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Blockade of nicotine reward and reinstatement by activation of alpha-type peroxisome proliferator-activated receptors

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Abstract

Background—Recent findings indicate that inhibitors of fatty acid amide hydrolase (FAAH) counteract the rewarding effects of nicotine in rats. FAAH inhibition increases levels of several endogenous substances in the brain, including the endocannabinoid anandamide and the non-cannabinoid fatty-acid ethanolamides oleoylethanolamide (OEA) and palmitoylethanolamide (PEA), which are ligands for alpha-type peroxisome proliferator-activated nuclear receptors (PPAR-α). Here, we evaluated whether directly-acting PPAR-α agonists can modulate reward-related effects of nicotine.

Methods—We combined behavioral, neurochemical and electrophysiological approaches to evaluate effects of the PPAR-α agonists WY14643 and methOEA (a long-lasting form of OEA) on: (1) nicotine self-administration in rats and squirrel monkeys; (2) reinstatement of nicotine-seeking behavior in rats and monkeys; (3) nicotine discrimination in rats; (4) nicotine-induced electrophysiological activity of VTA dopamine neurons in anesthetized rats; and (5) nicotine-induced elevation of dopamine levels in the nucleus accumbens shell of freely-moving rats.

Results—PPAR-α agonists dose-dependently decreased nicotine self-administration and nicotine-induced reinstatement in rats and monkeys, but did not alter food- or cocaine-reinforced operant behavior or the interoceptive effects of nicotine. PPAR-α agonists also dose-dependently decreased nicotine-induced excitation of dopamine neurons in the ventral tegmental area (VTA)

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and nicotine-induced elevations of dopamine levels in the nucleus accumbens shell of rats. The ability of WY14643 and methOEA to counteract the behavioral, electrophysiological, and neurochemical effects of nicotine was reversed by the PPAR-α antagonist MK886.

Conclusions—These findings indicate that PPAR-α might provide a valuable new target for anti-smoking medications.

Keywords

Nicotine; PPAR-α; reinstatement; reward; OEA; PEA; FAAH

Introduction

It has recently been recognized that peroxisome proliferator-activated receptors (PPAR) — which are known to be involved in metabolism and other cellular functions in many internal organs — also comprise a cannabinoid-like signaling system in the brain (1). Like the endogenous cannabinoid anandamide, the fatty acid amides oleoylethanolamide and palmitoylethanolamide (OEA and PEA) are endogenous ligands for the alpha subtype of the PPAR receptor (PPAR-α), are synthesized on demand, and are primarily degraded by fatty acid amide hydrolase (FAAH). Drugs that selectively inhibit FAAH prevent degradation and increase brain levels of anandamide, OEA, and PEA (2,3). But, unlike anandamide, OEA and PEA are devoid of action at cannabinoid receptors (4-6).

We recently reported that a FAAH-inhibiting drug can counteract addiction-related effects of nicotine in several animal models (7-9). In rats, FAAH inhibition suppressed the development of nicotine-induced conditioned place-preference and nicotine self-administration, widely-used animal models of nicotine's habit-forming effects (9). FAAH inhibition also suppressed reinstatement of nicotine-seeking, an animal model of relapse (7). In addition to these behavioral effects, we found that FAAH inhibition prevented the neurochemical and electrophysiological effects of nicotine on reward circuits of the brain that underlie addictive behavior. That is, FAAH inhibition prevented nicotine-induced elevations of the neurotransmitter dopamine in the nucleus accumbens shell (9), and it attenuated nicotine-induced excitation of dopamine neurons in the ventral tegmental area (VTA) (8). Surprisingly, the latter effect did not appear to be mediated by cannabinoid receptors since it was not mimicked *in vivo* by intravenous (i.v.) or intracerebroventricular (i.c.v.) administration of the cannabinoid methanandamide (a hydrolysis-resistant analog of anandamide), but rather by i.c.v. administration of the non-cannabinoid FAAH substrate, OEA (8). In addition, in vitro activation of VTA dopamine neurons by nicotine in brain slices was prevented by both OEA and PEA, but not by methanandamide (8). OEA and PEA, are potent agonists of PPAR-α, which is expressed in many areas of the rat brain [including cortex, VTA, midbrain, medulla, hippocampus, substantia nigra and olfactory tubercle (10-14)] and might regulate cholinergic neurotransmission and learning and memory processes (15,16). These findings suggest that FAAH inhibition counteracts the rewarding effects of nicotine by activating PPAR-α.

In the present study, we combined behavioral, neurochemical and electrophysiological approaches to determine whether directly-acting PPAR-α agonists can counteract several reward- and dependence-related effects of nicotine: (1) nicotine self-administration in rats and squirrel monkeys; (2) reinstatement of nicotine-seeking precipitated by re-exposure to nicotine in rats and squirrel monkeys after a period of abstinence; (3) the interoceptive effects of nicotine in a drug-discrimination procedure in rats; (4) electrophysiological effects of nicotine on the activity of VTA dopamine neurons in anesthetized rats; and (5) nicotine-induced elevations in dopamine levels in the nucleus accumbens shell of freely-moving rats.

Methods and Materials

Animals

Male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA; Harlan-Nossan, Milan, Italy) weighing 300-350g were housed in temperature- and humidity-controlled rooms on a 12-hr light/dark cycle. Experiments were conducted during the light phase. For self-administration experiments, food intake was limited to 20 g/day. For drug discrimination experiments, food was restricted to maintain weight at ≥85% of the subject's highest recorded weight.

