Title: Behavioural And Pharmacological Characterization Of A Novel Cannabinomimetic Adamantane-Derived Indole, Apica, And Considerations On The Possible Misuse As A Psychotropic Spice Abuse, In C57bl/6 J Mice

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BEHAVIOURAL AND PHARMACOLOGICAL CHARACTERIZATION OF A NOVEL CANNABINOMIMETIC ADAMANTINE-DERIVED INDOLE, APICA, AND CONSIDERATIONS ON THE POSSIBLE MISUSE AS A PSYCHOTROPIC SPICE ABUSE, IN C57BL/6J MICE

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Highlights

- The novel adamantanyl-derived indole APICA induces cannabimimetic effects in mice.
- APICA induced hypothermia, analgesia, and catalepsy through CB1 receptor activation.
- Single exposure to APICA dose-dependently elicits memory impairment in mice.
- APICA can be included in the Italian Consolidated Test of Drugs (D.P.R. 309/90).

BEHAVIOURAL AND PHARMACOLOGICAL CHARACTERIZATION OF A NOVEL CANNABINOMIMETIC ADAMANTANE-DERIVED INDOLE, APICA, AND CONSIDERATIONS ON THE POSSIBLE MISUSE AS A PSYCHOTROPIC SPICE ABUSE, IN C57BL/6J MICE

Abstract

The novel adamantane derivative APICA (N-(adamtan-1-yl)-1-pentyl-1H-indole-3-carboxamide) was recently identified as a cannabinomimetic indole of abuse. Despite its novel structure, APICA recalls cannabinomimetic indoles, such as representative member JWH-018.

In present study, the effects of APICA (0 - 1 - 3 mg/Kg, i.p.) were tested in C57BL/6J mice, in the Tetrad task which includes the assessment of: body temperature; locomotor activity and behavioural reactivity; nociception; motor coordination; declarative memory. Furthermore, pre-treatment with the CB1 antagonist AM251 (3 mg/Kg, i.p.) or the CB2 antagonist AM630 (3 mg/Kg, i.p.) was carried out to characterize APICA activity.

Our results show that APICA was able to dose-dependently decrease locomotor activity and behavioural reactivity in the open field, whereas only the highest dose was able to induce hypothermia, analgesia, motor incoordination and recognition memory impairment, with respect to vehicle ($p<0.01$; $p<0.001$).

The pretreatment with the CB1 antagonist AM251 elicited an increase in body temperature, total distance travelled in the open field, latency to fall down in the Rotarod, and a decrease in tail flick latency ($p<0.05$; $p<0.01$). On the other hand, pretreatment with AM630 did not induced significant differences on APICA effects.

This study supports preliminary reports on APICA cannabinomimetic properties, extending its detrimental effects on cognitive function. Moreover, these properties can be attributed to the CB1 receptor activity, indicating APICA as a selective CB1 receptor agonist.

Keywords

Synthetic Cannabinoid, CB1 Agonist, New Psychotropic Substances, APICA

Introduction

The alarming issue of the emergence of New Psychotropic Substances (NPS) represents one of current challenges in toxicology. Among them, synthetic cannabinoids (SC) are the most widespread in U.S.A. and Europe since 2000: they share similar pharmacological properties as cannabis sativa's derivatives, D9-THC, cannabinol and cannabidiol, and endocannabinoids, anandamide and 2 arachidonoylglycerol. These compounds act on specific receptors: CB 1 receptors,are localized in the central nervous system and their activation modulate many superior functions as learning and memory, mood, stress reactivity and locomotor activity. CB 2 receptors are located peripherally in immune cells, spleen and thymus and are involved in anti-inflammatory and cytotoxic mechanisms. Despite the similarities SC are not easily recognizable. Indeed, these molecules are constantly considered to escape the current controls and legal limitations and to strengthen the psychotropic effects.
They can be identified by gas chromatography in association with mass spectrometry and are involved in many forensic issue as safety at work, road safety, work tasks at risk but also in many cases of hospital admissions for acute intoxication [31 – 32].
In front of these considerations Europe and Italy have actuated enforcement and prevention of NPS and SC use [34]. However, the ongoing changes in the active cannabinoids contained in legal highs still represent a persisting difficulty for forensic and law enforcement personnel [1-3]. In this regard, the structure of the adamantanyl derivative N-(adamtan-1-yl)-1-pentyl-1H-indole-3-carboxamide) (APICA) recalls JWH-018, the representative member of cannabinomimetic indoles. In 2012, APICA was detected in products obtained by the National Institute of Health Sciences in Japan and recently its synthesis and preliminar pharmacological characterization have been published [4]. The emerging spreading of synthetic cannabinoids highlights the need for further evaluation of the *in vivo* properties and a further pharmacological characterization of indole-derived cannabinoids.
Therefore, the purpose of the present paper was to determine whether the indole-derived compound APICA produces cannabinomimetic behavioural activity in mice, by using the tetrad task, which is a widely used assay to investigate in vivo effects of THC and other cannabinoids, including antinociception, catalepsy, hypothermia, and hypomobility [5]. Other measures of cannabinoid activity, including effects on anxiety-like behaviour and on declarative memory were also assessed. The further goal of this research was to characterize the pharmacological profile of APICA, by using CB1 and CB2 receptor antagonist.

Materials and Methods

Animals and housing conditions

25 Female C57BL/6J mice (6 weeks), obtained from Charles River (Italy) were housed in groups of five in standard cages (Tecniplast, Italy) and maintained in a temperature- (22 ± 2 °C) and humidity- (55 ± 5 %) controlled room, with *ad libitum* access to water and food (Mucedola, Italy). The colony was maintained on 12 h light/dark cycle (light on from 08:00 to 20:00) and mice were gently handled for 3 min per day for a week before the experimental procedures. On the test days, animals were brought into the laboratory 60 minutes before the experimental sessions to acclimatize. The experiments were carried out in a sound isolated room between 9:00 and 14:00. A two-week interval was given between the experiments. Animal performance was recorded on videotape and monitored in an adjacent room. To ensure that mouse's behaviour was not affected by the detection of another mouse's scent, all the devices were thoroughly cleaned 10 min before the animal's entry into the cages. All the experiments were conducted in accordance with the regulations of the Committee for the Protection and Use of Animals of the University of Palermo, in accordance with current Italian legislation on animal experimentation (D.L. 116/92) and the European directives (2010/63/EU) on care and use of laboratory animals. Every effort was made to minimize the number of animals used and their surrow.

