

## RESEARCH PAPER

# The selective anandamide transport inhibitor VDM11 attenuates reinstatement of nicotine seeking behaviour, but does not affect nicotine intake

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## BACKGROUND AND PURPOSE

The endocannabinoid system appears to play a pivotal role in mediating the rewarding and reinforcing effects of nicotine. Recent studies have shown that the inhibition of fatty acid amide hydrolase (FAAH) attenuates reinstatement of nicotine-seeking induced by nicotine priming and nicotine-associated cues. FAAH hydrolyses the endogenous endocannabinoid anandamide, as well as other non-cannabinoid ligands such as oleoylethanolamide (OEA) and palmitoylethanolamide (PEA). As OEA and PEA can attenuate both nicotine-taking and nicotine-seeking behaviour, the specific role of anandamide remains unclear. In this study, we have tested the selective anandamide uptake inhibitor, VDM11, which elevates anandamide levels without affecting levels of OEA/PEA, on nicotine-taking and nicotine-seeking behaviour.

## EXPERIMENTAL APPROACH

We used a nicotine intravenous self-administration model in rats to assess the effect of VDM11, given i.p., on nicotine taking using fixed and progressive ratio schedules of reinforcement as well as on reinstatement of nicotine-seeking induced by nicotine priming and nicotine-associated cues.

## KEY RESULTS

VDM11 did not affect levels of responding for nicotine under fixed-ratio and progressive-ratio schedules of reinforcement. In contrast, VDM11 dose-dependently attenuated reinstatement of nicotine-seeking behaviour induced by nicotine-associated cues and nicotine priming.

## CONCLUSIONS AND IMPLICATIONS

These results indicate that ligands elevating anandamide levels could have therapeutic value for preventing relapse into nicotine-seeking behaviour and should be tested in humans trying to quit smoking.

## Abbreviations

2AG, 2-arachidonoylglycerol; AM404, N-(4-hydroxyphenyl)-arachidonamide; ANOVA, analysis of variance, CRF, continuous reinforcement; FAAH, fatty acid amide hydrolase; FR fixed ratio; OEA, oleoylethanolamide; PEA, palmitoylethanolamide; PPAR, peroxisome proliferator-activated receptor; PR, progressive ratio; TO, time out; TRPV, transient receptor potential vanilloid; URB597, cyclohexyl carbamic acid 3'-carbamoyle-3-yl ester; VDM11,[(5Z,8Z,11Z,14Z)-N-(4-hydroxy-2-methylphenyl)-5,8,11,14-eicosatetraenamide]

## Introduction

The endocannabinoid system is involved in drug-taking behaviour and relapse for various drugs of abuse, including nicotine (De Vries and Schoffelmeer, 2005; Le Foll and Goldberg, 2005; Le Foll *et al.*, 2008). The endocannabinoid system consists of the endocannabinoids [mostly anandamide and 2-arachidonoylglycerol (2-AG)], the target receptors for those endocannabinoids (cannabinoid CB<sub>1</sub> and CB<sub>2</sub> receptors, but also non-cannabinoid targets for anandamide; receptor nomenclature follows Alexander *et al.*, 2009), enzymatic degradation systems (through fatty acid amide hydrolase (FAAH) for anandamide and monoacylglycerol lipase for 2-AG) and a putative transport uptake system (Di Marzo *et al.*, 1998; 2001; Mechoulam *et al.*, 1998; Piomelli *et al.*, 2000; Sugiura and Waku, 2002; Piomelli, 2003; 2005; Di Marzo, 2006). Preclinical and human clinical studies (Le Foll *et al.*, 2008) have indicated that blocking endocannabinoid transmission by CB<sub>1</sub> receptor antagonists/inverse agonists could be a useful strategy for the treatment of human smokers. Rimonabant, a CB<sub>1</sub> receptor inverse agonist used in Europe for the treatment of obesity (Scheen *et al.*, 2006; Burch *et al.*, 2009; Leite *et al.*, 2009), increased the smoking cessation rates in controlled trials (Le Foll *et al.*, 2008). Unfortunately, the use of rimonabant has been associated with increased risk of anxiety and depression (Moreira *et al.*, 2009; Nathan *et al.*, 2010) and hence, rimonabant was withdrawn from the European market at the end of 2008 (Le Foll *et al.*, 2009).

Interestingly, we have recently discovered that elevating endogenous anandamide levels could be an alternative therapeutic strategy. We first evaluated the effects of URB 597 (a FAAH inhibitor) (Fegley *et al.*, 2005) as pharmacological blockade of FAAH activity prolonged many behavioural and neurobiological effects of anandamide. We found that URB 597 administration reversed abuse-related behavioural and neurochemical effects of nicotine in rats (Scherma *et al.*, 2008; Forget *et al.*, 2009). URB 597 elevates not only brain levels of anandamide, but also those of the non-cannabinoid acylethanolamides, oleoylethanolamide (OEA) and palmitoylethanolamide (PEA) which act on  $\alpha$ -type peroxisome proliferator-activated receptors (PPAR $\alpha$ ) (Bond *et al.*, 1995; Fegley *et al.*, 2005; Astarita *et al.*, 2006; Mascia *et al.*, 2011). As the effects of URB 597 could be mediated by either elevated levels of anandamide, by elevated levels of OEA and PEA, or by combinations of these three endogenous ligands, there is a need to explore further the effects of anandamide alone on nicotine-taking and nicotine-seeking behaviour.