Ten adult male squirrel monkeys *(Saimiri sciureus)* weighing 0.9-1.1 kg were housed in individual cages in a temperature- and humidity-controlled room with unrestricted access to water. Monkeys were fed five high-protein biscuits/day (Lab Diet 5045, PMI Nutrition International, Richmond, Indiana) and two pieces of Banana Softies (Bio-Serv, Frenchtown, NJ). Fresh fruits, vegetables and environmental enrichment were provided daily. Three monkeys (441, 431, 577) self-administered nicotine. Three monkeys (70F7, 5045, 39B) self-administered cocaine. Four monkeys (34A, 27B, 30A, 1549) were used for food - reinforcement experiments.

Monkeys and rats at NIDA-IRP were maintained in facilities fully accredited by AALAC, and all procedures were approved by the NIDA IACUC and conducted in accordance with the Guidelines of the National Research Council (2003). Rats in the electrophysiology study were maintained at the University of Cagliari, where all procedures were conducted in accordance with the European Economic Community Council Directive (86/609; DL27/01/92, no. 116).

Drugs

Nicotine [(-)-nicotine hydrogen tartrate] (Sigma, USA) was dissolved in saline (pH corrected to 7.0) and injected s.c. or i.v. (-)-Cocaine HCl (RTI international, USA) and injected i.v. The PPAR-α agonist methOEA (methyl oleoylethanolamide; donated by Dr. Daniele Piomelli, University of California, Irvine) was dissolved in 2% Tween80, 2% ethanol, and sterile water. The PPAR-α agonist WY14643 ([[4-Chloro-6-[(2,3-dimethylphenyl)amino]-2-pyrimidinyl] thio]acetic acid; Tocris, USA) and PPAR-α antagonist MK886 (1-[(4-Chlorophenyl)methyl]-3-[(1,1-dimethylethyl)thio]-a,a-dimethyl-5-(1-methylethyl)-1H-Indole-2-propanoic acid; Tocris, USA) were dissolved in 2-4% Tween 80, 30% DMSO, and sterile water. MethOEA, WY14643 and MK886 were injected i.p. (1 ml/kg) in rats and i.m. (0.3 ml/kg) in monkeys, except for electrophysiology, where methOEA was injected i.v. Dose selection was based on previous studies using WY14643 (8,16,17), methOEA (KDS5104) (18) and MK886 (8,16).

Self administration (rats)

General procedure and apparatus described previously (9,19). Self-administration sessions (2 hr/day) under a one-response fixed-ratio (FR1) schedule of i.v. nicotine injection (10 or 30 μg/kg/injection) began 7-10 days after catheterization, and increased to FR5 over 15-23 sessions. Responses in the left nose-poke hole produced nicotine and pulsed the houselight (5 Hz) for a 20 s timeout. Responses in the other, inactive, hole had no scheduled effect. Once a criterion was reached (>9 nicotine injections/session for three consecutive sessions), rats received a pretreatment injection (either drug or vehicle) before each subsequent session, with drugs tested only when the criterion had been met during the preceding three vehicle sessions. Doses of WY14643 (20 or 40 mg/kg i.p., 20 min before the session, tested in counterbalanced order) or methOEA (10 mg/kg i.m., 40 min before the session) were each tested for three consecutive sessions. Mean data from the three drug pretreatment sessions

for each dose were compared to the mean from the three preceding vehicle pretreatment sessions.

Reinstatement (rats)

Rats learned to self-administer nicotine (30 μg/kg/injection i.v.) as described above, and then were placed under extinction, during which responding had no programmed consequence. When there were <10 active responses per session for three consecutive sessions, reinstatement tests were conducted with a pretreatment injection (vehicle, 20 or 40 mg/kg WY14643, i.p., 20 min before the session) and a priming injection (vehicle or 0.2 mg/kg nicotine, s.c., 10 min before the session). Rats were required to meet the response-cessation requirement prior to each test. Nine rats were tested at both doses of WY14643, and some (2 rats at 20 mg/kg and 6 rats at 40 mg/kg WY14643) were only tested with one dose of WY14643. During the reinstatement test session, responding in the active hole pulsed the houselight for 20 seconds; to increase sensitivity of the reinstatement test, only a single response was required to produce this cue. This reinstatement procedure combines nicotine- and cue-induced reinstatement, has been used in several previous studies (20-23), and has several advantages. First, when the cues are removed during extinction, the response-cessation criterion is met more rapidly (mean ± SEM. = 5.2 ±0.8 sessions in the present study without the stimulus, versus 11.3 ± 1.5 in a pilot group with the stimulus). Second, reinstatement by the combination of nicotine and cues is more robust than when only nicotine or only the cues are presented. This was important because, consistent with the findings of others (20), we have found that a substantial number of rats fail to show reinstatement when given nicotine alone. Finally, the combination of nicotine and cues may be a more relevant and stringent model of the human relapse situation than cues alone or nicotine alone.

Self administration (monkeys)

General procedure and apparatus described previously (24,25). At the start of the session, the houselight was extinguished and a green stimulus light was presented. In the presence of the green light, 10 responses on the lever (FR10) produced a 0.2-s, 0.2-ml, 30 μg/kg injection of nicotine, extinguished the green light and illuminated the amber stimulus light for 2 s. Each reinforcement was followed by a 60-s timeout period, during which the chamber was dark and lever presses had no programmed consequences. One-hour sessions were conducted five days/week.