Drugs

The syntetic indole-derived APICA (N-1-adamantyl-1-pentyl-3H-indole-3-carboxamide) was synthesized by the Department of Pharmaceutical Sciences of the University of Milan.

N-(adamantan-1-yl)-1-pentyl-1*H*-indole-3-carboxamide

1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC, 762 mg, 3.99 mmol), hydroxybenzotriazole (HOBt, 539 mg, 3.99 mmol) and N,N-Diisopropylethylamine (DIPEA, 1.39 mL, 7.99 mmol) were added to a solution of 1-pentylindole-3-carboxylic acid (840 mg, 3.63 mmol) diluted in DMF (11 mL). After 0.5 h at room temperature 1-aminoadamantane (549 mg, 3.63 mmol) was added to this solution. Stirring was continued for 2 h at room temperature and at the end the solvent was concentrated. The resultant residue was dissolved in dichloromethane (20 mL) and washed twice with water (10 mL). The organic phase was separated, dried (Na_2S0_4) and concentrated. The resultant residue was treated with ethanol to quantitatively precipitate the product as white solid (mp 141 °C). Melting point was determined by DSC analysis and corresponded to the peak maximum. ^{1}H NMR spectra was recorded operating at 300 MHz and ^{13}C NMR at 75 MHz. Chemical shifts are reported in ppm relative to residual solvent ($CHCl_3$) as internal standard. Signal

multiplicity is designed according to the following abbreviations: s singlet, d doublet, t triplet, m multiplet, br s broad singlet. Coupling constants are reported in Hertz (Hz).
^{1}H-NMR (300 MHz, CDCl3): δ 7.87 (1H, d, J = 7.5 Hz), 7.63 (1H, s), 7.38 (1H, d, J = 8 Hz,), 7.25-7.20 (2H, m) 5.7 (1H, br s, NH) 4.17 (2H, t, J = 7.3 Hz), 2.20-2.18 (9H, br s), 1.82 (2H, m), 1.78 (6H, br s) 1.40-1.22 (4H, m), 0.84 (3H, t, J = 7.0 Hz)
^{13}C-NMR (75 MHz): δ 164.5, 136.7, 131.7, 124.8, 122.2, 121.8, 120.1, 112.0, 110.1, 52.0, 46.3, 42.2, 36.2, 30.0, 29.3, 22.1, 14.0.
EIMS: *m/z* [ion] (rel. int.%) 364 $[M]^{+}$ (50), 347 (29), 307 (60), 264 (18), 214 (100), 144 (25).(Fig. 1).
The selective CB1 receptor antagonist AM251 and the selective CB2-receptor antagonist AM630 were obtained from Sigma Aldrich, Italy.
Drugs were initially dissolved in 3% ethanol, then 3% Tween 20 was added, and then 94% physiological saline was added [4]. All drugs were injected intraperitoneally (i.p.) at a volume of 1 mL/kg.
APICA and vehicle were administered 30 min before testing, while pre-treatment with the antagonists AM251 and AM630 occurred 30 min before APICA or vehicle administration.
In Experiment 1 (tetrad task), APICA was administered at 1 and 3 mg/kg..
Mice in Experiment 2 were pretreated with vehicle, AM251 at 3.0 mg/kg or AM630 at 3.0 mg/kg. The doses of the CB1- and CB2- receptor antagonists were based on previous research on reversal of tetrad effects induced by other CB1 agonists [8, 9].

Tetrad-task

Open field test
Locomotor activity and behavioural reactivity in a novel environment were measured in an open field with an automatic video-tracking system, Any Maze (Ugo Basile, Italy), in a mean light intensity (100 lx) illuminated chamber. The apparatus is a Plexiglas square box, 44 cm long, 44 cm wide, and 20 cm high. The system produces a qualitative mapping of the motor patterns, measuring different parameters simultaneously: total distance travelled (TDT), as a measure of locomotor activity, number of transitions (NCT) from peripheral to central squares of the arena, and amount of time spent on the central areas (TSC) as measures of explorative behaviour. Each experimental session lasted 5 min. The animal motor patterns were recorded 1 min after the mice were placed in the box, and displayed on a PC.

Body temperature
Body temperature was measured using a rectal probe in conscious mice and was monitored by a thermalert monitoring thermometer. Temperature was the mean of 3 measurements every 20 sec, over 1 min. for each dosage. Body temperature measurement was repeated at 1h, 2h and 3h.

Rotarod test
Motor coordination was assessed by using a Rota-rod unit (Ugo Basile, Italy). Animals were required to walk against a rotating drum at increasing speed from 4 revolutions per min (rpm) to 40 rpm [8]. The latency to fall off the rotating drum was recorded (seconds). No cut-off latency was employed in the Rota-rod test, to ensure that the detection of subthreshold motor coordination would not be masked [9]. Rota-rod training took place on the two days prior to the test day. Animals were given a minimum of three practice trials on both training days. Practice trials terminated when the animal fell off the rotating drum. Training trials in which the animal failed to remain on the rotating drum for a minimum of 10 sec were re-run. On the test day, reliability testing was performed. Animals that did not remain on the rotating drum for 30 sec in two separate trials failed to meet the criteria, and were dropped from the experiment. Rota-rod latency to fall was measured after 45 min from the drug injection, and reported as the mean of two trials.

Hot-water immersion tail-flick test
Nociception was explored by measuring tail-flick latencies in the hot-water immersion tail flick test [10].
Briefly, two centimeters of the tail were immersed in a water bath apparatus (Instruments srl, Bernareggio, MI, Italy) maintained at 52±0.5 °C. Latency to response was determined by a vigorous tail flick. A cut-off time of 10 s was imposed to minimize tissue damage.