In this study, we evaluated the effect of the selective anandamide uptake inhibitor, VDM11 on nicotine-taking and nicotine-seeking behaviour. VDM11 elevates anandamide levels with minimal effects on levels of OEA, PEA or 2-AG (the other endogenous cannabinoid ligand) both *in vitro* (De Petrocellis *et al.*, 2000) and *in vivo* (Van der Stelt *et al.*, 2006). Our aim was to study the effect of selectively elevating levels of anandamide in the brain on i.v. nicotine self-administration under fixed-ratio (FR) and progressive-ratio (PR) schedules of reinforcement, as well as nicotine-seeking behaviour induced by nicotine-associated cues and nicotine priming. The anandamide uptake inhibitors were of potential therapeutic benefit in the treatment of pain, motor impairments and

anxiety (Fernandez-Espejo *et al.*, 2004; Bortolato, *et al.*, 2006; La Rana *et al.*, 2006). If proven to modulate reinstatement of nicotine-seeking behaviour, this class of ligands could have a potential therapeutic benefit as smoking-cessation therapy.

## Methods

### Animals

All animal care and experimental procedures described in this report complied with the guidelines of the Canadian Council on Animal Care (compatible with NIH guidelines), and were reviewed and approved by the institutional Animal Care Committee. Male Long Evans rats (Charles River, Lachine, PQ, Canada), experimentally naive at the start of the study and initially weighing 250–275 g, were used. All rats were individually housed in a temperature-controlled environment on a 12 h reverse light/dark cycle (lights off from 07:00 h to 19:00 h). Before any experimental manipulation, animals were given a minimum of 7 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 pellets or 20 g daily and had free access to water.

### Apparatus

Nicotine i.v. self-administration studies were carried out in commercially available experimental chambers (Med Associates, St Albans, VT, 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 the animals, the left lever was the active lever and for the other half, the right lever was the active lever.

### Experimental procedures

**Food-maintained behaviour.** Techniques for initial acquisition of food-maintained behaviour and surgery were similar to those already reported (Corrigall and Coen, 1989; Forget *et al.*, 2009; Gamaledin *et al.*, 2011; Khaled *et al.*, 2010). Animals learned to lever press for food reinforcement on a continuous reinforcement 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 5 days. Once food-maintained behaviour was acquired, i.v. catheters were surgically implanted.

**Implantation of i.v. catheters.** Surgical procedures for implantation of chronic i.v. catheters were similar to those already reported (Corrigall *et al.*, 1989; Forget *et al.*, 2009; Khaled *et al.*, 2010). Briefly, catheters were implanted into the jugular vein, exiting between the scapulae. Surgery was performed under anaesthesia induced by xylazine (10 mg·kg<sup>-1</sup>, i.p.) and ketamine hydrochloride (90 mg·kg<sup>-1</sup>, i.p.). Incision sites were infiltrated s.c. with a local anaesthetic, marcaine (0.125%). Buprenorphine was given for post-operative analgesia (0.03 mg·kg<sup>-1</sup>, s.c.), and a single dose of penicillin (30 000 units, i.m.) was administered at the completion of surgical

procedures. Animals were allowed to recover for 1 week before drug self-administration sessions were begun.

**Self-administration procedures.** Acquisition of nicotine self-administration was performed under an FR schedule of reinforcement at a unit dose of  $30 \mu\text{g}\cdot\text{kg}^{-1}$  per infusion of nicotine base. Session duration was 60 min. The start of each 60 min session was signalled by illumination of the house light. In the presence of the illuminated house light, 1–5 active lever presses resulted in the delivery of a nicotine infusion. Each infusion was followed by a time out 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). Response requirements were then gradually increased to reach a final value of FR5, by which time self-administration behaviour was stable and the animals had a 15–20 day history of nicotine self-administration. Self-administration sessions occurred mostly 5 days a week.

**Testing under the FR5 schedule of 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 i.p. injections of vehicle (tocrisolve) to habituate them to the injection procedure for an additional 3 days. Rats ( $n = 18$ ) were then tested using i.p. injections of vehicle or VDM11 at doses of 1, 3 and  $10 \text{ mg kg}^{-1}$  given 30 min before the start of the session, in a counterbalanced, within-subject design.

**Testing under the PR schedule of reinforcement.** The same group of animals ( $n = 14$ ) (four animals were excluded due to blocked catheters) was used to self-administer nicotine ( $30 \mu\text{g}\cdot\text{kg}^{-1}$  per infusion) under the FR schedule and then was directly switched to a PR schedule where the response requirement increased with each successive injection. The response requirement progression was based on the formula  $5e^{(0.25 \times [\ln(\text{number} + 3)])}$ , 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 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 the pharmacological compounds began. Testing of VDM11 ( $1\text{--}10 \text{ mg}\cdot\text{kg}^{-1}$ , 30 min before the session) was performed using a counterbalanced within-subject design.

**Extinction.** Reinstatement testing was performed on another group of animals (this experiment started with 18 rats, but only 14 rats completed testing on all doses of VDM11 on cue-induced reinstatement due to attrition). After acquisition of nicotine self-administration as described previously, 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 lever 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 in order to conduct testing. All animals reached extinction criteria within an average of eight extinction sessions. Both extinction and reinstatement sessions lasted for 60 min

**Effects of VDM11 on cue-induced reinstatement of nicotine-seeking behaviour.** All tests were carried out in a counterbalanced, within-subject design. After each test, extinction was re-established until extinction criteria were obtained for at least two consecutive days. Rats were pretreated 30 min before the session with vehicle or 1, 3 and  $10 \text{ mg}\cdot\text{kg}^{-1}$  VDM11 in a counterbalanced order to measure the effects of VDM11 on cue-induced reinstatement of nicotine-seeking behaviour. 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 1 h.

**Effects of VDM11 on nicotine-induced reinstatement of nicotine-seeking behaviour.** A new group of animals ( $n = 7$ ) were given same training and extinction and was subsequently used to determine the effects of VDM11 (1, 3, and  $10 \text{ mg}\cdot\text{kg}^{-1}$  i.p. 30 min before the session) on nicotine-induced reinstatement. Nicotine priming was performed as described by Forget *et al.*, (2010a,b) by administering  $0.15 \text{ mg}\cdot\text{kg}^{-1}$  nicotine s.c., 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.