After 4-5 sessions of vehicle pretreatment in which responding was stable (<15% variability), drug pretreatments were given for 5 consecutive sessions. Data from the 5 drug pretreatment sessions were compared to the 3 preceding vehicle pretreatment sessions. The drug pretreatments were WY14643 (10, 20 and 40 mg/kg i.m., 20 min before the session), MK886 (1 mg/kg i.m., 45 min before the session), MK886 (1 mg/kg) plus WY14643 (20 mg/kg), and methOEA (10 mg/kg i.m., 40 min before the session). WY14643 pretreatments (20 and 40 mg/kg i.m., 20 min before the session) were also tested in two additional groups of monkeys self-administering cocaine (30 μg/kg/injection) or food pellets under the same fixed-ratio schedule.

Reinstatement (monkeys)

Monkeys trained to self-administer nicotine (30 μg/kg/injection, i.v.), as described above, were placed under extinction by substituting vehicle for nicotine but maintaining the response-dependent presentation of the nicotine-paired stimulus. When responding reached a low, stable level (<10 injections/session, with no obvious increasing or decreasing trend), priming injections (vehicle or 0.1 mg/kg i.v. nicotine, immediately before the session) were given after pretreatment with WY14643 (20 or 40 mg/kg) or WY14643 (20 or 40 mg/kg)

plus MK886 (1 mg/kg). Pretreatments were given in the home cage, and primes were given while the monkey was in the training chamber. Each test was performed for a single session followed by extinction sessions with no pretreatment.

Nicotine discrimination (rats)

General procedure and apparatus described previously (26). Rats were trained under a discrete-trial schedule of food-pellet delivery (FR10, 45-s timeout) to respond on one lever after a subcutaneous (s.c.) injection of a training dose of 0.4 mg/kg nicotine (10 min before the session) and on the other lever after an injection of saline. Sessions lasted for 20 reinforcements or 30 min. WY14643 (40 mg/kg) was substituted for the training dose of nicotine and was also administered together with various doses of nicotine (0.01-0.4 mg/kg) to assess possible alterations of the nicotine dose-response curve.

Electrophysiology (anesthetized rats)

General procedure described previously (8). Single-unit activity of VTA neurons was recorded extracellularly with glass micropipettes filled with 2% pontamine sky blue dissolved in 0.5 M sodium acetate (impedance 2-5 MΩ). Single units were isolated and identified according to previously published criteria (27,28). All neurons were antidromically identified as projecting to the nucleus accumbens shell by antidromic spikes elicited by stimulation of the shell of the NAc. An antidromic response was defined as the ability of evoked spikes to follow stimulation frequencies of >250 Hz, displaying constant latency and collision with spontaneously occurring spikes (29). Nicotine (0.2 mg/kg) was administered i.v. after 5-10 min of baseline recording. MethOEA (0, 5 or 10 mg/kg i.v.) was injected 4 min before nicotine. WY14643 (20 or 40 mg/kg i.p.) was injected ~30 min before the start of recordings, MK886 (3 mg/kg i.p.) was injected 15 min before WY14643. Only one cell was recorded per rat.

Microdialysis (freely-moving rats)

General procedure described previously (30). Rats were surgically implanted with a concentric dialysis probe aimed at the shell of the nucleus accumbens [anterior +2.0 and lateral 1.1 from bregma, vertical −8.0 from dura] (31). Experiments were performed on freely-moving rats 20-24 h after the surgical implant. Ringer's solution (147.0 mM NaCl, 2.2 mM $CaCl_2$, 4.0 mM KCl) was delivered at a constant flow rate of 0.5 μl/min. Collection of dialysate samples (10 μl) started after 90 min, with samples collected every 20 min and immediately analyzed by an HPLC system coupled to electrochemical detection in order to quantify dopamine. Rats were treated only after stable dopamine values (<10% variability) were obtained for at least three consecutive samples. Probe location in the nucleus accumbens shell was determined histologically after each experiment and only data from rats with correct probe placement were analyzed. WY14643 (0, 20 or 40 mg/kg i.p.) was injected 20 min before nicotine (0.4 mg/kg s.c.) or cocaine (3 mg/kg i.p.), and methOEA (0, 5 or 10 mg/kg i.p.) was injected 40 min before nicotine (0.4 mg/kg s.c.). MK886 (3 mg/kg i.p.) was injected i.p. 20 min before WY14643 or methOEA.

Statistical analysis

All results are presented as group means (± SEM). For experiments comparing only two conditions, Student's t-test was performed. Self-administration and reinstatement data were analyzed with Proc Mixed (SAS Institute, Cary NC) with Tukey-Kramer comparisons. For self-administration, the dependent variable was injections/session and the independent variables were pretreatment (dose) and session type (baseline sessions vs. pretreatment sessions), allowing each pretreatment condition to be compared to the most recent baseline. For selected conditions where consecutive sessions are illustrated (Figures 1B, 2B, and 2C),

additional analyses were performed using session as a factor. For reinstatement in rats, the factors were nose-poke hole (active vs. inactive), prime (saline vs. nicotine), and pretreatment (dose of WY14643); for monkeys, separate one-way ANOVAs were conducted for the three doses of WY14643. Electrophysiology and microdialysis data were analyzed using analysis of variance (ANOVA) with Dunnett or Student-Newman-Keuls comparison procedures, respectively. Experiment-wise significance levels of 0.05 were maintained in all analyses.

