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AM404 attenuates reinstatement of nicotine seeking induced by nicotine-associated cues and nicotine priming but does not affect nicotine- and food-taking

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Abstract

Multiple studies suggest a pivotal role of the endocannabinoid system in the regulation of the reinforcing effects of various substances of abuse. Different approaches have been used to modulate endocannabinoid neurotransmission including the use of endogenous cannabinoid anandamide reuptake inhibitors. Previously, the effects of one of them, N-(4-hydroxyphenyl)-arachidonamide (AM404), have been explored in rodents trained to self-administer ethanol and heroin, producing some promising results. Moreover, AM404 attenuated the development and reinstatement of nicotine-induced conditioned place preference (CPP). In this study, we used the nicotine intravenous self-administration procedure to assess the effects of intraperitoneal administration of 0, 1, 3 and 10 mg/kg AM404 on nicotine-taking and food-taking behaviors under fixed-ratio and progressive-ratio schedules of reinforcement, as well as on reinstatement of nicotine-seeking induced by nicotine priming and by presentation of nicotine-associated cues. The

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ability of AM404 to produce place preference was also evaluated. AM404 did not produce CPP and did not modify nicotine-taking and food-taking behaviors. In contrast, AM404 dose-dependently attenuated reinstatement of nicotine-seeking behavior induced by both nicotine-associated cues and nicotine priming. Our results indicate that AM404 could be a potential promising therapeutic option for the prevention of relapse to nicotine-seeking in abstinent smokers.

Keywords

Nicotine; nicotine taking; nicotine seeking; endocannabinoids; AM404

Introduction

Accumulating evidence suggests that the rewarding effects of nicotine as well as nicotine-taking and nicotine-seeking behaviors are modulated by the endocannabinoid system (Le Foll and Goldberg 2005). The CB1 inverse agonist/antagonist rimonabant, was found to inhibit both intravenous nicotine self-administration behavior (Cohen et al., 2002), nicotine-induced conditioned place preference (CPP) in rats (Forget et al., 2005; Le Foll and Goldberg, 2004), motivation for nicotine assessed by a progressive-ratio (PR) schedule of reinforcement and nicotine-seeking induced by presentation of nicotine associated cues and nicotine priming (Forget et al., 2009). In agreement with the preclinical findings, clinical studies demonstrated that CB1 receptor blockade with rimonabant produced some positive outcomes as a smoking cessation therapy (Le Foll et al., 2008). However, emergent concerns about notably increased rates of depression, anxiety and suicidality related to rimonabant therapy (Moreira et al., 2009, Nathan et al., 2011) led to the withdrawal of the rimonabant from the market.

A possible pharmacological alternative to CB1 receptor blockade is the indirect alteration of CB receptor signaling by modulating the levels of endogenous cannabinoid ligands. The endogenous cannabinoid, anandamide (AEA), is synthesized on demand and is degraded by fatty acid amide hydrolase (FAAH). Studies with the FAAH inhibitor cyclohexyl carbamic acid 3′-carbamoyl-3-yl ester (URB597) showed that it increased brain AEA levels in rats and magnified AEA's physiological effects (Fegley et al., 2005). Moreover, while demonstrating no rewarding effects per se, URB597 prevented the development of nicotine-induced CPP and inhibited nicotine-taking and nicotine–seeking behaviors (Scherma et al., 2008). The surprising similarity between rimonabant and URB597 may be explained by the ability of both compounds to reduce nicotine-induced elevations of dopamine levels in the nucleus accumbens shell (Cohen et al., 2002; Scherma et al., 2008).

A promising alternative approach to FAAH inhibition is to inhibit the uptake of AEA into cells where it undergoes FAAH-mediated hydrolysis (Freund et al., 2003; Moore et al., 2005). N-(4-hydroxyphenyl)-arachidonamide (AM404) is the most studied synthetic inhibitor of AEA reuptake that has been shown to potentiate the effects of AEA in vitro (Beltramo et al., 1997) and in vivo (Beltramo et al., 2000; Giuffrida and Piomelli, 2000), and also activate transient receptor potential vanilloid (TRPV1) receptors at concentrations

similar to or lower than those that inhibit AEA transport (De Petrocellis et al., 2000; Zygmunt et al., 2000). Several studies have investigated the effects of AM404 on the reinforcing properties of substances of abuse as alcohol, heroin and cocaine, producing mixed results (Cippitelli et al., 2007; Solinas et al., 2005 Vlachou et al., 2008). Recently we used a nicotine-induced CPP procedure in rats to demonstrate the inhibitory effects of AM404 on both development and reinstatement of extinguished CPP (Scherma et al., 2012). To the best of our knowledge, no study has explored the impact of AM404 on nicotine self-administration and reinstatement of nicotine seeking behavior as well as on food self administration under fixed-ratio (FR) and PR schedules of reinforcement.

Here, we evaluated the effect of AM404 on nicotine-taking and nicotine-seeking behavior, food-taking behavior and CPP in rats.

Material and methods

Animals

Male Long Evans rats, experimentally naive at the start of the study and initially weighing 250–275 g, were used (Charles River, Lachine, Province du Quebec, Canada). All rats were individually housed in a temperature-controlled environment on a 12 h reverse light/dark cycle (lights off from 07:00–19:00). Prior to any experimental manipulation, animals were given a minimum of seven days to habituate to the colony room, during which they were weighed, handled and received unlimited access to both food and water. After habituation, all rats were diet restricted to five food pellets or 20 g daily and had free access to water. All animals were housed under standard non-enriched conditions. All the experimental procedures described in this report were carried out at the Centre for Addiction and Mental Health (CAMH) in compliance with the guidelines of the Canadian Council on Animal Care (compatible with National Institutes of Health guidelines) and were reviewed and approved by the CAMH, Animal Care Committee.