## Data analysis

The number of active and inactive lever presses or nicotine infusions was recorded and analysed. To analyse difference in responses between the active and inactive levers along time in the acquisition of nicotine self-administration under FR and PR schedules and during extinction, we used two-way ANOVA. To analyse the effects of VDM11 on the number of nicotine infusions earned under the FR and the PR schedule of reinforcement, one-way ANOVA was performed. For reinstatement studies, one-way repeated measures ANOVA was used to assess the reinstatement effect and the effects of VDM11 on reinstatement induced by nicotine priming and nicotine-associated cues.

## Materials

(-)-Nicotine hydrogen tartrate (Sigma-Aldrich, St Louis, MO, USA) was dissolved in saline, the pH was adjusted to 7.0 ( $\pm 0.2$ ) and the solution was filtered through a 0.22  $\mu\text{m}$  syringe filter (Fisher Scientific, Pittsburgh, PA, USA) for sterilization purposes. All nicotine doses are reported as free base

concentrations. Nicotine was administered i.v. in a volume of  $100 \mu\text{L} \cdot \text{kg}^{-1}$  per injection for self-administration studies or was administered s.c. at the dose of  $0.15 \text{ mg} \cdot \text{kg}^{-1}$  for reinstatement studies.

VDM11[(5Z,8Z,11Z,14Z)-N-(4-hydroxy-2-methylphenyl)-5,8,11,14-eicosatetraenamide] (Tocris Bioscience, Ellisville, MO, USA) was dissolved in Tocrisolve and injected i.p. at a volume of  $1 \text{ mL} \cdot \text{kg}^{-1}$  30 min before the start of the session.

## Results

### Acquisition of nicotine self-administration behaviour under FR schedule of reinforcement

During the first week of acquisition, responding on the active lever, which had previously been reinforced by food and was now reinforced by nicotine infusion, decreased to relatively low levels. When response requirement was then increased to reach a final value of FR5 over the next 2 weeks, responding on the active lever that was reinforced by nicotine infusion increased to the high levels previously maintained by food, while responding on the inactive lever remained low (Figure 1A). ANOVA revealed a significant effect of time ( $F_{14,644} = 13.35$ ;  $P < 0.0001$ ), a significant effect of lever (i.e. active vs. inactive;  $F_{1,46} = 44.16$ ;  $P < 0.0001$ ) and a significant interaction between time and lever ( $F_{14,644} = 7.77$ ;  $P < 0.0001$ ).

### Acquisition of nicotine self-administration behaviour under PR schedule of reinforcement

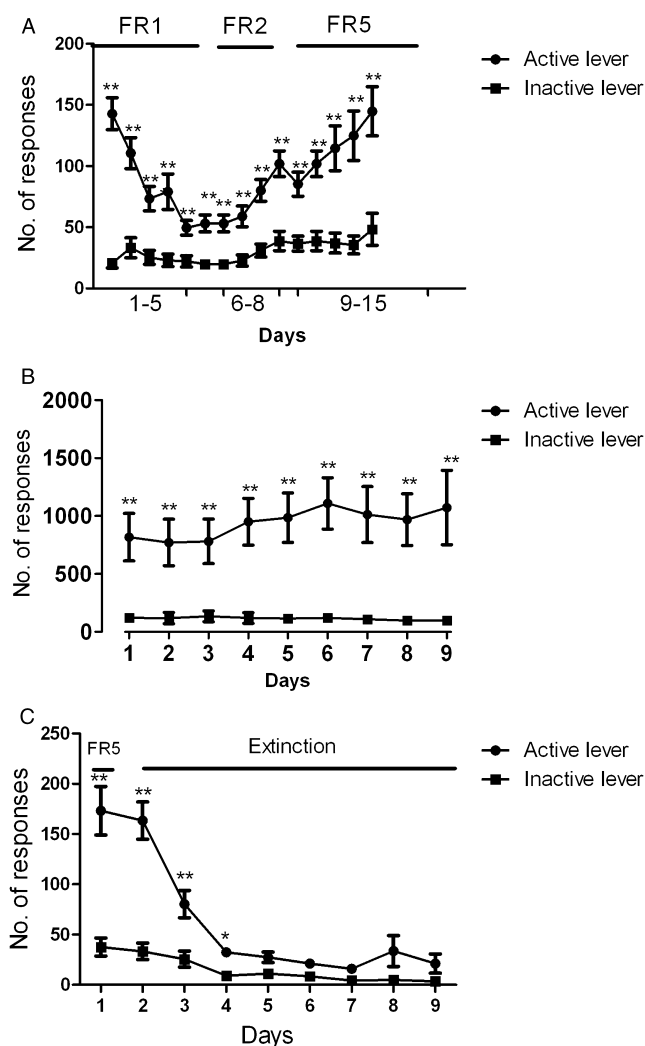
Animals achieved stable self-administration under the PR schedule of reinforcement within 9 days, after which subsequent testing with VDM11 started. ANOVA performed on acquisition of nicotine self-administration under PR schedule of reinforcement showed a non-significant effect of time ( $F_{8,208} = 1.08$ ;  $P = 0.37$ ), a significant effect of lever (i.e. active vs. inactive levers,  $F_{1,26} = 27.36$ ;  $P < 0.0001$ ) and no significant interaction between time and lever ( $F_{8,208} = 1.17$ ;  $P = 0.31$ ) (Figure 1B).

### Extinction

The data presented in Fig 1C reflect the extinction pattern for the group of animals ( $n = 16$ ) used in the cue-induced reinstatement testing (only 14 animals completed testing on cue-induced reinstatement). Animals were subjected to extinction sessions that lasted for 8 days, during which most of the animals reached extinction criteria and testing with VDM11 on reinstatement was started (extinction training was pursued for the remaining rats until they reach the extinction criteria). ANOVA performed on the 8 days of extinction showed a significant effect of time ( $F_{8,240} = 48.19$ ;  $P < 0.0001$ ), a significant effect of lever ( $F_{1,30} = 27.10$ ;  $P < 0.0001$ ) and a significant interaction between time and lever ( $F_{8,240} = 27.10$ ;  $P < 0.0001$ ). LSD *post hoc* analysis showed no significant difference between responses on the active and inactive levers after the third day of extinction ( $P = 0.65$ ).