Results

PPAR-α activation suppressed nicotine self-administration in rats and monkeys

The PPAR-α agonists WY14643 and methOEA significantly decreased ongoing nicotine self-administration in both rats (Fig. 1A: main effect of WY14643, $F(1,11)=5.4$, $p<.05$; main effect of session $F(1,11)=41.8$, $p<.0001$; for 0.01 mg/kg nicotine baseline, t-test, $t_5=5.019$, $p<.05$; methOEA: $t_4=5.4$, $p<.006$) and monkeys (WY14643 in Fig. 2A: interaction of WY14643 and session $F(2,4)=8.25$, $p<.05$; methOEA in Fig. 2A: $t_2=25.8$, $p<.01$). At the most effective doses, self-administration behavior was decreased significantly throughout the course of PPAR-α agonist treatment (Fig. 1B: $F(3,27)=7.99$, $p<.001$; Fig. 2B: $F(5,10)=11.04$, $p<.001$; Fig 2D: $F(5,10)=6.23$, $p<.007$) and rapidly returned to higher levels when treatment was discontinued. In rats, response rates in the inactive hole occurred at a fairly constant percentage of response rates in the active hole regardless of pretreatment (mean percentage±SEM=26±5 under vehicle treatment, 21±3 under 20 mg/kg WY14643, and 27±9 under 40 mg/kg WY14643). The specificity of WY14643's effects was verified by giving the PPAR-α antagonist MK886 as a pretreatment in monkeys. MK886 reversed the decreases in nicotine self-administration produced by WY14643, but had no effect on nicotine self-administration when given alone. On the final day of treatment with the most effective dose of WY14643 (40 mg/kg), nicotine intake was decreased by 35% in rats and 76% in monkeys.

PPAR-α activation suppressed reinstatement when abstinent rats and monkeys were re-exposed to nicotine

When relapse was modeled using a reinstatement procedure, the nicotine-seeking response (nose poking in rats, lever pressing in monkeys) was reinstated by a non-contingent priming injection of nicotine before the session (Fig. 3). WY14643 significantly reduced this reinstatement in rats (Fig. 3A: main effect of active vs. inactive nose-poking hole, $F(1,14)=20.4$, $p<.0005$; interaction of WY14643 dose and nicotine, $F(2,13)=7.7$, $p<.01$) and monkeys (Fig. 3B: 20 mg/kg WY14643: $F(3,6)=15.4$, $p<.005$; 40 mg/kg WY14643: $F(3,6)=93.5$, $p<.001$). WY14643 alone did not reinstate drug-seeking. In rats, nicotine also increased responding in the inactive hole, and WY14643 prevented this increase. However, it should be noted that responding in the active hole remained higher than the inactive hole under all testing conditions, indicating that the nicotine-induced increases in active-hole responding were due to reward rather than nonspecific increases in locomotor activity. Pretreatment of monkeys with the PPAR-α antagonist MK886 prevented the effects of WY14643 in this model of relapse, demonstrating the receptor-specificity of these effects (Fig. 3B).

PPAR-α activation did not alter nicotine's interoceptive effects or produce a general depression of operant behavior

The ability of WY14643 to reduce nicotine self-administration and reinstatement was not due to a nonspecific disruption of operant behavior. WY14643 had no effect on cocaine- or food-reinforced responding in squirrel monkeys under testing conditions identical to those used with nicotine (Fig. 2D), and it did not alter food-reinforced responding by rats as

measured in the drug discrimination procedure (even when combined with intraperitoneal nicotine injection; Fig. 4B) or rats' ability to detect that they had received nicotine (Fig. 4A) in a drug-discrimination procedure.

PPAR-α activation prevented nicotine-induced changes in dopamine-cell firing in the ventral tegmental area of rats

In single-unit *in-vivo* recording experiments in anesthetized rats, i.v. injection of 0.2 mg/kg nicotine enhanced firing rate (Fig.5A: $F(6,7)=6.99$, $p<.0001$;) and burst firing (Fig. 5B: $F(6,7)=2.837$; $p<.05$) of VTA dopamine neurons that were antidromically identified as projecting to the nucleus accumbens. At doses of WY14643 that significantly reduced nicotine self administration and nicotine-induced reinstatement in the behavioral experiments, WY14643 and methOEA did not alter spontaneous firing rate (Fig. 5A,C; basal mean Hz ±SEM.; controls: 3.24±0.2; WY14643 20 mg/kg: 3.2±0.6; WY14643 40 mg/kg: 3.3±0.6; methOEA 5 mg/kg: 3.18±0.5; MK886+WY1464340 mg/kg: 3.6±0.8; methOEA 10 mg/kg: 3.11±0.4) or burst firing (Fig. 5B,D; basal mean % of spikes/bursts ± SEM; controls: 12.9±3.72; WY14643 20 mg/kg: 13.6±9.88; WY14643 40 mg/kg: 15.8±6.1; MK886+WY1464340 mg/kg: 8.0±6.4; methOEA 5 mg/kg: 8.7±3.57; methOEA 10 mg/kg: 8.9±1.61) of VTA dopamine neurons when given alone. However, when given before nicotine, 20 mg/kg of WY14643 partially blocked and 40 mg/kg of WY14643 completely blocked nicotine-induced excitation of dopamine neurons (Fig. 5A,B: effect of WY14643 on firing rate, $F(1,48)=20.36$, $p<.001$; and burst firing, $F(1,48)=5.98$, $p<.05$). Intravenous administration of methOEA (5 and 10 mg/kg) also completely prevented excitation of dopamine neurons by nicotine (Fig. 5C,D: effects of two doses of methOEA on firing rate: $F(1,48)=5.02$, $p<.05$, and $F(1,48)=5.24$, $p<.05$; and burst firing: $F(1,52)=6.34$, $p<.05$, and $F(1,52)=4.73$, $p<.05$). Pretreatment with the PPAR-α antagonist MK886 completely reversed WY14643's blockade of nicotine-induced increases in firing rate (Fig. 5A) and burst firing (Fig. 5B: MK886+WY14643 vs. WY14643, $F(1,60)=16.57$, $p<.01$; $F(1,60)=18.24$, $p<.05$, for firing rate and burst firing, respectively).