Apparatus

Nicotine intravenous self-administration studies were carried out in commercially available experimental chambers (Med Associates, St. Albans, Vermont, USA) located in sound-attenuating boxes and equipped with two levers, a house light, and two cue lights, one located above each lever. For half of the animals, the left lever was the active lever and for the other half the right lever was the active lever.

Food-maintained behavior

Techniques for initial acquisition of food-maintained behavior and surgery were similar to those already reported (Corrigall and Coen, 1989). Animals learned to lever press for food reinforcement on a continuous reinforcement (CRF) schedule in which each press on the active lever resulted in the delivery of a 45 mg food pellet. During these acquisition sessions, the house light was on with no illumination of the cue lights above the levers. Daily 1 h acquisition sessions were conducted for five days. Once food-maintained behavior was acquired, intravenous catheters were surgically implanted.

Intravenous catheterization

Surgical procedures for implantation of chronic intravenous catheters were similar to those already reported (Corrigall and Coen, 1989; Forget et al., 2010; Khaled et al., 2010). Briefly, catheters were implanted into the jugular vein, exiting between the scapulae. Surgery was performed under anesthesia induced by intraperitoneal (IP) xylazine (10 mg/kg) and ketamine hydrochloride (90 mg/kg). Incision sites were infiltrated with the subcutaneous (SC) local anesthetic marcaine (0.125%). Buprenorphine was given for post-operative analgesia (0.03 mg/kg, SC) and a single dose of penicillin (30,000 units, intramuscular) was administered at the completion of surgical procedures. Animals were allowed to recover for a one-week period before drug self-administration sessions were begun.

Nicotine self-administration procedures

Acquisition of nicotine self-administration was performed under a FR schedule of reinforcement at a unit dose of 30 μg/kg/infusion of nicotine base. Session duration was 60 min. The start of each 60 min session was signaled by illumination of the house light. In the presence of the illuminated house light, one to five active lever presses resulted in the delivery of a nicotine infusion. Each infusion was followed by a time out (TO) period of 60 s, during which the house light was dimmed, the cue light above the active lever illuminated, and lever press responses had no programmed consequences. During the first week of acquisition, response requirements were FR1 (i.e. each active lever press during the time-in period resulted in the delivery of a nicotine infusion). After completing five days of FR1, response requirements were increased for FR2 for three days and then increased to reach a final value of FR5 for 10 days, by which time self-administration behavior was stable and the animals had a 15–20 day history of nicotine self-administration. Self-administration sessions occurred mostly five days a week.

Testing under the FR5 schedule of nicotine reinforcement

Animals were considered to have acquired stable nicotine self-administration when they pressed the active lever more than twice the number of times they pressed the inactive lever, received a minimum of 10 infusions per 60 min session, and had less than 20% variation in the number of infusions earned per session during two consecutive sessions. Once stability was reached, the animals were given IP injections of vehicle (dimethylsulfoxide, Tween 80 and distilled water in a ratio of 10:10:80), to habituate them to the injection procedure for an additional three days. Rats (n=10) were then tested using IP injections of vehicle or AM404 at doses of 1, 3 and 10 mg/kg in a counter-balanced, within-subject design. Injections were given 30 min before the start of the session. Throughout all the experiments, animals were assigned to vehicle, 1, 3 and 10 mg/kg of AM404 using a Latin square design where each animal had an equal chance of receiving any of the different doses of AM404.

Testing under the PR schedule of nicotine reinforcement

Another group of animals (n=10) acquired nicotine (30 ug/kg/infusion) self-administration behavior under the FR schedule and was then directly switched to a PR schedule where the response requirement increased with each successive injection. The response requirement progression was based on the formula $5e^{(0.25\times[\text{inj.number}+3])}$, with the first two values

replaced by 5 and 10 (modified from Roberts and Bennett, 1993). Thus, the response requirements for successive injections were 5, 10, 17, 24, 32, 42, 56, 73, 95, 124, 161, 208, etc. The break point (BP) was defined as the highest ratio completed prior to the first 30 min period without a response on the active lever. PR sessions lasted a maximum of 4 h. The animals were allowed 10 days of nicotine self-administration under the PR schedule before testing with AM404 began. Testing of AM404 (1–10 mg/kg, 30 min before the session) was performed using a counterbalanced within-subject design.

Testing under FR and PR schedules of food reinforcement

Animals (n=12) were trained to press a specific lever to receive food pellets. Training followed the same criteria as for nicotine (except the reinforcer was food). Animals tested on operant responding for food pellets started to receive injections of the AM404 vehicle before each session starting on the first day at FR5 in order to habituate them to the injection procedure before the start of actual AM404 testing. AM404 testing was started once the animal reached stable responding (i.e. the difference between the numbers of active lever presses by each animal was less than 20% for two consecutive sessions). Thirty minutes before the start of the session AM404 (1, 3 and 10 mg/kg) was administered IP at a volume of 10 μl/100gm. After completing testing on the FR5 schedule, the animals were switched to the PR schedule with the same response requirement progression, session duration and testing criteria as in the nicotine self-administration experiment described above.

Extinction

After acquisition of nicotine self-administration, as described above, an extinction phase was conducted by withholding nicotine and its associated cues (house light stayed on and cue lights stayed off throughout the session). Responses on the active and inactive levers were recorded, but had no programmed consequences. An extinction criterion was established for each animal individually and was defined as total active lever responses being less than 20 presses. The extinction criteria had to be maintained for two consecutive days before reinstatement testing started. All animals reached extinction criteria within an average of eight extinction sessions. Both extinction and reinstatement sessions lasted for 60 min.

