sodium-calcium exchange, inward rectifier potassium current, and residual beta-adrenergic responsiveness. *Circ. Res.* **2001**, *88*, 1159–1167. [CrossRef] [PubMed]
96. Bers, D.M. Cardiac excitation-contraction coupling. *Nature* **2002**, *415*, 198–205. [CrossRef] [PubMed]
97. Moore, R.L.; Binger, C.A. The response to respiratory resistance: A comparison of the effects produced by partial obstruction in the inspiratory and expiratory phases of respiration. *J. Exp. Med.* **1927**, *45*, 1065–1080. [CrossRef]
98. Fanta, C.H.; Watson, J.W.; Lacouture, P.G.; Drazen, J.M. In vivo bronchodilator activity of nifedipine in the guinea pig. *Am. Rev. Respir. Dis.* **1987**, *136*, 76–79. [CrossRef] [PubMed]
99. Flores-Soto, E.; Reyes-García, J.; Sommer, B.; Montaño, L.M. Sarcoplasmic reticulum Ca(2+) refilling is determined by L-type Ca(2+) and store operated Ca(2+) channels in guinea pig airway smooth muscle. *Eur. J. Pharmacol.* **2013**, *721*, 21–28. [CrossRef] [PubMed]
100. Rubini, A.; Catena, V.; Del Monte, D.; Bosco, G. The effects of nifedipine on respiratory mechanics investigated by theend-inflation occlusion method in the rat. *J. Enzyme Inhib. Med. Chem.* **2017**, *32*, 1–4. [CrossRef]
101. Watanabe, K.; Kimura, Y.; Abiko, Y. Nifedipine increases oxygen saturation level of myoglobin in the rat heart during hypoxia. *Arch. Int. Pharmacodyn. Ther.* **1986**, *284*, 297–312. [PubMed]
102. Al-Majed, A.A.; Bakheit, A.H.H.; Abdel Aziz, H.A.; Alajmi, F.M.; AlRabiah, H. Propranolol. *Profiles Drug Subst. Excip. Relat. Methodol.* **2017**, *42*, 287–338. [CrossRef] [PubMed]

103. Vaile, J.C.; Fletcher, J.; Al-Ani, M.; Ross, H.F.; Littler, W.A.; Coote, J.H.; Townend, J.N. Use of opposing reflex stimuli and heart rate variability to examine the effects of lipophilic and hydrophilic beta-blockers on human cardiac vagal control. *Clin. Sci.* **1999**, *97*, 585–593. [CrossRef]
104. Randall, M.D.; Harris, D.; Kendall, D.A.; Ralevic, V. Cardiovascular effects of cannabinoids. *Pharmacol. Ther.* **2002**, *95*, 191–202. [CrossRef]
105. Santosh, P.J.; Bell, L.; Lievesley, K.; Singh, J.; Fiori, F. Paradoxical physiological responses to propranolol in a Rett syndrome patient: A case report. *BMC Pediatr.* **2016**, *16*, 194. [CrossRef]
106. Harrison, D.C.; Griffin, J.R.; Fiene, T.J. Effects of beta-adrenergic blockade with propranolol in patients with atrial arrhythmias. *N. Engl. J. Med.* **1965**, *273*, 410–415. [CrossRef]
107. Zilm, D.H.; Jacob, M.S.; MacLeod, S.M.; Sellers, E.M.; Ti, T.Y. Propranolol and chlordiazepoxide effects on cardiac arrhythmias during alcohol withdrawal. *Alcohol. Clin. Exp. Res.* **1980**, *4*, 400–405. [CrossRef]
108. Morganroth, J.; Horowitz, L.N.; Anderson, J.; Turlapaty, P. Comparative efficacy and tolerance of esmolol to propranolol for control of supraventricular tachyarrhythmia. *Am. J. Cardiol.* **1985**, *56*, 33F–39F. [CrossRef]
109. Kubik, M.M.; Gill, B.; Dawes, P.M. Propranolol in cardiac arrhythmias: A comparative study of the conventional and long-acting formulations. *Curr. Med. Res. Opin.* **1986**, *10*, 215–220. [CrossRef]
110. Bhagat, B.D. Mechanism of the antihypertensive effect of propranolol. *Gen. Pharmacol.* **1979**, *10*, 291–296. [CrossRef]