PPAR-α activation prevented nicotine-induced increases in dopamine levels in the nucleus accumbens shell of rats

In-vivo microdialysis experiments in freely-moving rats showed that systemic injection of 0.4 mg/kg nicotine increased extracellular dopamine levels in the nucleus accumbens shell by ~100% (Fig. 6A: $F(12,48)=16.23$, $p<.0001$; Fig. 6B: $F(13,65)=58.61$; $p<.0001$). WY14643 alone did not alter dopamine levels, but it significantly reduced nicotine-induced elevations in dopamine levels in a dose-related manner (Fig. 6A: time–treatment interaction, $F(18,108)=3.01$, $p<.001$; basal level, expressed as mean fmoles/10 μL sample ±SEM. for controls: 31±2.7; WY14643 40 mg/kg: 31.8 ± 5.2;). Administration of methOEA also did not alter dopamine levels by itself (Fig. 6D), but markedly reduced nicotine-induced elevations in dopamine levels (Fig. 6B: time–treatment interaction, $F(26,169)=5.95$, $p<.0001$). The PPAR-α antagonist MK886 had no effect when given alone but completely reversed WY14643's (40 mg/kg) blockade of nicotine-induced elevations in dopamine levels (Fig. 6C: time–treatment interaction, $F(26,169)=4.06$, $p<.05$). Similarly, MK866 prevented the effects of 10 mg/kg of methOEA (Fig. 6D: time–treatment interaction, $F(28,182)=3.06$, $p<.01$). In contrast, WY14643 did not alter the effects of cocaine on dopamine levels in the nucleus accumbens shell. Basal levels of dopamine in dialysates, expressed as mean fmoles/10 μL sample ± SEM, did not differ between groups prior to injections (Fig. 5A,C; vehicle +nicotine: 31.2±5.2; WY14643 40 mg/kg+saline: 31.8±5.2; WY14643 20 mg/kg+nicotine: 30.8±4.5; WY14643 40 mg/kg+nicotine: 36.0±5.9; MK886+vehicle+saline: 41.0±6.0; MK886+WY14643 40 mg/kg+nicotine: 32.0±4.8; Fig. 5B,D; vehicle+nicotine: 30.8±2.9; methOEA 5 mg/kg+nicotine: 31.5±1.3; methOEA 10 mg/kg+nicotine: 33.8±4.2;

MK886+vehicle+saline: 32.5±3.2; MK886+methOEA 10 mg/kg+nicotine: 30.4±3.3; Fig. 5E; vehicle+cocaine:34.9±5.8; cocaine+WY14643: 36.6±5.4).

Discussion

These findings indicate that activation of PPAR-α receptors can counteract addiction-related effects of nicotine on the brain and behavior. In both rats and monkeys, the PPAR-α agonists WY14643 and methOEA significantly decreased nicotine self-administration and suppressed reinstatement of nicotine seeking, which models relapse, the main obstacle to smoking cessation. At the doses that produced these effects with nicotine self-administration, there was no indication that PPAR-α ligands had any effect on food- or cocaine maintained behavior. The reduction of nicotine self-administration and reinstatement by PPAR-α agonists was most likely due to these drugs' ability to prevent nicotine-induced excitation of dopaminergic transmission in reward-related areas of the brain. Specifically, PPAR-α agonists prevented nicotine-induced increases in firing rate and burst firing in dopamine neurons in the VTA, and they prevented nicotine-induced (but not cocaine-induced) elevations of dopamine levels in the shell of the nucleus accumbens. These potentially therapeutic behavioral, electrophysiological, and neurochemical effects of PPAR-α agonists were reversed by the PPAR-α antagonist MK886, verifying that they were indeed due to PPAR-α activation.

The fact that the PPAR-α agonist WY14643 did not alter the interoceptive effects of nicotine in the drug-discrimination procedure is consistent with previous findings that nicotine's reward-related dopaminergic effects are not well-captured by this procedure (32). For example, even though the cannabinoid CB_1 receptor antagonist rimonabant can block nicotine reward (i.e., self-administration, conditioned place preference) and nicotine-induced increases of dopamine levels in the nucleus accumbens, rimonabant does not alter nicotine discrimination (26,33). Similarly, the dopamine D_3 antagonist BP897 blocks nicotine-induced conditioned place preference, but does not alter nicotine discrimination (34). The finding that WY14643 blocked nicotine's effects on dopamine but did not alter its discriminative effects is consistent with previous data suggesting that nicotine discrimination can be mediated by nicotine's indirect effects on serotonin, cannabinoid, and glutamate receptors (35).