Testing the effects of AM404 on cue-induced reinstatement of nicotine-seeking behavior

All tests were carried out in a counter-balanced within-subject design. After each test, extinction was re-established until extinction criteria were obtained for at least two consecutive days. Rats (n=10) were pretreated 30 min before the session with vehicle or 1, 3 and 10 mg/kg AM404 in a counterbalanced order to measure the effects of AM404 on cue-induced reinstatement of nicotine-seeking behavior. Cue-induced reinstatement tests were conducted under conditions identical to that of self-administration, except that responses on the active lever (on an FR5 schedule) resulted in contingent presentation of the cues (light above the active lever on and house-light off for 60 s) without nicotine availability (no infusions). Responses on the inactive lever were recorded but had no programmed consequences. The testing sessions lasted one hour.

Testing the effects of AM404 on nicotine-induced reinstatement of nicotine seeking

Another group of animals (*n*=9) underwent the same training and extinction and was subsequently used to determine the effects of AM404 (1, 3 and 10 mg/kg, IP 30 min before the session) on nicotine-induced reinstatement. Nicotine priming was performed as in (Forget et al., 2010b) by administering 0.15 mg/kg nicotine SC, 10 min before the test session. During the extinction and nicotine-induced reinstatement testing sessions, the cue light above the active lever was always off.

CPP

The procedure was adapted from (Bortolato et al., 2006). The floor of each open-field (eight open-fields total) was covered with a removable floor texture made from wire mesh or rough Plexiglas. The CPP procedure consisted of three consecutive phases: (a) Baseline preference session: one session of 15 min duration in which half of the open field was covered with wire mesh and half was covered with rough Plexiglas (to evaluate baseline preference); (b) Conditioning phase: this lasted 12 days and consisted of six alternated presentations of AM404 (1, 3 and 10 mg/kg) or vehicle (1 mg/kg) before daily conditioning sessions. On odd days, the animals received one of the doses of the test drug and these effects were paired with one floor texture. On even days the animals received vehicle, and these effects were paired with the other floor texture. Each conditioning session lasted for 1 h and the floor was covered with only one type of floor texture during each session; (c) Postconditioning test: on the subsequent test day, the rats received no treatment and were placed in the open field in which half of the open field was covered with wire mesh and half was covered with rough Plexiglas. Drug-induced effects were assessed from differences in the times spent between post-conditioning and pre-conditioning tests. Drug–texture pairings were counterbalanced so that, within each treatment group (*n*=8), the drug was associated with the wire mesh floor for half of the rats and with the Plexiglas floor for the other half of the rats (unbiased design). Unlike the self administration/reinstatement experiments, animals in the CPP experiments were tested once using a single dose of AM404.

Data analysis

The number of active and inactive lever presses and nicotine infusions were recorded and analyzed. To analyze the effects of AM404 on the number of nicotine infusions and food pellets earned under the FR and the PR schedules of reinforcement, one-way analysis of variance (ANOVA) analysis was performed where the independent variable was the dose of AM404 and dependent variable was the number of nicotine infusions. For reinstatement studies, one-way ANOVA analysis was used to assess the reinstatement effect and the effects of AM404 on reinstatement induced by nicotine priming and nicotine-associated cues followed by post-hoc Newman–Keuls test. In the reinstatement experiments, the independent variable was the dose of AM404 and the dependent variable was the number of active and inactive levers. For the CPP experiment two-way ANOVA was used to assess the effects of the AM404 doses.

Drugs

(−)Nicotine hydrogen tartrate (Sigma-Aldrich, St Louis, Missouri, USA) was dissolved in 0.9% NaCl, the pH was adjusted to 7.0 (±0.2), and the solution was filtered through a 0.22 mm syringe filter (Fisher Scientific, Pittsburgh, Pennsylvania, USA) for sterilization purposes. All nicotine doses are reported as free base concentrations. Nicotine was administered IV in a volume of 100 μL/kg/injection for self-administration studies or was administered SC at the dose of 0.15 mg/kg at a volume of 1 mL/kg for the nicotine induced reinstatement studies. AM404 was dissolved in dimethylsulfoxide, Tween 80 and distilled water in a ratio of 10: 10: 80 and injected intraperitoneally at a volume of 1 mL/kg, 30 min before the start of the session. Doses of 1, 3 and 10 mg/kg were tested.

Results

Effects of AM404 on nicotine self - administration under the FR5 schedule

ANOVA showed no effect of AM404 pre-treatment on the number of nicotine infusions ($F_{3,27}$= 0.3872, p>0.05). AM404 (1, 3 and 10 mg/kg) did not affect the number of nicotine infusions received during the session (Figure 1(a)).

Effects of AM404 on nicotine self-administration under the PR schedule

ANOVA showed no effect of AM404 pretreatment on the number of nicotine infusions ($F_{3,21}$=0.9576, p>0.05). AM404 (1, 3 and 10 mg/kg) at the various doses tested failed to produce any change in the break point values, as compared to vehicle (Figure 1(b)).