111. Karnani, N.G.; Reisfield, G.M.; Wilson, G.R. Evaluation of chronic dyspnea. *Am. Fam. Physician* **2005**, *71*, 1529–1537. [PubMed]
112. Kupper, N.; Bonhof, C.; Westerhuis, B.; Widdershoven, J.; Denollet, J. Determinants of dyspnea in chronic heart failure. *J. Card. Fail.* **2016**, *22*, 201–209. [CrossRef]
113. Antonelli-Incalzi, R.; Pedone, C. Respiratory effects of beta-adrenergic receptor blockers. *Curr. Med. Chem.* **2007**, *14*, 1121–1128. [CrossRef]
114. Khambatta, H.J.; Stone, J.G.; Askanazi, J.; Khan, E. Propranolol increases oxygen utilization during hypoxia. *Br. J. Anaesth.* **1987**, *59*, 1171–1176. [CrossRef]
115. Narkiewicz, K.; Kjeldsen, S.E.; Oparil, S.; Hedner, T. Beta-blockers as sub-optimal treatment for hypertension: Time for first-line therapy revision? *Blood Press.* **2006**, *15*, 323–324. [CrossRef] [PubMed]
116. DAWN. *The DAWN. Report: Drug-Related Emergency Department Visits Involving Synthetic Cannabinoids*; Substance Abuse and Mental Health Services Administration (SAMHSA), Center for Behavioral Health Statistics and Quality: Rockville, MD, USA, 2012.
117. Fogel, J.S.; Kelly, T.H.; Westgate, P.M.; Lile, J.A. Sex differences in the subjective effects of oral Δ9-THC in cannabis users. *Pharmacol. Biochem. Behav.* **2017**, *152*, 44–51. [CrossRef] [PubMed]
118. Nia, A.B.; Mann, C.; Kaur, H.; Ranganathan, M. Cannabis use: Neurobiological, behavioral, and sex/gender considerations. *Curr. Behav. Neurosci. Rep.* **2018**, *5*, 271–280. [PubMed]
119. Wiley, J.L.; Walentiny, D.M.; Wright, M.J., Jr.; Beardsley, P.M.; Burston, J.J.; Poklis, J.L.; Lichtman, A.H.; Vann, R.E. Endocannabinoid contribution to Δ9-tetrahydrocannabinol discrimination in rodents. *Eur. J. Pharmacol.* **2014**, *737*, 97–105. [CrossRef] [PubMed]
120. Wiley, J.L.; Lefever, T.W.; Marusich, J.A.; Craft, R.M. Comparison of the discriminative stimulus and response rate effects of Δ9-tetrahydrocannabinol and synthetic cannabinoids in female and male rats. *Drug Alcohol. Depend.* **2017**, *172*, 51–59. [CrossRef] [PubMed]
121. Jouanjus, E.; Raymond, V.; Lapeyre-Mestre, M.; Wolff, V. What is the current knowledge about the cardiovascular risk for users of cannabis-based products? A Systematic Review. *Curr. Atheroscler. Rep.* **2017**, *19*, 26. [CrossRef]
122. Zuurman, L.; Ippel, A.E.; Moin, E.; van Gerven, J.M. Biomarkers for the effects of cannabis and THC in healthy volunteers. *Br. J. Clin. Pharmacol.* **2009**, *67*, 5–21. [CrossRef]
123. Faul, F.; Erdfelder, E.; Lang, A.G.; Buchner, A. G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav. Res. Methods* **2007**, *39*, 175–191. [CrossRef]
124. Cohen, J. Statistical power analysis for the behavioral sciences. *SAGE Encycl. Res. Des.* **1969**, *26*, 588. [CrossRef]
125. Weinstein, A.M.; Rosca, P.; Fattore, L.; London, E.D. Synthetic cathinone and cannabinoid designer drugs pose a major risk for public health. *Front. Psychiatry* **2017**, *8*, 156. [CrossRef]
126. Reagan-Shaw, S.; Nihal, M.; Ahmad, N. Dose translation from animal to human studies revisited. *FASEB J.* **2008**, *22*, 659–661. [CrossRef]