The nicotine-related behavioral, electrophysiological, and neurochemical effects of PPAR-α agonists in the present study are similar to the effects obtained earlier with the FAAH inhibitor URB597 (7,9). The results obtained here with the PPAR-α agonist WY14643 are also consistent with the finding that URB597 does not alter nicotine discrimination (36). All of these findings converge to suggest that URB597 modulates the rewarding effects of nicotine by increasing levels of the endogenous PPAR-α ligands OEA and PEA, rather than by increasing the endocannabinoid anandamide.

The mechanism by which PPAR-α agonists and FAAH inhibition exert these unanticipated anti-addictive actions is not completely understood. However, the following points are well-established. Mesolimbic dopamine plays a pivotal role in nicotine dependence, and the VTA and nucleus accumbens shell are critical brain areas for nicotine's rewarding effects (37-39). Nicotinic receptors in the VTA are located both on cell bodies of dopaminergic neurons and on presynaptic nerve endings of glutamatergic neurons which descend from the medial prefrontal cortex and impinge on these cell bodies (40). Nicotine facilitates dopaminergic neurotransmission and dopamine release in the nucleus accumbens shell by directly stimulating nicotinic receptors on cell bodies of dopaminergic neurons and by indirectly stimulating glutamate release, which in turn stimulates VTA dopaminergic neuron firing and dopamine release in the nucleus accumbens shell. Our data show that activation of PPAR-α,

either indirectly through FAAH inhibition or directly by administration of a PPAR-α agonist, prevents nicotine-induced increases in firing rate and burst firing in dopamine neurons in the VTA and as a consequence prevents nicotine-induced elevations of dopamine levels in the shell of the nucleus accumbens. The mechanism underlying these effects was elucidated by our recent in vitro findings showing that activation of PPAR-α produces a non-genomic (rapid) modulation of nicotinic receptors on cell bodies of dopaminergic neurons in the VTA by promoting their phosphorylation by tyrosine kinases (8). Phosphorylated nicotinic receptors show diminished ionic conductance (41) and are rapidly internalized (42), reducing or abolishing the responses of dopamine neurons to nicotine, and we have demonstrated that the general tyrosine kinase inhibitor genistein reverses OEA's ability to block nicotine-induced excitation of VTA dopamine neurons (8). Additionally, we have found that the β2 subunit of nicotinic receptors is critical for PPAR-α effects, since deletion of this subunit abolished the effects of PPAR-α compounds, whereas its selective re-expression in VTA dopamine neurons restores both the behavioral effects of nicotine (motor activity) and PPAR-α actions (43). Taken together, all of these findings suggest a mechanism by which PPAR-α may modulate the reward-related dopaminergic effects of nicotine that provide a basis for nicotine self-administration.

In conclusion, our findings demonstrate that nicotine's ability to have rewarding effects and reinstate drug-seeking behavior after a period of abstinence are suppressed by PPAR-α activation, accomplished directly by PPAR-α agonists or indirectly by FAAH inhibition. These behavioral effects appear to be due to modulation of nicotine's excitatory effects on reward-related mesolimbic dopamine transmission. Notably, PPAR-α agonists and FAAH inhibitors appear to suppress nicotine reward and reinstatement much like the cannabinoid inverse agonist/antagonist rimonabant, but do not share the adverse psychoactive effects produced by medications that target cannabinoid CB1 receptors (3,9,19). Thus, PPAR-α is a promising new molecular target for the treatment of the devastating problem of tobacco dependence.

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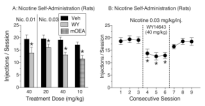

Figure 1.

The PPAR-α agonists WY14643 (20 and 40 mg/kg) and methOEA (10 mg/kg) reduced nicotine self-administration in rats. PPAR-α agonists were given i.p. 20 minute (WY14643) or 40 min (methOEA) before three consecutive sessions in which rats self-administered nicotine (0.01 or 0.03 mg/kg/injection) under a FR5 schedule. **(A)** Average rate of injection over three test sessions, compared to average of three sessions of vehicle treatment. **(B)** Rates of nicotine self-administration during individual sessions under baseline conditions (sessions 1-3), after treatment with 40 mg/kg WY14643 (sessions 4-6), and after return to baseline conditions (sessions 7-9). N=6 for rats at the 0.01 mg/kg/injection nicotine dose; n=12 for rats at the 0.03 mg/kg/injection nicotine dose, except for methOEA, where n=5. Asterisks indicate a significant difference from vehicle treatment. Data are represented as group means ± SEM.