Effects of AM404 on reinstatement of nicotine-seeking induced by nicotine-associated cues

ANOVA analysis performed on active lever presses indicated a main effect of cues per se on reinstatement of nicotine seeking compared to baseline conditions (p<0.05). ANOVA performed on the active lever presses indicated a main effect of AM404 ($F_{4,32}$=18.19; p<0.0001) and Newman–Keuls post-hoc analysis showed that pretreatment with 10 mg/kg AM404, 30 min before the start of the session, attenuated the reinstatement induced by nicotine-associated cues (p<0.05), as compared to reinstatement under vehicle pretreatment. Neither presentation of nicotine-associated cues, nor AM404 administration, had a significant effect on responding on the inactive lever ($F_{4,32}$= 1.773; p>0.05) (Figure 2(a)).

Effects of AM404 on reinstatement of nicotine-seeking induced by nicotine priming

ANOVA analysis performed on active lever presses indicated a main effect of 0.15 mg/kg nicotine priming on nicotine-seeking behavior, as compared to baseline conditions (p<0.05). ANOVA analysis performed on the active lever presses indicated a main effect of AM404 ($F_{4,32}$ = 5.603; p<0.05) and Newman–Keuls post-hoc analysis showed that pretreatment with 3 and 10 mg/kg AM404, 30 min before the start of the session, attenuated the effect of nicotine priming (p<0.05), as compared to reinstatement under vehicle pretreatment. Neither priming injections of nicotine, nor AM404 (1, 3 and 10 mg/kg) administration, had a significant effect on responding on the inactive lever ($F_{4,32}$ = 1.372; p>0.05) (Figure 2(b)).

Effects of AM404 on operant food responding under the FR5 schedule

ANOVA showed no effect of AM404 pre-treatment on the number of food pellets level indicated that pre-administration of AM404 (1, 3 and 10 mg/kg) did not affect the number of food pellets received during the session ($F_{3, 33}$ = 1.373, p>0.05) (Figure 3(a)).

Effects of AM404 on operant food responding under the PR schedule

ANOVA showed no effect of AM404 pretreatment on the number of food pellets received during the session ($F_{3,33}$=0.1897, p>0.05). AM404 (1, 3 and 10 mg/kg) at the various doses tested failed to produce any change in the break point values, as compared to vehicle (Figure 3(b)).

Effects of AM404 on development of CPP

All doses of AM404 (1, 3 and 10 mg/kg) failed to produce significant CPP. Two-way ANOVA analysis did not show any significant effect of time ($F_{1,28}$ = 0.34; p>0.05) and dose ($F_{3,28}$ = 0.21; p>0.05) (Figure 4).

Discussion

The results of the present study demonstrate that pretreatment with AM404 dose-dependently attenuated the effects of a priming dose of nicotine and nicotine-associated cues on reinstatement of nicotine-seeking behavior. However, AM404 did not affect the number of nicotine infusions received under the FR schedule of reinforcement and did not alter the break point values under the PR schedule of reinforcement. Furthermore, AM404 demonstrated no effect on food self-administration under both FR and PR schedules of reinforcements, nor did it show any reinforcing properties using CPP procedure.

These findings further validate the results we obtained previously with VDM11 (AM404 analogue). VDM11 produced significant attenuation of reinstatement induced by both nicotine-associated cues and by nicotine priming but had no effect on nicotine self-administration under FR or PR schedules of reinforcement (Gamaleddin et al., 2011). The results from both the AM404 and VDM11 experiments are in apparent agreement with our previous results with URB597, that demonstrated an inhibitory effect on nicotine-induced reinstatement of nicotine seeking in both the nicotine self-administration and CPP models (Scherma et al., 2008). URB597 was able to attenuate the acquisition of nicotine self-administration behavior under a FR schedule (Scherma et al., 2008) but it had no effect on established self-administration behavior under a PR schedule (Forget et al., 2009). Here, we demonstrated that AM404, produced effects similar to URB597. However, URB597 appears to modulate the rewarding effects of nicotine by also affecting levels of oleylethanolamide (OEA) and palmitoylethanolamide (PEA) (Mascia et al., 2011; Melis et al., 2008) which possess a structural similarity to AEA but do not functionally activate cannabinoid receptors (Fegley et al., 2005) and, instead, are endogenous ligands for peroxisome proliferator-activated receptors-a (PPAR-a) (Astarita et al., 2006; Fu et al., 2003). Thus, the inhibitory effects of AM404 on reinstatement of nicotine seeking are probably due to its ability to increase AEA levels, rather than OEA or PEA levels, in the brain.

It should be noted that the enzyme FAAH (fatty acid amide hydrolase) has been cloned, yet the proteins responsible for the membrane transport of endocannabinoids have not. However, several lines of evidence and indirect observations support the existence of an endocannabinoid membrane transport protein that is independent from the intracellular metabolism mediated by degradation enzymes: (a) There are selective compounds that are able to inhibit the cellular uptake of AEA without affecting the activity of FAAH enzyme (Di Marzo et al., 2002; Lopez-Rodriguez et al., 2003; Ortar et al., 2003); (b) AEA accumulation inside the cells is inhibited by AEA uptake inhibitors and increased by some FAAH inhibitors (Kathuria et al., 2003); (c) AEA uptake can still be demonstrated in cells lacking the expression of FAAH (Deutsch et al., 2001, Di Marzo et al., 1999). It has been suggested that AM404 is not only an AEA inhibitor but is also considered an inhibitor of 2-arachidonoylglycerol (2-AG), the other main endocannabinoid in the brain and that they both share the same transport system (Bisogno et al., 2001; Di et al., 2005; Hajos et al., 2004). Thus, at the dose range we used in vivo, 2-AG may have contributed to the effects we observed with AM404. The findings that we obtained in this study appear to be specific to nicotine and not generalizable to other substances of abuse. It has been shown that AM404 attenuated alcohol self-administration while failing to modulate reinstatement of alcohol-seeking behavior (Cippitelli et al., 2007). The same study further demonstrated that the effect produced by AM404 was neither CB1 receptor nor TRPV1 receptor mediated as it was not reversed by CB1 and TRPV1 receptor antagonists respectively. Furthermore, Solinas et al. (2005) showed that AM404 attenuated heroin self-administration under a PR schedule of reinforcement. In contrast, the CB receptor agonists, delta-9-tetrahydrocannabinol and WIN 55,212-2 increased the break point for heroin respectively (Solinas et al., 2005). On the other hand, AM404 has shown to increase the threshold for intracranial self-stimulation and reverse the decrease in intracranial self-stimulation threshold produced by cocaine. So, it could be concluded that AM404 reduced the rewarding properties of cocaine in addition to reversing the locomotor hyperactivity observed after administration of cocaine (Vlachou et al., 2008). On the other hand, in our experiments with operant responding for food, AM404 had no effect on food intake which can be explained by the difference in reinforcing properties of natural rewards compared to those of drugs of abuse.