Novel Object recognition task
This test was carried out in the open field Plexiglas square box, in a mean light intensity (100 lx) illuminated chamber. APICA at 3 mg/kg, i.p was administered 30 min before the experiment: mice were subjected to a 5-min training session when they were presented two identical, non-toxic objects (i.e. two metal cans) placed against a wall in the open field arena. To prevent coercion to explore the objects, mice were released against the opposite wall with the back to the objects. The time spent on exploring each object was recorded using ANY MAZE Video Tracking System, (Ugo Basile, Italy); a 2-cm^2 area surrounding the object was defined such that nose entries were recorded as time exploring the object. After the training session, animals were placed in their home cage for a 24 h retention interval. Then, they were returned to the arena containing two objects: one was identical to the familiar one but previously unused (to prevent

olfactory cues and the necessity to wash objects during experimentation), the other was a novel object (metal, glass or hard plastic items). Time spent on exploring each object was recorded along 5-min session. Objects were randomized and counterbalanced across animals. The objects and arena were thoroughly cleaned at the end of each experimental session. The recognition index (RI), which is the time spent on investigating the novel object, divided by the total amount of exploration time of the novel and familiar objects, [RI = TN/(TN + TF)], is a measure of novel object recognition and the main index of retention [11, 12]. If RI percentage is higher than 50%, it indicates more time spent on enquiring into the novel object, whereas less than 50% indicates that time was prevailingly spent on exploring the familiar object, and 50% indicates a null preference.

Experiment 1 - Effects of APICA on the tetrad task

Following the administration of vehicle, 1 or 3 mg/kg APICA ,i.p., animals (n = 5 per group) were tested for locomotor activity, and then for body temperature, motor coordination, and analgesia. Doses listed above were administered i.p. 30 min prior to the start of the locomotor activity assay.

Experiment 2 - Duration of the effects of APICA on thermoregulation, nociception and motor coordination

The duration of the effects of APICA (3 mg/kg, i.p n=5 per group).) was assessed on body temperature, motor coordination, and then analgesia, monitoring the animal parameters (along three consecutive time points (T1: 1h; T2: 2h; T3: 3h).

Experiment 3 - Pharmacological characterization of APICA effects in the tetrad task

The assessment of the pharmacological profile of APICA was carried out by pre-treating a separate groups of mice (n = 5 per group) with the CB1 receptor antagonist AM251 or CB2 receptor antagonist AM630, 20 min prior to APICA administration. Each animal was tested for locomotor activity in the Open field test, as well as for thermoregulation, nociception in the Tail-flick test and motor coordination in the Rotarod test.

Experiment 4 - Effects of APICA on recognition memory in the novel object recognition test

After a two-week washout, the effect of APICA (1 - 3 mg/kg, i.p., n = 5 per group) on declarative memory was assessed by using the novel object recognition test. The drug was administered 30 min before the presentation of the novel object.

Data analysis

Data were analysed by using GraphPad Prism v. 6.1 (La Jolla, CA). One-way analysis of variance (ANOVA) was performed for assessing the main effect of treatment; two-way ANOVA for repeated measures was employed for assessing the effects of treatment, considered as the between-subject factor, and time, as the within-subject factor. Bonferroni post-hoc test was used, when necessary. Statistical difference was considered for $p<0.05$.

Results

Experiment 1- Effects of APICA on the tetrad task

Open field test

The animals were subjected to the open field test 30 minutes after the administration of APICA (1 - 3 mg/kg) to evaluate the effect of different doses on locomotor activity and behavioral reactivity.

The results of one-way ANOVA on total distance travelled (TDT) show a significant effect of treatment (F: 2, 12 = 14.78; p = 0.0006). Administration of APICA reduced locomotor activity in a dose-dependent manner. In particular, the Bonferroni post hoc test highlights, and to a greater extent for the dose of a significant reduction in TDT at 1 mg / kg (t = 3.194, p <0.05), and a further decrease at 3 mg / kg (t = 5.407, p <0.001), with respect to controls (Fig. 2A).

Administration of APICA also reduced behavioral reactivity exploration of the central area of open field. In fact, the analysis of data on the number of transitions in the center of the arena (NCT) shows a significant effect of treatment (F (2, 12) = 10.07; p = 0.0027). In detail, the Bonferroni post hoc test indicates a significant reduction of the NCT at 1 mg/kg (t = 3.704; p <0.01) and at 3 mg/kg (t = 4.048; p <0.01), compared to vehicle (Fig. 2B). Consistently, we observed a significant effect of treatment on time spent in the center of the arena (TSC): (F (2, 12) = 8,986; p = 0.0041), in a dose-dependent manner (1 mg / kg: t = 3.591; p <0.05; 3 mg / kg t = 3.747; p <0.01), compared to vehicle (Fig. 2C).

Body temperature

The body temperature of the animals was measured at 35 minutes after APICA injection (1 - 3 mg / kg) to evaluate the effect of the different doses used on thermoregulation.

The one-way ANOVA shows a significant effect of treatment (F (2, 12) = 13:32; p = 0.0009). In detail, the post hoc analysis shows a significant reduction in body temperature in the group treated with 3 mg/kg with respect to vehicle (t = 4.600, P <0.01) and compared to the group treated with 1 mg/kg (t = 4,328; p <0.01) (Fig. 2D).

Hot water- tail flick test

The animals were subjected to the tail flick test 40 minutes after the administration of APICA (1 - 3 mg / kg) to evaluate the effect of different doses on nociception.

Statistical analysis performed on tail flick latency shows a significant effect of treatment (F (2, 12) = 21.71; p = 0.0001). The administration of APICA had an antinociceptive effect. In particular, the Bonferroni post hoc test indicates a significant increase in tail flick latency in the group treated with 3 mg / kg compared to vehicle (t = 6.290, P <0.001) and the group treated with 1 mg / kg (t = 4.848 ; p <0.01) (Fig. 2E).