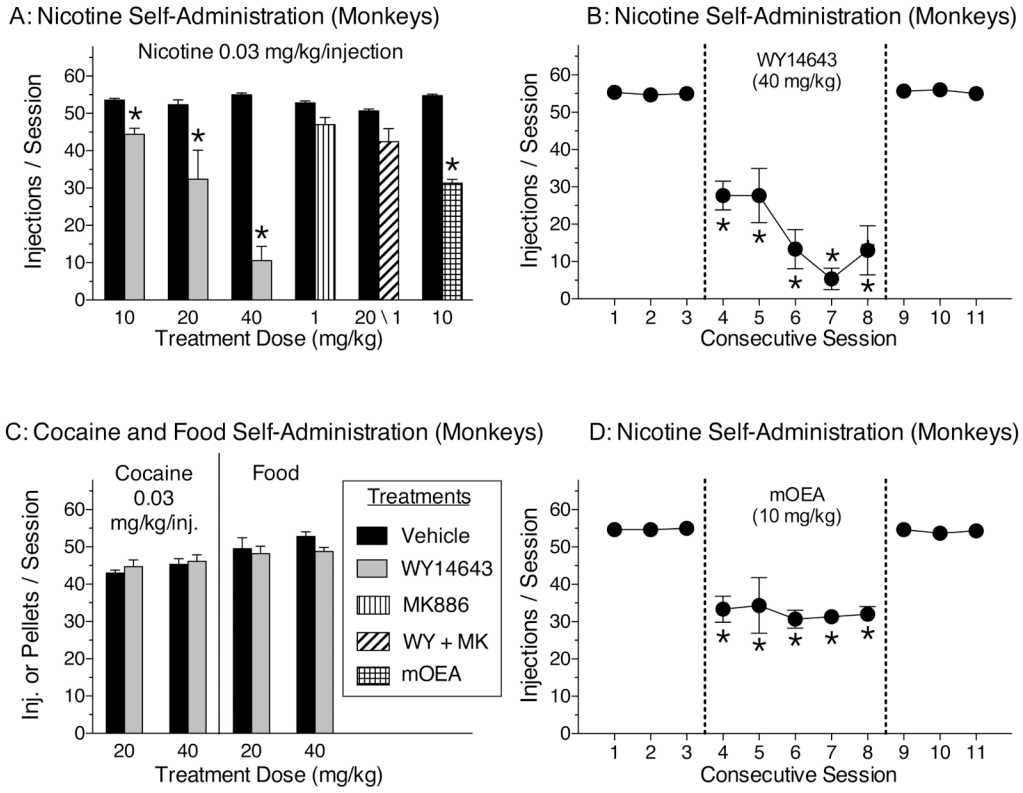

Figure 2.

The PPAR-α agonists WY14643 (10, 20 and 40 mg/kg i.m., 20 min before session for 5 consecutive sessions) and methOEA (10 mg/kg i.m., 40 min before session) significantly reduced the rate of nicotine injections self-administered by squirrel monkeys under a FR10 schedule at a nicotine dose of 30 μg/kg/injection. The effects of WY14643 (20 mg/kg) were reversed by pretreatment with the PPAR-α antagonist MK866 (1 mg/kg i.m., 45 min before session), which had no significant effect when given alone. **(A)** Average rate of injection over five test sessions, compared to average of five sessions of vehicle treatment. **(B, D)** Rates of nicotine self-administration during individual sessions under baseline conditions (sessions 1-3), after treatment with 40 mg/kg WY14643 (sessions 4-8) or 10 mg/kg methOEA, and after return to baseline conditions (sessions 9-11). **(C)** WY14643 (20 or 40 mg/kg i.m., 20 min before session) did not alter the number of 30 μg/kg cocaine injections self administered or the number of food pellets obtained under an identical FR10 schedule in squirrel monkeys. N=3 for monkeys under all conditions except food reinforcement, where n=4. Asterisks indicate a significant difference from vehicle treatment. Data are represented as group means ± SEM.

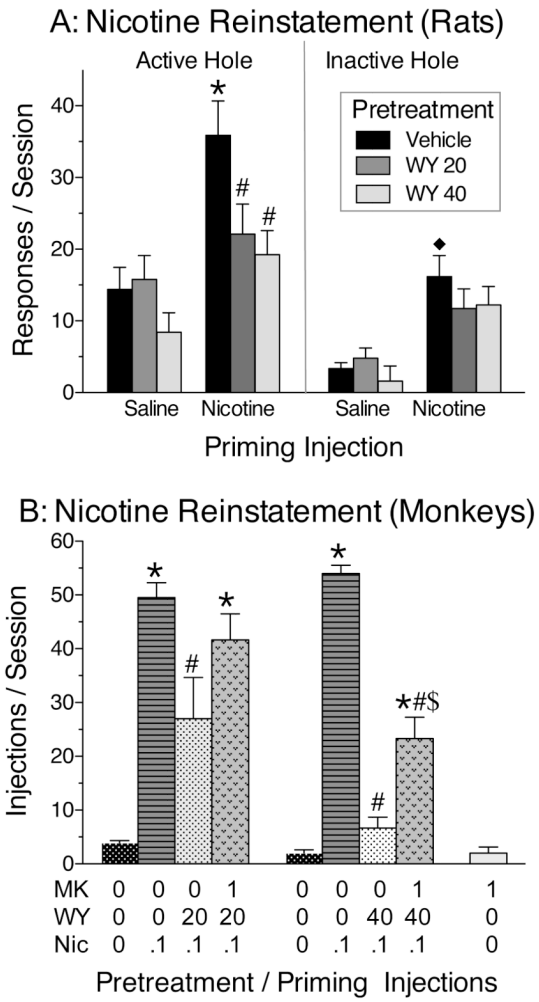

Figure 3.