Here we used a dose range of AM404 that has been shown to be effective in modulating the reinforcing effects of different drugs of abuse such as heroin, cocaine and alcohol (Cipitelli et al., 2007; Solinas et al., 2005; Vlachou et al., 2008).

Using the CPP procedure, AM404 failed to produce CPP at any dose studied, which is in agreement with a previous study, in which AM404 displayed significant shift in preference toward the environment associated with AM404 in rats that were housed in enriched conditions but not in rats that were housed in standard conditions (Bortolato et al., 2006). The fact that Scherma and colleagues observed the development of a CPP at a dose of 10 mg/kg AM404 may be due to use of a different study design and conditions, and use of a different strain of rats (Scherma et al., 2012).

One possible limitation of our findings is the fact that the effects could be modulated by non-specific effects on motor response. Beltramo and colleagues have previously reported

that intracerebroventricular injection of AM404 induced mild hypokinesia as shown by the increase in immobility time and decreased motor behavior stimulated by dopamine D_2 receptor agonists (Beltramo et al., 2000). However, such effects are unlikely to mediate the effects observed here with AM404 as we found no significant effect on the ability of the animals to lever press for nicotine under both FR and PR schedules.

Another limitation to our findings is that we did not explore the precise mechanism by which AM404 produces the effects observed on reinstatement of nicotine seeking. The effect of AM404 on AEA reuptake is not exclusive, as AM404 has shown its ability to inhibit reuptake of the other main endocannabinoid in the brain, 2-AG (Bisogno et al., 2001). Therefore, an inhibitor of monoacylglycerol lipase (MAGL), an enzyme that degrades 2-AG, could be used to selectively increase the levels of 2-AG in nicotine-administering rodents. Finally, AM404 has been shown to activate vanilloid receptors TRPV1 (Zygmunt et al., 2000). However, the fact that VDM11, which has negligible agonist activity at TRPV1 receptors (De Petrocellis et al., 2000), produced effects identical to AM404, allows us to conclude that the results we observed were not mediated by activation of vanilloid receptors.

To summarize, our results suggest that AM404 can reduce relapse to nicotine-seeking behavior induced by both nicotine-associated cues and by exposure to a priming dose of nicotine, without affecting food intake. While this set of characteristics is in favor of the possible use of AM404 and compounds from the same class for preventing relapse in abstinent smokers, further studies are required to clarify the underlying mechanism of action.

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References

Astarita G, Di Giacomo B, Gaetani S, et al. Pharmacological characterization of hydrolysis-resistant analogs of oleoylethanolamide with potent anorexiant properties. J Pharmacol Exp Ther. 2006a; 318:563–570. [PubMed: 16702440]

Beltramo M, de Fonseca FR, Navarro M, et al. Reversal of dopamine D(2) receptor responses by an anandamide transport inhibitor. J Neurosci. 2000; 20:3401–3407. [PubMed: 10777802]

Beltramo M, Stella N, Calignano A, et al. Functional role of high-affinity anandamide transport, as revealed by selective inhibition. Science. 1997; 277:1094–1097. [PubMed: 9262477]

Bisogno T, MacCarrone M, De Petrocellis L, et al. The uptake by cells of 2-arachidonoylglycerol, an endogenous agonist of cannabinoid receptors. Eur J Biochem. 2001; 268:1982–1989. [PubMed: 11277920]

Bortolato M, Campolongo P, Mangieri RA, et al. Anxiolytic-like properties of the anandamide transport inhibitor AM404. Neuropsychopharmacology. 2006; 31:2652–2659. [PubMed: 16541083]

Cippitelli A, Bilbao A, Gorriti MA, et al. The anandamide transport inhibitor AM404 reduces ethanol self-administration. Eur J Neurosci. 2007; 26:476–486. [PubMed: 17650118]

Cohen C, Perrault G, Voltz C, et al. SR141716, a central cannabinoid (CB(1)) receptor antagonist, blocks the motivational and dopamine-releasing effects of nicotine in rats. Behav Pharmacol. 2002; 13:451–463. [PubMed: 12394421]

Corrigall WA, Coen KM. Nicotine maintains robust self-administration in rats on a limited-access schedule. Psychopharmacology (Berl). 1989; 99:473–478. [PubMed: 2594913]

De Petrocellis L, Bisogno T, Davis JB, et al. Overlap between the ligand recognition properties of the anandamide transporter and the VR1 vanilloid receptor: Inhibitors of anandamide uptake with negligible capsaicin-like activity. FEBS Lett. 2000; 483:52–56. [PubMed: 11033355]