Rotarod test

The animals were subjected to the Rotarod test 45 minutes after the administration of APICA (0 - 1 - 3 mg / kg) to evaluate the effect of different doses on the motor coordination.

The one-way ANOVA shows a significant effect of treatment on latency to fall (F (2, 12) = 9,971; p = 0.0028). The administration of APICA reduced motor coordination of the animals. In particular, the Bonferroni post hoc test shows a reduction of down time in the group treated with 3 mg/kg compared to vehicle (t = 4.268, P <0.01) and compared to the group treated with 1 mg / kg (t = 3.273; p <0.05) (Fig. 2F).

Experiment 2 - Duration of the effects of APICA on thermoregulation, nociception and motor coordination

Body temperature

In order to assess the duration of APICA activity, thermoregulation, nociception and motor coordination were sequentially evaluated every 60 minutes (T1, T2, T3) from APICA injection at the dose of 3 mg/kg.

The results of a two-way ANOVA for repeated measures, carried out considering "treatment" as between subject factor and "time" as within subject factor, show a significant effect of time (F (2, 16) = 11.91; p = 0.0007), treatment (F (1, 8) = 8,871, p = 0.0176) and their interaction (F (2, 16) = 10:38; p = 0.0013) on body temperature. The administration of APICA had a maximum hypothermic effect at T1, yet at T2 but not T3. In detail, the Bonferroni post hoc test indicates a significant reduction in body temperature in the APICA group with respect to vehicle, at T1 (t = 4.355, p = 0.0006) and T2 (t = 2.882, p = 0.0246) (Fig. 3A).

Hot water - tail flick test

The results of two-way ANOVA for repeated measures on data from the tail flick test showed a significant treatment effect (F (1, 6) = 25.23; p = 0.0024). The administration of APICA had a maximum antinociceptive effect at T1, yet at T2 and T3. In particular, the Bonferroni post hoc test shows a statistically significant increase in tail flick latency at T1 (t = 4.365, p = 0.0011), T2 (t = 4,762, p = 0.0005) and T3 (t = 3.163, p = 0.0162), with respect to vehicle (Fig. 3B).

Rotarod test
The results of two-way ANOVA for repeated measures on descent latency in the Rotarod test show a significant effect of time ($F(2, 12) = 7.821$; $p = 0.0067$) and treatment ($F(1, 6) = 17:28$; $p = 0.006$). Also in this case, the impairment of motor coordination determined by the administration of APICA was no longer present at T3. In particular, the Bonferroni post hoc shows a significant decrease in descent latency at T1 ($t = 4.486$, $p = 0.0009$) and T2 ($t = 2.54$; $p = 0.0392$) in the group treated with APICA respect to the vehicle (Fig. 3C).

Experiment 3 - Pharmacological characterization of APICA effects in the tetrad task

In order to evaluate the pharmacological activity of APICA, animals were pre-treated with the selective CB1 antagonist AM251 or with the CB2 receptor antagonist AM630, before receiving the administration of APICA, and tested for locomotor activity, body temperature, nociception and motor coordination.

Open Field Test
The one-way ANOVA on the data of TDT in the open field test shows a significant effect of treatment ($F(3, 16) = 4.211$; $p = 0.0225$). Specifically, the pre-treatment with AM251 resulted in a significant increase in the TDT with respect to the group treated with APICA ($t = 3.365$, $p < 0.05$), while the pre-administration of AM630 had no effect compared to APICA (Fig. 4A). No differences were observed with respect to vehicle-treated mice.

Body Temperature
In addition, the statistical analysis on the data of body temperature shows a significant effect of treatment ($F(3, 16) = 43.30$; $p < 0.001$). AM251 resulted in a significant increase in body temperature compared to the group treated with APICA ($t = 6.803$, $p < 0.001$), while the pre-administration of AM630 has had no effect compared to APICA (Fig. 4B). no differences in temperature were observed with respect to vehicle.

Hot water - tail flick test
Consistently, the one-way ANOVA performed on data from tail flick latency shows a significant effect of treatment ($F(3, 16) = 12.38$; $p = 0.0002$). In particular, the pre-treatment with AM251 resulted in a significant reduction in the tail flick latency compared to the group treated with APICA ($t = 3.170$, $p < 0.05$), while AM630 did not exert significant effects compared to APICA (Fig. 4C). No differences were observed with respect to vehicle-treated mice.

Rotarod test
The one-way ANOVA on data of descent latency in the Rotarod test shows a significant effect of treatment ($F(3, 16) = 21.03$; $p < 0.001$). In particular, the pre-treatment with AM251 resulted in a significant reduction in descent latency compared to the group treated with APICA ($t = 4.383$, $p < 0.01$), while AM630 had no statistically significant effect compared to APICA (Fig.4D). No differences were observed with respect to vehicle-treated mice.

Experiment 4 - Effect of APICA on declarative memory in the novel object recognition test

The animals were subjected to the novel object recognition test, 30 minutes after administration of APICA (1 - 3 mg/kg), to evaluate the effect of different doses on declarative memory, assessed by the Recognition Index (RI)%. The results of one-way ANOVA show a significant effect of treatment ($F(2, 12) = 8,959$; $p = 0.0042$). Administration of APICA resulted in a dose-dependent impairment of declarative memory: the post hoc analysis shows a statistically significant reduction of RI% both for the dose of 1 mg / kg ($t = 2.783$, $p < 0.05$) and for 3 mg / kg ($t = 4.152$; $p < 0.01$) compared with vehicle (Fig. 5).

Discussion

The purpose of the present paper was to investigate the cannabinomimetic properties of a novel adamantly-indole-derived compound, APICA, in a battery of tests known as Tetrad task, which is highly sensitive to the effects of psychoactive cannabinoids [5]. It includes the assessment of locomotor activity, nociception (tail flick assay), motor coordination and body temperature. Our results show that, when administered to mice at the dose of 1 and 3 mg/kg, APICA showed behavioural effects typical of cannabinoid agonists, such as suppression of spontaneous locomotion, antinociception, hypothermia, and loss of motor coordination. These effects seemed to be played by the interaction with CB1 receptors. Moreover APICA was also able to disrupt declarative memory in the Novel object recognition task.