127. Psychonautwiki. JWH-018. Available online: https://psychonautwiki.org/wiki/JWH-018 (accessed on 12 January 2023).
128. Riva, E.; Hearse, D.J. Anti-arrhythmic effects of amiodarone and desethylamiodarone on malignant ventricular arrhythmias arising as a consequence of ischaemia and reperfusion in the anaesthetised rat. *Cardiovasc. Res.* **1989**, *23*, 331–339. [CrossRef]
129. Vulliemoz, Y.; Verosky, M. Effect of clonidine on myocardial cyclic GMP content of clonidine in the mouse-activation of central and peripheral alpha adrenoceptors. *J. Pharmacol. Exp. Ther.* **1989**, *251*, 884–887.
130. Pozsgai, G.; Bodkin, J.V.; Graepel, R.; Bevan, S.; Andersson, D.A.; Brain, S.D. Evidence for the phatophysiological relevance of TRPA1 receptors in the cardiovascular system in vivo. *Cardiovas. Res.* **2010**, *87*, 760–768. [CrossRef]
131. Altamirano, F.; Valladares, D.; Henríquez-Olguín, C.; Casas, M.; López, J.R.; Allen, P.D.; Jaimovich, E. Nifedipine treatment reduces resting calcium concentration, oxidative and apoptotic gene expression, and improves muscle function in dystrophic mdx mice. *PLoS ONE* **2013**, *8*, e81222. [CrossRef]

132. Deten, A.; Volz, H.C.; Holzl, A.; Briest, W.; Zimmer, H.G. Effect of propranolol on cardiac cytokine expression after myocardial infarction in rats. *Mol. Cell Biochem.* **2003**, *251*, 127–137. [CrossRef]
133. Ossato, A.; Bilel, S.; Gregori, A.; Talarico, A.; Trapella, C.; Gaudio, R.M.; De-Giorgio, F.; Tagliaro, F.; Neri, M.; Fattore, L.; et al. Neurological, sensorimotor and cardiorespiratory alterations induced by methoxetamine, ketamine and phencyclidine in mice. *Neuropharmacology* **2018**, *141*, 167–180. [CrossRef]
134. Foti, F.; Marti, M.; Ossato, A.; Bilel, S.; Sangiorgi, E.; Botrè, F.; Cerbelli, B.; Baldi, A.; De-Giorgio, F. Phenotypic effects of chronic and acute use of methiopropamine in a mouse model. *Int. J. Leg. Med.* **2019**, *133*, 811–820. [CrossRef] [PubMed]
135. Bilel, S.; Tirri, M.; Arfè, R.; Sturaro, C.; Fantinati, A.; Cristofori, V.; Bernardi, T.; Boccuto, F.; Cavallo, M.; Cavalli, A.; et al. In vitro and in vivo pharmaco-toxicological characterization of 1-cyclohexyl-x-methoxybenzene derivatives in mice: Comparison with tramadol and PCP. *Int. J. Mol. Sci.* **2021**, *22*, 7659. [CrossRef] [PubMed]
136. Bilel, S.; Azevedo Neto, J.; Arfè, R.; Tirri, M.; Gaudio, R.M.; Fantinati, A.; Bernardi, T.; Boccuto, F.; Marchetti, B.; Corli, G.; et al. In vitro and in vivo pharmaco-dynamic study of the novel fentanyl derivatives: Acrylfentanyl, Ocfentanyl and Furanylfentanyl. *Neuropharmacology* **2022**, *209*, 109020. [CrossRef] [PubMed]