Deutsch DG, Glaser ST, Howell JM, et al. The cellular uptake of anandamide is coupled to its breakdown by fatty-acid amide hydrolase. J Biol Chem. 2001; 276:6967–6973. [PubMed: 11118429]

Di Marzo V, De Petrocellis L, Bisogno T, et al. Metabolism of anandamide and 2-arachidonoylglycerol: An historical overview and some recent developments. Lipids. 1999; 34:S319–S325. [PubMed: 10419192]

Di Marzo V, Griffin G, De Petrocellis L, et al. A structure/activity relationship study on arvanil, an endocannabinoid and vanilloid hybrid. J Pharmacol Exp Ther. 2002; 300:984–991. [PubMed: 11861807]

Di S, Boudaba C, Popescu IR, et al. Activity-dependent release and actions of endocannabinoids in the rat hypothalamic supraoptic nucleus. J Physiol. 2005; 569:751–760. [PubMed: 16239276]

Fegley D, Gaetani S, Duranti A, et al. Characterization of the fatty acid amide hydrolase inhibitor cyclohexyl carbamic acid 3′-carbamoyl-biphenyl-3-yl ester (URB597): Effects on anandamide and oleoylethanolamide deactivation. J Pharmacol Exp Ther. 2005; 313:352–358. [PubMed: 15579492]

Forget B, Coen KM, Le Foll B. Inhibition of fatty acid amide hydrolase reduces reinstatement of nicotine seeking but not break point for nicotine self-administration--comparison with CB(1) receptor blockade. Psychopharmacology (Berl). 2009; 205:613–624. [PubMed: 19484221]

Forget B, Hamon M, Thiebot MH. Cannabinoid CB1 receptors are involved in motivational effects of nicotine in rats. Psychopharmacology (Berl). 2005; 181:722–734. [PubMed: 15986197]

Forget B, Wertheim C, Mascia P, et al. Noradrenergic alpha1 receptors as a novel target for the treatment of nicotine addiction. Neuropsychopharmacology. 2010; 35:1751–1760. [PubMed: 20357760]

Freund TF, Katona I, Piomelli D. Role of endogenous cannabinoids in synaptic signaling. Physiol Rev. 2003; 83:1017–1066. [PubMed: 12843414]

Fu J, Gaetani S, Oveisi F. Oleylethanolamide regulates feeding and body weight through activation of the nuclear receptor PPAR-alpha. Nature. 2003; 425:90–93. [PubMed: 12955147]

Gamaleddin I, Guranda M, Goldberg SR, et al. The selective anandamide transport inhibitor VDM11 attenuates reinstatement of nicotine seeking behaviour, but does not affect nicotine intake. Br J Pharmacol. 2011; 164:1652–1660. [PubMed: 21501143]

Giuffrida A, Piomelli D. The endocannabinoid system: A physiological perspective on its role in psychomotor control. Chem Phys Lipids. 2000; 108:151–158. [PubMed: 11106788]

Hajos N, Kathuria S, Dinh T, et al. Endocannabinoid transport tightly controls 2-arachidonoyl glycerol actions in the hippocampus: Effects of low temperature and the transport inhibitor AM404. Eur J Neurosci. 2004; 19:2991–2996. [PubMed: 15182306]

Kathuria S, Gaetani S, Fegley D, et al. Modulation of anxiety through blockade of anandamide hydrolysis. Nat Med. 2003; 9:76–81. [PubMed: 12461523]

Khaled MA, Farid Araki K, Li B, et al. The selective dopamine D3 receptor antagonist SB 277011-A, but not the partial agonist BP 897, blocks cue-induced reinstatement of nicotine-seeking. Int J Neuropsychopharmacol. 2010; 13:181–190. [PubMed: 19995481]

Le Foll B, Goldberg SR. Rimonabant, a CB_1 antagonist, blocks nicotine-conditioned place preferences. Neuroreport. 2004; 15:2139–2143. [PubMed: 15486497]

Le Foll B, Goldberg SR. Cannabinoid CB_1 receptor antagonists as promising new medications for drug dependence. J Pharmacol Exp Ther. 2005; 312:875–883. [PubMed: 15525797]

Le Foll B, Forget B, Aubin HJ, et al. Blocking cannabinoid CB1 receptors for the treatment of nicotine dependence: Insights from pre-clinical and clinical studies. Addict Biol. 2008; 13:239–252. [PubMed: 18482433]

Lopez-Rodriguez ML, Viso A, Ortega-Gutierrez S, et al. Design, synthesis, and biological evaluation of new inhibitors of the endocannabinoid uptake: Comparison with effects on fatty acid amidohydrolase. J Med Chem. 2003; 46:1512–1522. [PubMed: 12672252]

Mascia P, Pistis M, Justinova Z, et al. Blockade of nicotine reward and reinstatement by activation of alpha-type peroxisome proliferator-activated receptors. Biol Psychiatry. 2011; 69:633–641. [PubMed: 20801430]

Melis M, Pillolla G, Luchicchi A, et al. Endogenous fatty acid ethanolamides suppress nicotine-induced activation of mesolimbic dopamine neurons through nuclear receptors. The Journal of Neuroscience : the official journal of the Society for Neuroscience. 2008; 28:13985–13994. [PubMed: 19091987]

Moore SA, Nomikos GG, Dickason-Chesterfield AK, et al. Identification of a high-affinity binding site involved in the transport of endocannabinoids. Proc Natl Acad Sci U S A. 2005; 102:17852–17857. [PubMed: 16314570]