APICA administration dose-dependently decreased total distance traveled in the open field test, This result is consistent with preliminary evaluation of APICA cannabinomimetic profile, which showed that APICA dose-dependently induced hypothermia and reduced heart rate at the maximal dose of 10 mg/kg, a similar potency as Δ^9-THC, but lower than JWH-018, the representative member of cannabinomimetic indoles [4].

Since CB1 agonists have been shown to exert anxiogenic effect [13], we also evaluated APICA activity on behavioural reactivity in the open field. Our results show that APICA strongly decreased the time spent in the center of the arena, as well as the number of transitions in the central area, and this observation is consistent with an overall reduction of locomotor activity and exploration. However, a cannabinoid-like modulation of emotionality cannot be ruled out. Indeed, it has been clearly demonstrated that cannabinoid signaling is involved in the control of anxiety, albeit it has been difficult to define the exact role of this signaling. In particular, both anxiolytic- and anxiogenic-like effects have been reported by using cannabinoid receptor agonists at low and high doses, respectively [14], and also by CB1 receptor antagonists [15, 16]. These opposite observations might be explained by the distribution of CB1 receptors in the brain. Rey et al. [13] showed a biphasic effects of the synthetic CB1 cannabinoid receptor agonist CP-55,940, demonstrating that the anxiogenic effect exerted by the high doses was mediated via the CB1 receptor activation on the GABAergic terminals. Indeed, the broad distribution of CB1 receptors in the central nervous system [17], together with its modulatory role on neurotransmission [18], account for the controversial activity of cannabinoids on anxiety modulation. Our results are consistent with data from McLaughlin et al. [6] showing that another adamantyl cannabinoid agonist, AM 411, reduced both outside (thigmotaxic movements) and inside line crossings in the open field task, indicating an anxiogenic profile for adamantyl-derived compounds. Our finding represents a preliminary report on APICA-induced reduction of behavioural reactivity and explorative behaviour; a further characterization of APICA effects on anxiety-like behaviour would benefit from more specific behavioural paradigms.

In order to gather information on the pharmacokinetic profile of APICA, thermoregulation, motor coordination and nociception parameters were sequentially evaluated at 30 min, 1h, 2h and 3 h from APICA injection. Our data show that APICA induced reduction of body temperature, suppression of motor coordination, and nociception in a dose dependent manner. The hypothermic effect and motor incoordination lasted for 1 h, while the analgesic effect was observed up to 2h from the first measurement. It is worth noting that, in contrast with previous findings in rats [4], in our study APICA effects were reversible at 3 h post-administration. Because other pharmacological compounds (i.e. dopamine D2 antagonists) produce cannabinoid-like effects on the tetrad task [19], the further aim of this study was to pharmacologically characterize the effects of APICA on the behavioural patterns examined. Indeed APICA activity was verified by reversing its behavioural effects by a CB1-selective antagonist/inverse agonist such as AM 251 [20] and a CB2-selective antagonist AM 630 [21]. Our results show that APICA effects on the Tetrad task depend on CB1 receptor activation: indeed the pretreatment with the AM251 was able to revert the effect of APICA on locomotor activity, body temperature, nociception and motor coordination, and no significant differences were observed with respect to control group. Banister and colleagues [4] showed that APICA is a full cannabinoid agonist with comparable affinity for both CB1 and CB2 cannabinoid receptors in vitro, and that CB2 receptors may modulate pain in inflammatory [23] and non-inflammatory states [24], via local peripheral mechanisms. Over the last decade, accumulating evidence has shown that CB2 receptors are also widely present in both microglia and neurons [25-27]. However, when AM630 was administered before APICA, the significant decrease in locomotor activity in the open field, body temperature and motor coordination in the Rotarod, together with the increased tail flick latency, were not modified. Our data rules out the involvement of CB2 receptor in APICA effects on the tetrad task, suggesting that they are centrally- and CB1-mediated.

Endogenous and exogenous CB receptor agonists modulate learning and memory mainly through the activation of CB1 receptor [28, 29]. Thus, the final aim of this study was the evaluation of APICA effects on declarative memory in the novel object recognition test. Our results show that the administration of APICA before the presentation of the novel object was able to decrease the recognition index in a dose dependent manner. This is the first report of a detrimental effect on memory processes in the rat, and since APICA was detected in 2012 in Japan as new designer drug [30] its detrimental effect on declarative memory raises relevant health concerns about its widespread illicit recreational use. Future studies should assess the effects of repeated administration of this compound, as well as the extent of recovery.

Conclusions

In the present study, we report that systemic exposure to the novel adamantanyl-derived indole, APICA in mice produces a large spectrum of effects that is typically indicative of cannabinomimetic activity. In particular the tetrad assay reveals that it shares some physical properties of THC and other cannabinoids, including (1) antinociception, (2) incoordination, (3) hypothermia, and (4) hypomobility [5]. The observations that AM251, but not AM630, antagonizes APICA-induced behavioural signs indicate a CB1 receptor-related mechanism of action. Moreover, we also report that a single exposure to APICA dose-dependently elicits memory impairment in mice, raising further concerns about its recreational use.
APICA is used as drug of abuse for its psychotropic effects. In Italy, the above-mentioned law (L.D. 122 on may 16, 2011), includes JWH 018, JWH 122 and JWH 250 in the Consolidated Test of Drugs (D.P.R. 309/90 – table A). We assume that according to the structural analogies between APICA and JWH 018, APICA should be recognized as an illicit compound.