### Effects of VDM11 on nicotine self-administration under the FR5 schedule

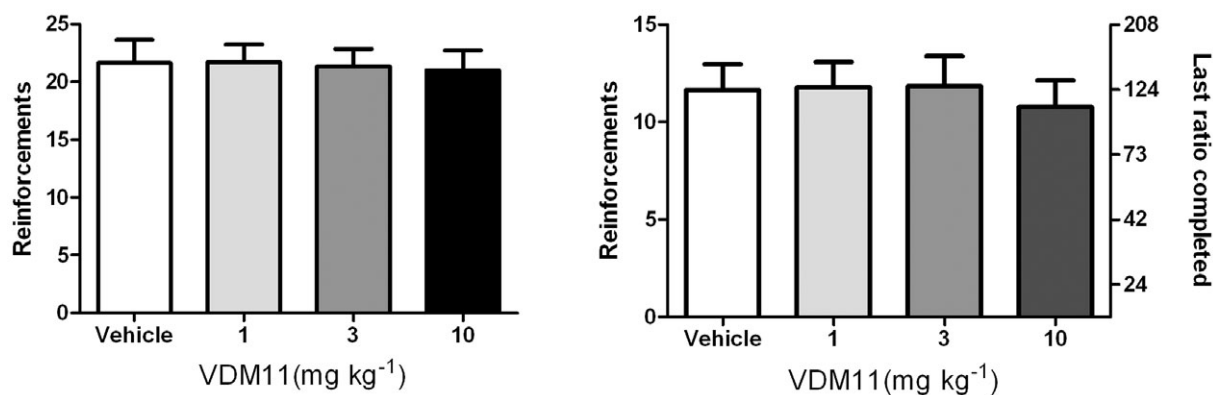
ANOVA showed no effect of VDM11 pretreatment on the number of nicotine infusions ( $F_{3,51} = 0.07087$ ,  $P = 0.97$ ), and



**Figure 1**

(A) Acquisition of nicotine ( $30 \mu\text{g} \cdot \text{kg}^{-1}$  per infusion) self-administration under an FR schedule of reinforcement. A: the number of active and inactive lever presses (mean  $\pm$  SEM) received in each session under the different schedules of reinforcement (FR-1, FR-2, FR-5) ANOVA showed a significant effect of time ( $P < 0.0001$ ) and active versus inactive lever ( $P < 0.0001$ ), and a significant interaction between lever presses and time ( $P < 0.0001$ ). (B) Acquisition of nicotine ( $30 \mu\text{g} \cdot \text{kg}^{-1}$ /infusion) self-administration under PR schedule of reinforcement. B: the number of active and inactive lever presses (mean  $\pm$  SEM) received in each session under PR schedule of reinforcement. ANOVA showed no significant effect of time ( $P = 0.37$ ), a significant difference between active and inactive levers ( $P < 0.0001$ ), and no significant interaction between lever presses and time ( $P = 0.31$ ). (C) Extinction of nicotine self-administration behaviour before reinstatement testing. C: the number of active and inactive lever presses (mean  $\pm$  SEM) received in each extinction session. ANOVA performed on the 8 days of extinction showed a significant effect of time ( $P < 0.0001$ ) and lever ( $P < 0.0001$ ), and interaction between lever presses and time ( $P < 0.0001$ ). LSD *post hoc* analysis showed no significant difference between responses on the active and inactive levers after the 3rd day of extinction ( $P = 0.65$ ); \* $P < 0.05$ ; \*\* $P < 0.01$ .





**Figure 2**

Effect of VDM11 on nicotine self-administration under FR5 and PR schedules of reinforcement. (A) Effects of pre-treatment with VDM11 (1, 3 and 10 mg·kg<sup>-1</sup>, i.p.) on nicotine (30 µg·kg<sup>-1</sup> per infusion) self-administration under the FR5 schedule. Data are expressed as mean ± SEM of the number of infusions obtained during the 60 min session. VDM11 did not affect responding versus vehicle pre-treatment ( $n = 18$ ;  $P = 0.97$ ). (B) Effects of pre-treatment with VDM11 (1, 3 and 10 mg·kg<sup>-1</sup>, i.p.) on nicotine (30 µg·kg<sup>-1</sup> per infusion) self-administration under PR schedule. Data are expressed as mean ± SEM of the number of infusions obtained during the 4 h sessions. VDM11 did not affect BP  $P > 0.05$  versus vehicle pre-treatment ( $n = 14$ ;  $P = 0.15$ ).

pairwise comparisons with baseline level indicated that pre-treatment with VDM11 (1, 3 and 10 mg·kg<sup>-1</sup>) did not affect the number of nicotine infusions received during the session (Figure 2A).

### Effects of VDM11 on nicotine self-administration under the PR schedule

ANOVA showed no effect of VDM11 pretreatment on the number of nicotine infusions ( $F_{3,39} = 1.843$ ,  $P > 0.05$ ). VDM11 (1, 3 and 10 mg·kg<sup>-1</sup>) at the various doses tested failed to produce any change in the break point values, compared with vehicle (Figure 2B).

### Effects of VDM11 on reinstatement of nicotine seeking induced by nicotine-associated cues

ANOVA performed on active lever presses indicated a main effect of cues *per se* on reinstatement of nicotine seeking compared with baseline conditions ( $P < 0.001$ ). ANOVA performed on the active lever presses indicated a main effect of VDM11 ( $F_{4,52} = 14.22$ ;  $P < 0.05$ ) and Newman-Keuls *post hoc* analysis showed that pre-treatment with 3 and 10 mg·kg<sup>-1</sup> VDM11, 30 min before the start of the session, attenuated the reinstatement induced by nicotine-associated cues ( $P < 0.05$ ), as compared with reinstatement under vehicle pre-treatment. Neither presentation of nicotine-associated cues nor VDM11 administration had a significant effect on responding on the inactive lever ( $F_{4,52} = 1.019$ ;  $P = 0.40$ ) (Figure 3A).