Moreira FA, Grieb M, Lutz B. Central side-effects of therapies based on CB1 cannabinoid receptor agonists and antagonists: Focus on anxiety and depression. Best Pract Res Clin Endocrinol Metab. 2009; 23:133–144. [PubMed: 19285266]

Nathan PJ, O'Neill BV, Napolitano A, et al. Neuropsychiatric adverse effects of centrally acting antiobesity drugs. CNS Neurosci Ther. 2011; 17:490–505. [PubMed: 21951371]

Ortar G, Ligresti A, De Petrocellis L, et al. Novel selective and metabolically stable inhibitors of anandamide cellular uptake. Biochem Pharmacol. 2003; 65:1473–1481. [PubMed: 12732359]

Roberts DC, Bennett SA. Heroin self-administration in rats under a progressive ratio schedule of reinforcement. Psychopharmacology (Berl). 1993; 111:215–218. [PubMed: 7870955]

Scherma M, Justinova Z, Zanettini C, et al. The anandamide transport inhibitor AM404 reduces the rewarding effects of nicotine and nicotine-induced dopamine elevations in the nucleus accumbens shell in rats. Br J Pharmacol. 2012; 165:2539–2548. [PubMed: 21557729]

Scherma M, Panlilio LV, Fadda P, et al. Inhibition of anandamide hydrolysis by cyclohexyl carbamic acid 3′-carbamoyl-3-yl ester (URB597) reverses abuse-related behavioural and neurochemical effects of nicotine in rats. J Pharmacol Exp Ther. 2008; 327:482–490. [PubMed: 18725543]

Solinas M, Panlilio LV, Tanda G, et al. Cannabinoid agonists but not inhibitors of endogenous cannabinoid transport or metabolism enhance the reinforcing efficacy of heroin in rats. Neuropsychopharmacology. 2005; 30:2046–2057. [PubMed: 15870833]

Vlachou S, Stamatopoulou F, Nomikos GG, et al. Enhancement of endocannabinoid neurotransmission through CB1 cannabinoid receptors counteracts the reinforcing and psychostimulant effects of cocaine. Int J Neuropsychopharmacol. 2008; 11:905–923. [PubMed: 18377702]

Zygmunt PM, Chuang H, Movahed P, et al. The anandamide transport inhibitor AM404 activates vanilloid receptors. Eur J Pharmacol. 2000; 396:39–42. [PubMed: 10822052]

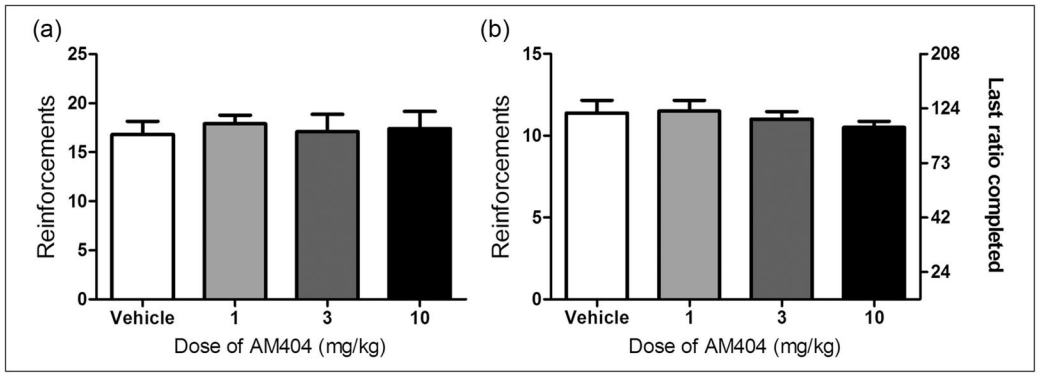

Figure 1.

Effects of AM404 on nicotine self administration: (a) effects of AM404 (1, 3 and 10 mg/kg, intraperitoneal (IP) 30 min pretreatment time (PTT)) on nicotine self-administration under a fixed-ratio (FR) 5 schedule of reinforcement. Data are expressed as the number of infusions (mean±standard error of the mean (SEM), n=10) obtained during the 60 min session. AM404 did not affect responding vs vehicle pretreatment (p>0.05); (b) effects of AM404 (1, 3 and 10 mg/kg, IP 30 min PTT) on nicotine self administration under a progressive-ratio (PR) schedule of reinforcement. Data are expressed as the number of infusions (mean±SEM, n=8) obtained during the 4 h sessions. AM404 did not affect break point vs vehicle pretreatment (p>0.05).

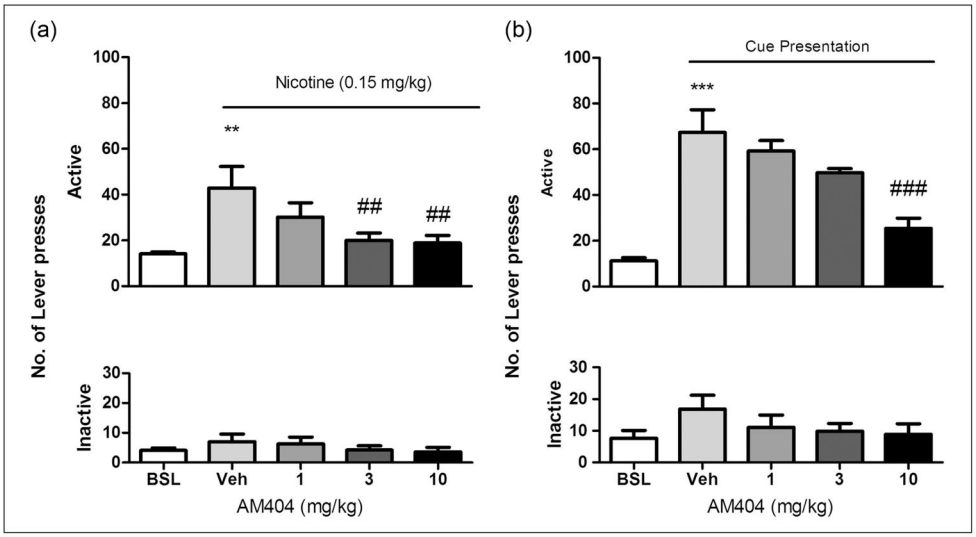

Figure 2.