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Figure 1

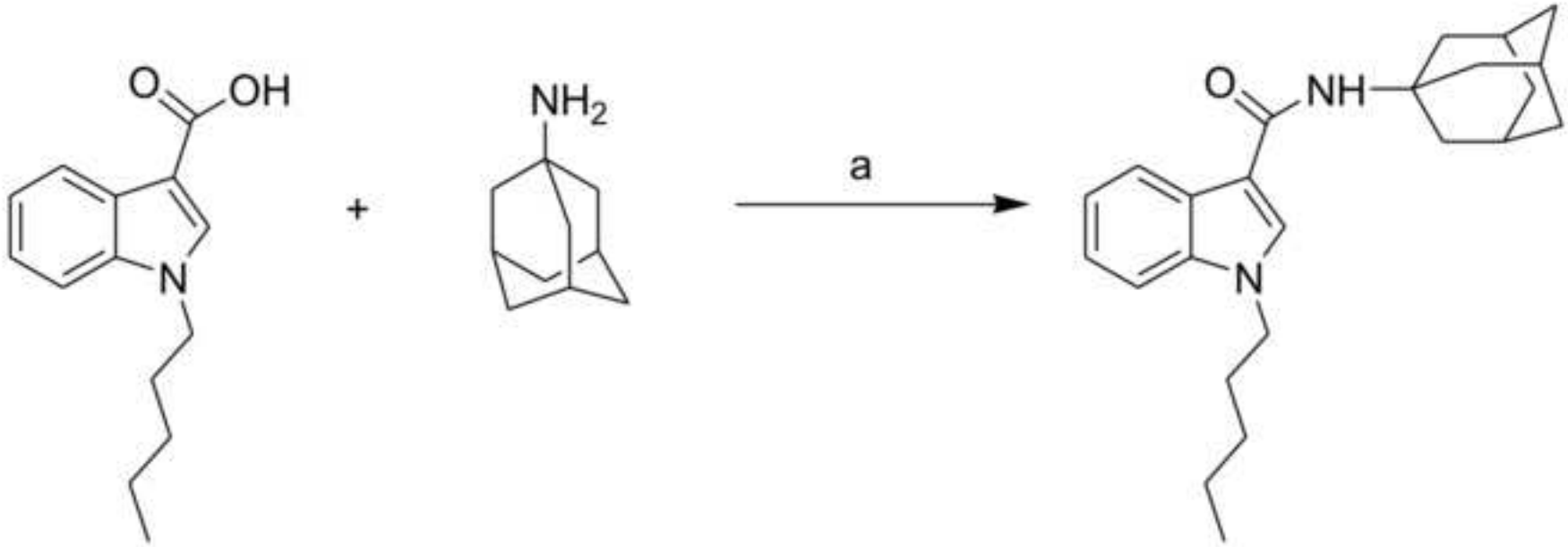


Figure 2

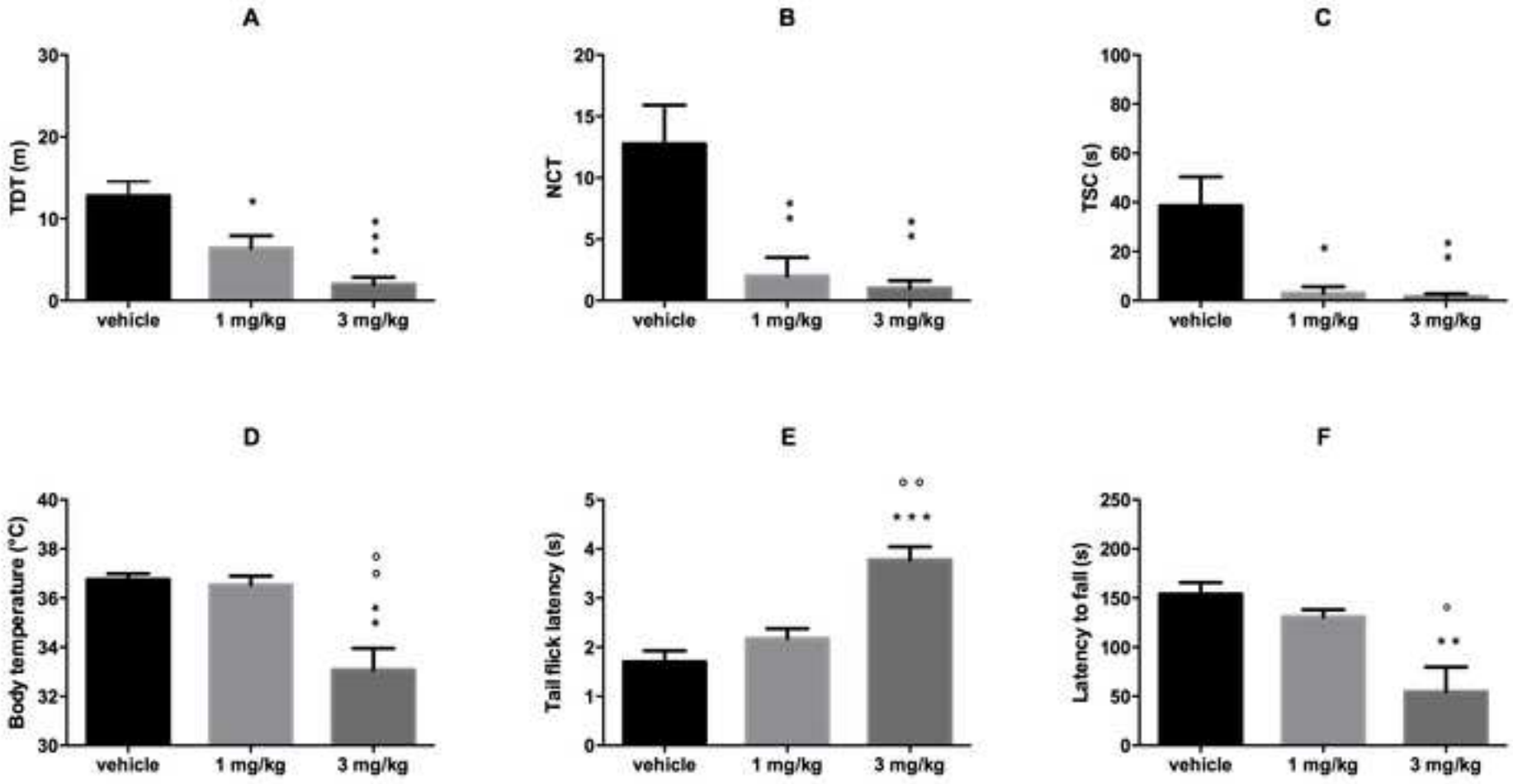

A
TDT (m)
30
20
10
0
vehicle
1 mg/kg
3 mg/kg
B
NCT
20
15
10
5
0
vehicle
1 mg/kg
3 mg/kg
C
TSC (s)
100
80
60
40
20
0
vehicle
1 mg/kg
3 mg/kg
D
Body temperature (°C)
40
38
36
34
32
30
vehicle
1 mg/kg
3 mg/kg
E
Tail flick latency (s)
5
4
3
2
1
0
vehicle
1 mg/kg
3 mg/kg
F
Latency to fall (s)
250
200
150
100
50
0
vehicle
1 mg/kg
3 mg/kg