### Effects of VDM11 on reinstatement of nicotine seeking induced by nicotine priming

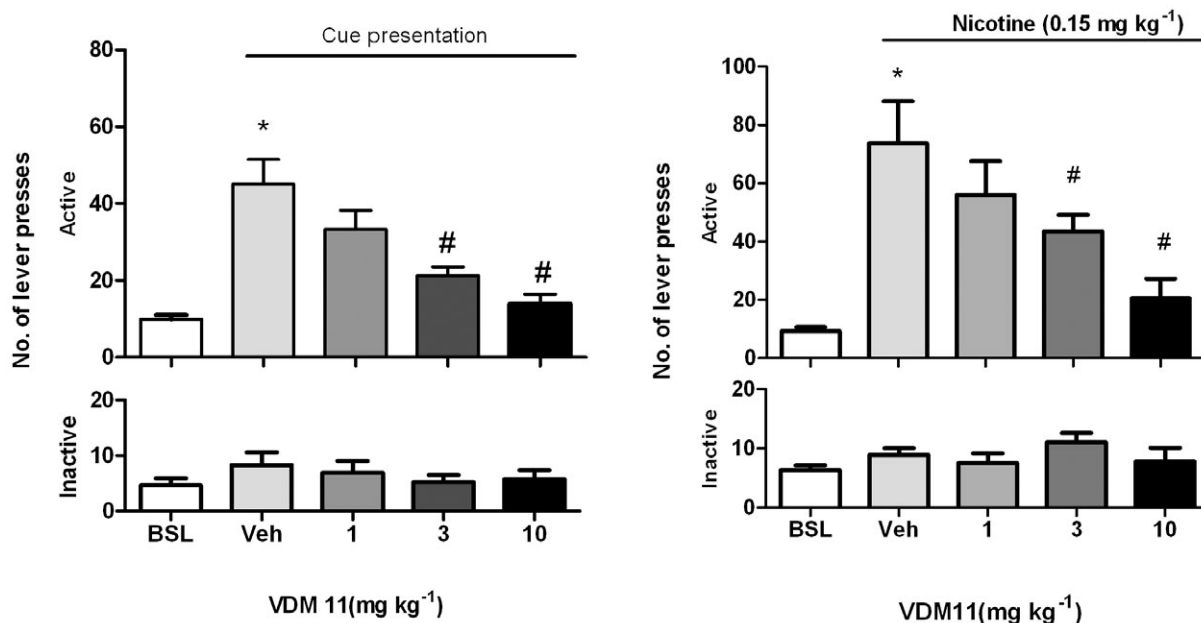
ANOVA performed on active lever presses indicated a main effect of priming with nicotine (0.15 mg·kg<sup>-1</sup>) on nicotine-seeking behaviour, as compared with baseline conditions ( $P < 0.001$ ; Figure 3B). ANOVA analysis of the active lever presses indicated a main effect of VDM11 ( $F_{4,24} = 9.963$ ;  $P < 0.05$ ) and

Newman-Keuls *post hoc* analysis showed that pre-treatment with 3 and 10 mg·kg<sup>-1</sup> VDM11, 30 min before the start of the session, attenuated the effect of nicotine priming ( $P < 0.05$ ), compared with reinstatement under vehicle pretreatment. Neither priming injections of nicotine nor VDM11 (1, 3 and 10 mg·kg<sup>-1</sup>) treatment had any significant effect on responding on the inactive lever ( $F_{4,24} = 0.947$ ;  $P = 0.43$ ) (Figure 3B).

## Discussion

Anandamide transport inhibitors represent a novel class of ligands that modulate endocannabinoid neurotransmission. Our results indicate that elevation of anandamide levels by the selective uptake inhibitor VDM11 reduced reinstatement of nicotine-seeking behaviour induced by either presentation of nicotine-associated cues or by nicotine priming. In contrast, VDM11 did not have an effect on responding for nicotine under FR or PR schedules of reinforcement.

Our findings with VDM11 are in agreement with our previous results with URB597. We reported that URB597 attenuated nicotine-induced reinstatement of nicotine seeking using both i.v. nicotine self-administration and conditioned place preference paradigms (Scherma *et al.*, 2008; Forget *et al.*, 2009). URB597 also attenuated acquisition of nicotine self-administration under an FR schedule (Scherma *et al.*, 2008). In contrast, URB597 did not alter nicotine self-administration behaviour under a PR schedule (Forget *et al.*, 2009). The present findings obtained with VDM11 indicates that this ligand has a similar profile; that is, it is able to disrupt nicotine seeking induced by both cues and priming, but does not affect established nicotine-taking behaviour. Further studies will be required to evaluate whether VDM11 can affect acquisition of nicotine self-administration behaviour, as it has been reported that URB597 significantly decreases acquisition of nicotine self-administration (Scherma *et al.*, 2008).