Effects of AM404 on reinstatement of nicotine-seeking behavior: (a) effects of AM404 (1, 3 and 10 mg/kg, intraperitoneal (IP) 30 min pretreatment time (PTT)) on reinstatement of nicotine-seeking behavior induced by a priming dose of nicotine. Data are expressed as the number of active and inactive lever presses (mean±standard error of the mean (SEM), n=9). Pretreatment with nicotine (0.15 mg/kg, subcutaneously (SC), 10 min PTT) produced a significant reinstatement of nicotine-seeking behavior (**p<0.01). Pretreatment with AM404 (3 and 10 mg/kg, IP 30 min PTT) significantly reduced nicotine-induced reinstatement of nicotine-seeking behavior (##p<0.01); (b) effects of AM404 (1, 3 and 10 mg/kg, IP 30 min PTT) on reinstatement of nicotine-seeking behavior induced by presentation of nicotine-associated cues. Data are expressed as the number of active and inactive lever presses (mean±SEM, n=9). Presentation of nicotine-associated cues alone produced a significant reinstatement of nicotine-seeking behavior (***p<0.001) compare to baseline behavior (BSL). Pretreatment with AM404 (10 mg/kg, IP, 30 min PTT) significantly reduced cue-induced reinstatement of nicotine-seeking behavior (###p<0.001).

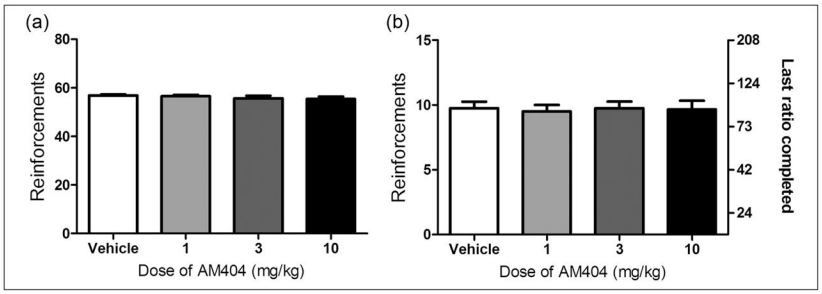

Figure 3.

Effects of AM404 on operant responding for food: (a) effects of AM404 (1, 3 and 10 mg/kg, intraperitoneal (IP), 30 min pretreatment time (PTT)) on operant responding for food under a fixed-ratio (FR) 5 schedule of reinforcement. Data are expressed as the number of food pellets (mean±standard error of the mean (SEM), n=12) obtained during the 60 min session. AM404 did not affect responding vs vehicle pretreatment (p>0.05); (b) effects of AM404 (1, 3 and 10 mg/kg, IP 30 min PTT) on operant responding for food under a progressive-ratio (PR) schedule of reinforcement. Data are expressed as the number of food pellets (mean ±SEM, n=12) obtained during the 4 h sessions. AM404 did not affect break point vs vehicle pretreatment (p>0.05).

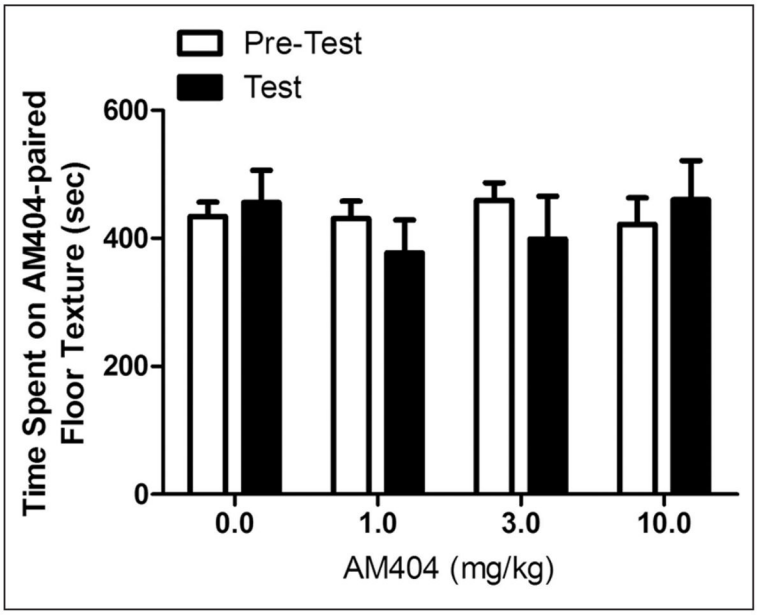

Figure 4.

Effects of AM404 (1, 3 and 10 mg/kg, IP 30 min pretreatment time (PTT)) on development of conditioned place preference (CPP). Data are expressed as time spent in seconds (mean ±standard error of the mean (SEM), n=8) in the nicotine-paired compartment during 15 min pre-test sessions (open bars) and 15 min test sessions (filled black bars). AM404 produced no significant CPP or aversion compared to the vehicle group (p>0.05).