Figure 3

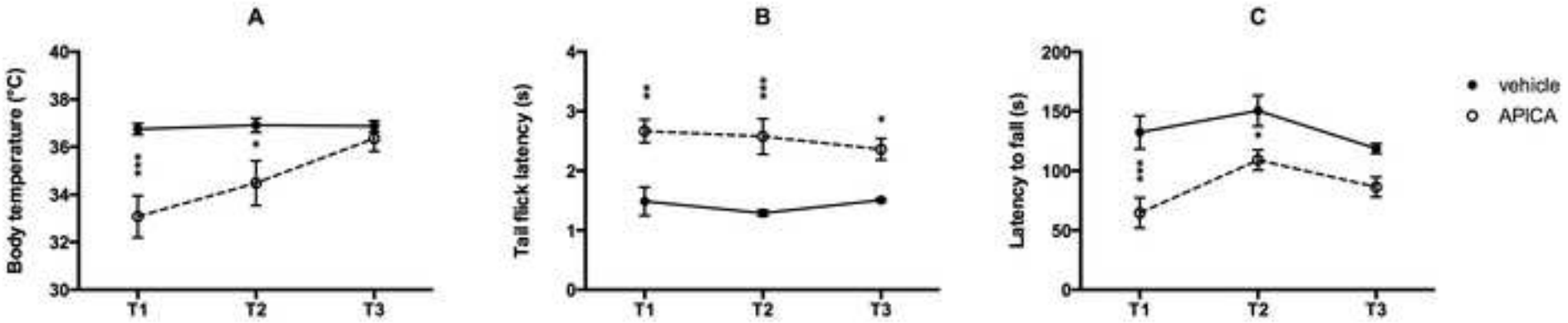
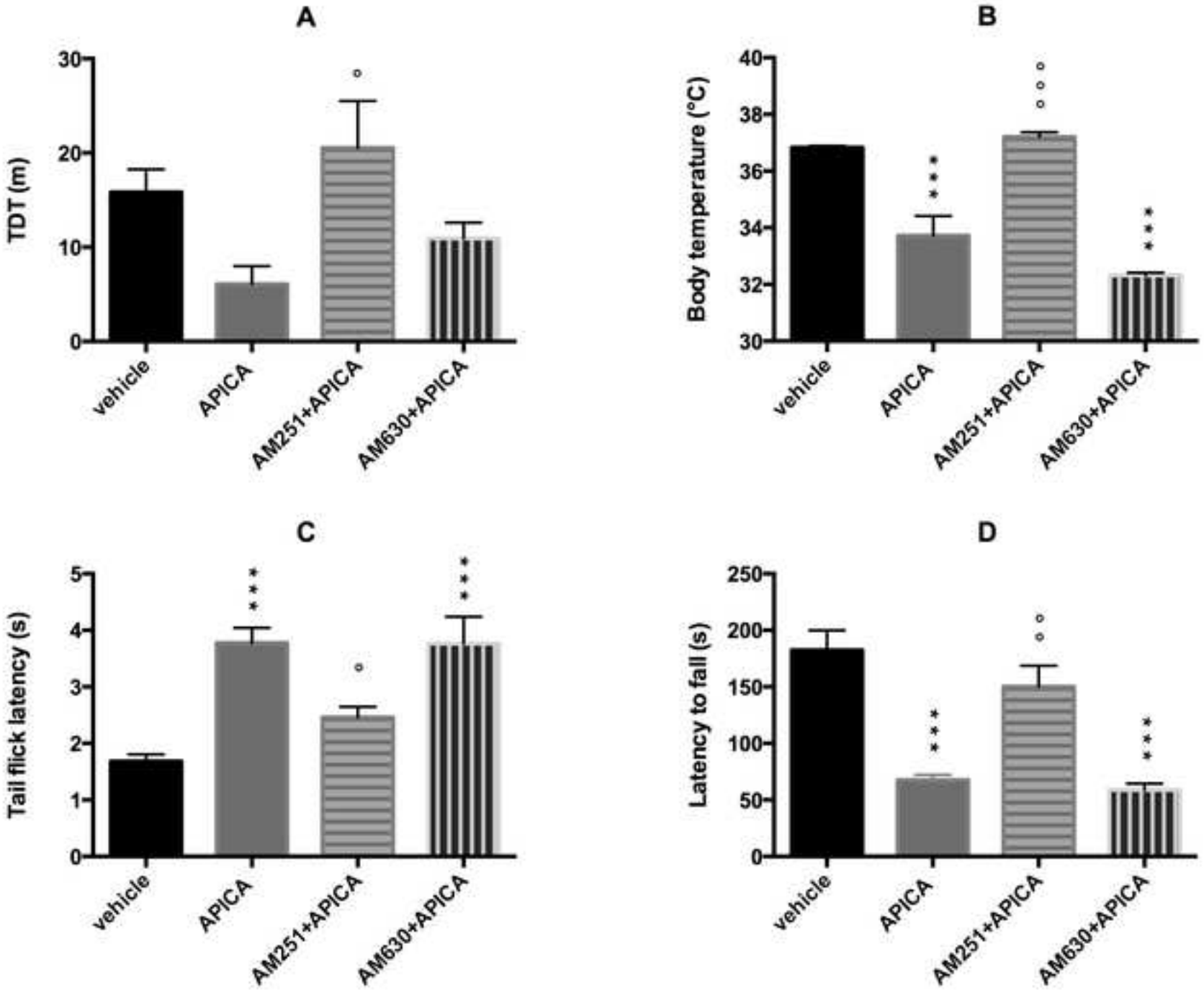