**Figure 3**

(A) Effects of VDM11 on reinstatement of nicotine-seeking behaviour induced by presentation of nicotine associated cues and by (B) priming doses of nicotine. (A) Effects of pretreatment with VDM11 (1, 3 and 10 mg·kg<sup>-1</sup>, i.p.) on cue-induced reinstatement of nicotine-seeking behaviour. A significant reinstatement of nicotine-seeking behaviour was produced by presentation of nicotine-associated cues alone (\**P* < 0.001). Newman–Keuls *post hoc* analysis after significant ANOVA showed that pre-treatment with VDM11 (3 and 10 mg·kg<sup>-1</sup>, i.p.) significantly reduced cue-induced reinstatement of nicotine-seeking behaviour (#*P* < 0.05), *n* = 14. (B) A Significant reinstatement of nicotine-seeking was also produced by pre-treatment with nicotine (0.15 mg·kg<sup>-1</sup>) (\**P* < 0.001). Newman–Keuls *post hoc* analysis after significant ANOVA showed that VDM11 (3 and 10 mg·kg<sup>-1</sup>, i.p.) significantly reduced the reinstatement of nicotine-seeking behaviour induced by a priming injection of 0.15 mg·kg<sup>-1</sup> nicotine administered 10 min before the session (#*P* < 0.05). Data are expressed as mean ± SEM of the number of lever presses: BSL, during extinction; Veh, vehicle (Veh) pre-treatment (visual cues); *n* = 7.

As mentioned earlier, inhibition of FAAH increases levels of several endogenous substances in the brain, including the endocannabinoid anandamide and the non-cannabinoid fatty acid acylethanolamides OEA and PEA, which are ligands for PPARα (Scherma *et al.*, 2008). We recently reported that ligands acting selectively as PPARα agonists can reduce reward-related effects of nicotine (Mascia *et al.*, 2011). In those studies, PPARα agonists dose-dependently decreased nicotine self-administration and nicotine-induced reinstatement, but did not alter food- or cocaine-reinforced operant behaviour (Mascia *et al.*, 2011). Therefore, the effects observed with URB597 could have been mediated by only PPARα activation by OEA and PEA. It is interesting to note that VDM11 exhibited a different profile from that of the PPARα agonists, being unable in the present experiments to affect established nicotine self-administration behaviour.

One possible limitation of our findings is the fact that the effects could be modulated by non-specific effects on motor response. Beltramo *et al.*, (2000) have previously reported that intracerebroventricular injection of AM404 (a VDM11 analogue) induced mild hypokinesia as shown by the increase in immobility time and decreased motor behaviour stimulated by dopamine D<sub>2</sub> receptor agonists. However, such effects are unlikely to mediate the effects observed here with VDM11 as we found no significant effect on the ability of the animals to lever press for nicotine under both FR and PR schedules (Figure 2A,B).

Another limitation of our study is that we tested only one anandamide uptake inhibitor. Further studies evaluating the ability of other anandamide uptake inhibitors, such as AM404, which also elevate anandamide levels selectively, are needed to validate our findings. Interestingly, it has been reported that AM404 administration reduces alcohol self-administration under an FR schedule of reinforcement but it did not affect reinstatement of alcohol-seeking, suggesting that either there may be differences between AM404 and VDM11, or that anandamide may cause differential effects on drug-taking and drug-seeking depending on the substance under study (Cippitelli *et al.*, 2007).

It is also possible that VDM11 could have an inhibitory effect on FAAH enzymatic activity *in vitro* (De Petrocellis *et al.*, 2000), however, such an effect has not been reported consistently. Any potential inhibitory effects of VDM11 on FAAH activity could be assay-related and also dependent on the source of the compound or the pH used in the assay, as well as the concentration of fatty acid-free BSA used in the experiment (Fowler *et al.*, 2004).

Anandamide and 2-AG are the two main endocannabinoids in the brain (Bisogno *et al.*, 2000; Hanus *et al.*, 2001; Huang *et al.*, 2002; Porter *et al.*, 2002; Di Marzo, 2006). Anandamide not only binds to cannabinoid CB<sub>1</sub> receptors with high affinity (K<sub>i</sub> = 52 nM) (Devane *et al.*, 1992; Felder *et al.*, 1993; Childers *et al.*, 1994; Terranova *et al.*, 1995), but also acts on CB<sub>2</sub> receptors and may also have non-cannabinoid-

mediated effects, notably through the transient receptor potential vanilloid (TRPV) ion channels (Di Marzo *et al.*, 2000). The present experiments do not provide information on the downstream mechanisms by which VDM11 acts to produce its effects on nicotine reinstatement. Activation at CB<sub>1</sub>, CB<sub>2</sub> and TRPV receptors are possible mechanisms. Interestingly, like anandamide, 2-AG also binds to cannabinoid CB<sub>1</sub> receptors, but with lower affinity ( $K_i = 15 \mu\text{M}$ ). 2-AG acts as a full agonist at CB<sub>1</sub> receptors (Devane *et al.*, 1992; Felder *et al.*, 1993; Childers *et al.*, 1994; Terranova *et al.*, 1995), whereas anandamide acts as a partial agonist (Sugiura *et al.*, 2002). Because 2-AG is a full agonist and anandamide is a partial agonist, it is possible that anandamide may oppose some effects of 2-AG (Maccarrone *et al.*, 2008). It may appear surprising that an experimental approach which increases endocannabinoid tone produces opposing effects. However, the administration of a full cannabinoid agonist, such as WIN 55,212-2, has been shown to increase motivation for nicotine and produce reinstatement of nicotine-seeking behaviour (Gamaledin *et al.*, in press). Moreover, such opposite effects have also been described in experiments utilising nicotine full agonists and partial agonists, with nicotine (full agonist) being able to induce nicotine-seeking (see Figure 3B and Scherma *et al.*, 2008), while varenicline (a nicotinic partial agonist) reducing the motivation to self-administer nicotine and decreasing cue-induced reinstatement of nicotine-seeking (Le Foll *et al.*, abstract SRNT 2011). These hypotheses will be evaluated through further ongoing studies that will investigate the separate roles of anandamide and 2-AG on nicotine-seeking behaviour.

Our results indicate that elevation of anandamide levels reduced reinstatement to nicotine seeking, induced by nicotine-associated cues as well as by exposure to a priming dose of nicotine. As the ligands elevating anandamide levels are likely to be devoid of the psychotropic side effects of the CB<sub>1</sub> inverse agonist rimonabant (Le Foll *et al.*, 2009), this class of ligands may have some therapeutic potential to prevent relapse in abstinent smokers.