The PPAR-α agonist WY14643 blocked reinstatement of nicotine self-administration after a period of abstinence in rats and monkeys. (**A**) In rats, WY14643 (20 mg/kg i.p, n=11; and 40 mg/kg i.p., n=15) dose-dependently reduced the reinstatement of extinguished nicotine-seeking responses produced by a priming injection of nicotine. (**B**) In squirrel monkeys, WY14643 (20 or 40 mg/kg i.m., 20 min before the session) dose-dependently reduced the reinstatement of extinguished nicotine-seeking responses produced by a priming injection of nicotine (0.1 mg/kg i.v.) before the session (n=3). This effect of WY14643 was prevented by pretreatment with the PPAR-α antagonist MK886 (1 mg/kg i.m., 45 min before session). Data are presented as group means ± SEM. Asterisks indicate a significant difference from vehicle pretreatment during a saline prime session. # signs represent a significant difference from vehicle pretreatment during a nicotine prime session. Diamond represents a significant difference from inactive-hole responding during a saline prime session.

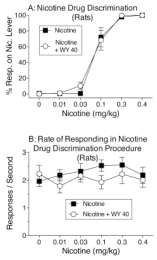

Figure 4.

The PPAR-α agonist WY14643 (40 mg/kg i.p., 20 min before session) did not alter the interoceptive effects of nicotine or the rate of food-maintained lever-pressing under a nicotine drug discrimination procedure in rats (n=12). When given alone or in combination with any dose of nicotine (0.01 to 0.4 mg/kg s.c.), WY14643 did not significantly affect the percentage of responses on the nicotine-appropriate lever (**A**) or the rate of lever responding (**B**). Data are presented as group means ± SEM.

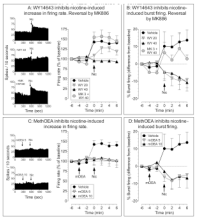

Figure 5.

PPAR-α agonists inhibited nicotine-induced activation of VTA dopamine neurons in anesthetized rats. Histograms show the stimulatory effects of nicotine (Nic, 0.2 mg/kg i.v., n=7) on discharge activity of an individual VTA dopamine neuron in a representative rat and the actions of PPAR-α agonists **(A, C)**. Line graphs show the time course of nicotine's effects. WY14643 (40 mg/kg, i.p. injected ~30 min before the start of recordings, n=7) significantly blocked nicotine-induced increases in firing rate (**A**) and burst firing (**B**). MK886 (3 mg/kg injected ~45 min before the start of recordings, n=5) significantly abolished the effects produced by WY14643 (**A, B**). MethOEA (5 and 10 mg/kg i.v. injected 4 min before nicotine, n=7 both) mimicked the effects of WY14643, significantly blocking nicotine-induced increases in firing rate (**C**) and burst firing (**D**). Results are presented as mean ± SEM of firing rates and burst firing, expressed as percentages of or differences from baseline values, respectively. Note that data for vehicle in panel a are repeated in panel c, and that data for vehicle in panel b are repeated in panel d. Arrows indicate time of drug injections. The following treatments significantly reduced the effects of nicotine on firing rate (*p*'s<.05, Dunnett's post-hoc comparisons): WY14643 40 mg/kg, methOEA 5 mg/kg, and methOEA 10 mg/kg. Burst firing was significantly reduced by WY14643 20 and 40 mg/kg, and by methOEA 5 and 10 mg/kg. Both firing rate and burst firing differed when WY14643 40 mg/kg was given with vs. without MK886 (*p*'s<.05).

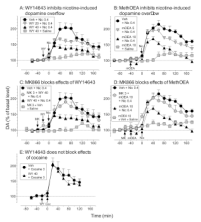

Figure 6.

PPAR-α agonists inhibited nicotine-induced elevations in dopamine levels in the nucleus accumbens shell of freely-moving rats. Pre-treatment with WY14643 (20 and 40 mg/kg i.p., n=5 both) or methOEA (10 mg/kg i.p., n=5) but not their vehicle (n=5 both), given 20 and 40 min, respectively, before nicotine (0.4 mg/kg s.c., n=6), significantly reduced the increase in extracellular dopamine levels produced by nicotine (**A, B**). The PPAR-α antagonist MK886 (3 mg/kg i.p.) injected 20 min before 40 mg/kg WY14643 (n=6) or 10 mg/kg methOEA (n=6) completely reversed the reduction of nicotine-induced elevations in dopamine levels produced by WY14643 (40 mg/kg i.p.) and methOEA (10 mg/kg i.p.) (**C, D**). The following treatments significantly reduced the effects of nicotine (Tukey post-hoc comparisons): WY14643 20 mg/kg, (p<.05); WY14643 40 mg/kg, (p<.001; methOEA 10 mg/kg (p<.001). MK886 (3 mg/kg i.p., n=4) had no significant effect when given with the vehicle of WY14643 and saline (**C**), and methOEA (10 mg/kg i.p, n=4) had no significant effect when given with the vehicles of MK886 and saline (D). The following treatments significantly reduced the effects of nicotine (Tukey post-hoc comparisons): WY14643 20 mg/kg, (p<.05); WY14643 40 mg/kg, (p<.001; methOEA 10 mg/kg (p<.001). WY14643 (40 mg/kg i.p., n=5) injected 20 min before cocaine (3 mg/kg i.p.; n=5) did not significantly alter the effects of cocaine (E). Note that data for vehicle + nicotine and for WY14643 + nicotine in panel a are repeated in panel c, and that data for vehicle + nicotine and for methOEA + nicotine in panel b are repeated in panel d. Arrows indicate time of drug or vehicle injection. Results are presented as group means ± SEM, expressed as percent of basal values.