Figure 4


A
TDT (m)
30
20
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vehicle
APICA
AM251+APICA
AM630+APICA
B
Body temperature (°C)
40
38
36
34
32
30
vehicle
APICA
AM251+APICA
AM630+APICA
C
Tail flick latency (s)
5
4
3
2
1
0
vehicle
APICA
AM251+APICA
AM630+APICA
D
Latency to fall (s)
250
200
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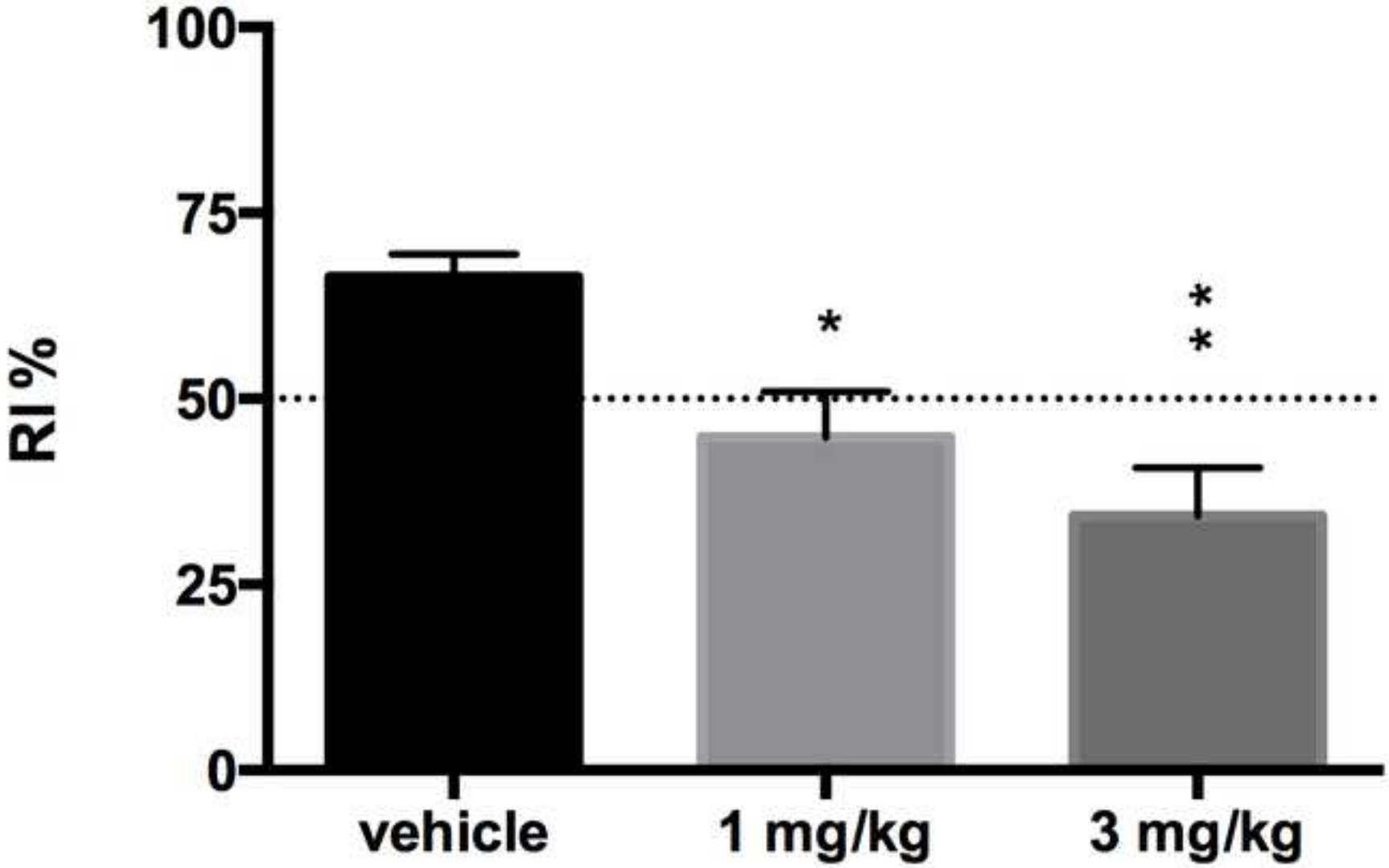
0
vehicle
APICA
AM251+APICA
AM630+APICA

Figure 5



Legend

Fig. 1. APICA synthesis. Reagents and conditions: (a) EDAC, HOBt, DIPEA, DMF, rt, 2.5 h.

Fig. 2. Effect of APICA on the tetrad task. APICA decreased locomotor activity (A) and behavioural reactivity (B, C); APICA induced hypothermia (D); antinociception (E) and motor incoordination (F). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with vehicle. ° $p < 0.05$; °° $p < 0.01$ compared with the dose of 1 mg / kg. Every value represents the mean ± SD of n=5 mice.

Fig. 3. Duration of the effects of APICA on body temperature (A), nociception (B) and motor incoordination (C). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with vehicle. Every value represents the mean ± SD of n=5 mice.

Fig. 4. Effect of the pre-treatment with the CB1 antagonist AM251 and the CB2 antagonist AM630 on APICA-induced hypolocomotion (A), hypothermia (B), analgesia (C) and motor incoordination (D). *** $p < 0.001$ compared with vehicle; ° $p < 0.05$, °° $p < 0.01$, °°° $p < 0.001$ compared with APICA. Every value represents the mean ± SD of n=5 mice.

Fig. 5. Effect of the administration of APICA on declarative memory. * $p < 0.05$, ** $p < 0.01$ compared with vehicle. Every value represents the mean ± SD of n=5 mice.