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## Conflict of interest

None.

## References

- Alexander SPH, Mathie A, Peters JA (2009). Guide to Receptors and Channels (GRAC), 4th edn. Br J Pharmacol 158 (Suppl. 1): S1–S254.
- Astarita G, Di Giacomo B, Gaetani S, Oveisi F, Compton TR, Rivara S *et al.* (2006). Pharmacological characterization of hydrolysis-resistant analogs of oleoylethanolamide with potent anorexiant properties. J Pharmacol Exp Ther 318: 563–570.
- Beltramo M, de Fonseca FR, Navarro M, Calignano A, Gorriti MA, Grammatikopoulos G *et al.* (2000). Reversal of dopamine D(2) receptor responses by an anandamide transport inhibitor. J Neurosci 20: 3401–3407.
- Bisogno T, Melck D, Bobrov M, Gretskey NM, Bezuglov VV, De Petrocellis L *et al.* (2000). N-acyl-dopamines: novel synthetic CB(1) cannabinoid-receptor ligands and inhibitors of anandamide inactivation with cannabimimetic activity *in vitro* and *in vivo*. Biochem J 351 (Pt 3) 817–824.
- Bond AR, Leff P, Johnson TD, Milano CA, Rockman HA, McMinn TR *et al.* (1995). Physiological effects of inverse agonists in transgenic mice with myocardial overexpression of the  $\alpha_2$ -adrenoreceptor. Nature 374: 272–276.
- Bortolato M, Campolongo P, Mangieri RA, Scattoni ML, Frau R, Trezza V *et al.* (2006). Anxiolytic-like properties of the anandamide transport inhibitor AM404. Neuropsychopharmacology 31: 2652–2659.
- Burch J, McKenna C, Palmer S, Norman G, Glanville J, Sculpher M *et al.* (2009). Rimonabant for the treatment of overweight and obese people. Health Technol Assess 13 (Suppl. 3): 13–22.
- Childers SR, Sexton T, Roy MB (1994). Effects of anandamide on cannabinoid receptors in rat brain membranes. Biochem Pharmacol 47: 711–715.
- Cippitelli A, Bilbao A, Gorriti MA, Navarro M, Massi M, Piomelli D *et al.* (2007). The anandamide transport inhibitor AM404 reduces ethanol self-administration. Eur J Neurosci 26: 476–486.
- Corrigall WA, Coen KM (1989). Nicotine maintains robust self-administration in rats on a limited-access schedule. Psychopharmacology (Berl) 99: 473–478.
- De Petrocellis L, Bisogno T, Davis JB, Pertwee RG, Di Marzo V (2000). 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 483: 52–56.
- De Vries TJ, Schoffelmeer AN (2005). Cannabinoid CB1 receptors control conditioned drug seeking. Trends Pharmacol Sci 26: 420–426.
- Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G *et al.* (1992). Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 258: 1946–1949.
- Di Marzo V (2006). Endocannabinoids: synthesis and degradation. Rev Physiol Biochem Pharmacol 160: 1–24.
- Di Marzo V, Melck D, Bisogno T, De Petrocellis L (1998). Endocannabinoids: endogenous cannabinoid receptor ligands with neuromodulatory action. Trends Neurosci 21: 521–528.
- Di Marzo V, Breivogel CS, Tao Q, Bridgen DT, Razdan RK, Zimmer AM *et al.* (2000). Levels, metabolism, and pharmacological activity of anandamide in CB(1) cannabinoid receptor knockout mice: evidence for non-CB(1), non-CB(2) receptor-mediated actions of anandamide in mouse brain. J Neurochem 75: 2434–2444.
- Di Marzo V, De Petrocellis L, Bisogno T (2001). Endocannabinoids Part I: molecular basis of endocannabinoid formation, action and

inactivation and development of selective inhibitors. *Expert Opin Ther Targets* 5: 241–265.

Fegley D, Gaetani S, Duranti A, Tontini A, Mor M, Tarzia G *et al.* (2005). 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* 313: 352–358.

Felder CC, Briley EM, Axelrod J, Simpson JT, Mackie K, Devane WA (1993). Anandamide, an endogenous cannabimimetic eicosanoid, binds to the cloned human cannabinoid receptor and stimulates receptor-mediated signal transduction. *Proc Natl Acad Sci USA* 90: 7656–7660.

Fernandez-Espejo E, Caraballo I, Rodriguez de Fonseca F, Ferrer B, El Banoua F, Flores JA *et al.* (2004). Experimental parkinsonism alters anandamide precursor synthesis, and functional deficits are improved by AM404: a modulator of endocannabinoid function. *Neuropsychopharmacology* 29: 1134–1142.

Forget B, Coen KM, Le Foll B (2009). 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)* 205: 613–624.

Forget B, Pushparaj A, Le Foll B (2010a). Granular insular cortex inactivation as a novel therapeutic strategy for nicotine addiction. *Biol Psychiatry* 68: 265–271.

Forget B, Wertheim C, Mascia P, Pushparaj A, Goldberg SR, Le Foll B (2010b). Noradrenergic alpha1 receptors as a novel target for the treatment of nicotine addiction. *Neuropsychopharmacology* 35: 1751–1760.

Fowler CJ, Tiger G, Ligresti A, Lopez-Rodriguez ML, Di Marzo V (2004). Selective inhibition of anandamide cellular uptake versus enzymatic hydrolysis – a difficult issue to handle. *Eur J Pharmacol* 492: 1–11.

Gamaledin I, Wertheim C, Zhu AZ, Coen KM, Vemuri K, Makryannis A *et al.* (2011). Cannabinoid receptor stimulation increases motivation for nicotine and nicotine seeking. *Addict Biol* doi: 10.1111/j.1369-1600.2011.00314.x. [Epub ahead of print].

Hanus L, Abu-Lafi S, Fride E, Breuer A, Vogel Z, Shalev DE *et al.* (2001). 2-arachidonyl glyceryl ether, an endogenous agonist of the cannabinoid CB1 receptor. *Proc Natl Acad Sci USA* 98: 3662–3665.

Huang SM, Bisogno T, Trevisani M, Al-Hayani A, De Petrocellis L, Fezza F *et al.* (2002). An endogenous capsaicin-like substance with high potency at recombinant and native vanilloid VR1 receptors. *Proc Natl Acad Sci USA* 99: 8400–8405.

Khaled MA, Farid Araki K, Li B, Coen KM, Marinelli PW, Varga J *et al.* (2010). 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* 13: 181–190.

La Rana G, Russo R, Campolongo P, Bortolato M, Mangieri RA, Cuomo V *et al.* (2006). Modulation of neuropathic and inflammatory pain by the endocannabinoid transport inhibitor AM404 [N-(4-hydroxyphenyl)-eicosa-5,8,11,14-tetraenamide]. *J Pharmacol Exp Ther* 317: 1365–1371.

Le Foll B, Goldberg SR (2005). Cannabinoid CB<sub>1</sub> receptor antagonists as promising new medications for drug dependence. *J Pharmacol Exp Ther* 312: 875–883.

Le Foll B, Forget B, Aubin HJ, Goldberg SR (2008). Blocking cannabinoid CB1 receptors for the treatment of nicotine dependence: insights from pre-clinical and clinical studies. *Addict Biol* 13: 239–252.

Le Foll B, Gorelick DA, Goldberg SR (2009). The future of endocannabinoid-oriented clinical research after CB1 antagonists. *Psychopharmacology (Berl)* 205: 171–174.

Le Foll B, Chatterjee M, Lev-Ran S, Barnes C, Gamaledin I, Pushparaj A *et al.* (2011). *The Nicotinic Partial Agonist Varenicline Blocks Motivation for Nicotine and Significantly Reduces Cue-Induced Reinstatement of Nicotine-Seeking in Rats*. SRNT Poster session: Ontario, Canada.

Leite CE, Mocelin CA, Petersen GO, Leal MB, Thiesen FV (2009). Rimonabant: an antagonist drug of the endocannabinoid system for the treatment of obesity. *Pharmacol Rep* 61: 217–224.

Maccarrone M, Rossi S, Bari M, De Chiara V, Fezza F, Musella A *et al.* (2008). Anandamide inhibits metabolism and physiological actions of 2-arachidonoylglycerol in the striatum. *Nat Neurosci* 11: 152–159.

Mascia P, Pistis M, Justinova Z, Panlilio LV, Luchicchi A, Lecca S *et al.* (2011). Blockade of nicotine reward and reinstatement by activation of alpha-type peroxisome proliferator-activated receptors. *Biol Psychiatry* 69: 633–641.

Mechoulam R, Fride E, Di Marzo V (1998). Endocannabinoids. *Eur J Pharmacol* 359: 1–18.

Moreira FA, Grieb M, Lutz B (2009). Central side-effects of therapies based on CB1 cannabinoid receptor agonists and antagonists: focus on anxiety and depression. *Best Pract Res Clin Endocrinol Metab* 23: 133–144.

Nathan PJ, O'Neill BV, Napolitano A, Bullmore ET (2010). Neuropsychiatric adverse effects of centrally acting antiobesity drugs. *CNS Neurosci Ther* doi: 10.1111/j.1755-5949.2010.00172.x. [Epub ahead of print].

Piomelli D (2003). The molecular logic of endocannabinoid signalling. *Nat Rev Neurosci* 4: 873–884.

Piomelli D (2005). The endocannabinoid system: a drug discovery perspective. *Curr Opin Investig Drugs* 6: 672–679.

Piomelli D, Giuffrida A, Calignano A, Rodriguez de Fonseca F (2000). The endocannabinoid system as a target for therapeutic drugs. *Trends Pharmacol Sci* 21: 218–224.

Porter AC, Sauer JM, Knierman MD, Becker GW, Borna MJ, Bao J *et al.* (2002). Characterization of a novel endocannabinoid, virodhamine, with antagonist activity at the CB1 receptor. *J Pharmacol Exp Ther* 301: 1020–1024.

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

Scheen AJ, Van Gaal LG, Despres JP, Pi-Sunyer X, Golay A, Hanotin C (2006). [Rimonabant improves cardiometabolic risk profile in obese or overweight subjects: overview of RIO studies]. *Rev Med Suisse* 2: 1916–1923.

Scherma M, Panlilio LV, Fadda P, Fattore L, Gamaledin I, Le Foll B *et al.* (2008). Inhibition of anandamide hydrolysis by URB597 reverses abuse-related behavioral and neurochemical effects of nicotine in rats. *J Pharmacol Exp Ther* 327: 482–490.

van der Stelt M, Mazzola C, Esposito G, Matias I, Petrosino S, De Filippis D *et al.* (2006). Endocannabinoids and beta-amyloid-induced neurotoxicity *in vivo*: effect of pharmacological elevation of endocannabinoid levels. *Cell Mol Life Sci* 63: 1410–1424.



Sugiura T, Waku K (2002). Cannabinoid receptors and their endogenous ligands. *J Biochem* 132: 7–12.

Sugiura T, Kobayashi Y, Oka S, Waku K (2002). Biosynthesis and degradation of anandamide and 2-arachidonoylglycerol and their possible physiological significance. *Prostaglandins Leukot Essent Fatty Acids* 66: 173–192.

Terranova JP, Michaud JC, Le Fur G, Soubrie P (1995). Inhibition of long-term potentiation in rat hippocampal slices by anandamide and WIN55212-2: reversal by SR141716 A, a selective antagonist of CB1 cannabinoid receptors. *Naunyn Schmiedebergs Arch Pharmacol* 352: 576–579.