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ORIGINAL INVESTIGATION

Anandamide-induced behavioral disruption through a vanilloid-dependent mechanism in rats

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Abstract
Rationale Endocannabinoids are involved in a variety of behavioral and physiological processes that are just beginning to be understood. In the five-choice serial reaction-time task, exogenous cannabinoids have been found to alter attention, but endocannabinoids such as anandamide have not been studied.
Objectives We used this task to evaluate the effects of anandamide in rats. Since anandamide is a ligand for not only cannabinoid receptors but also transient receptor potential vanilloid 1 (TRPV1) receptors, and as recently suggested, peroxisome proliferator-activated nuclear receptor-α (PPARα), we also determined whether anandamide's effects in this task were mediated by each of these receptors.
Materials and methods Whenever one of five holes was illuminated for 2 s, a food pellet was delivered if a response occurred in that hole during the light or within 2 s after the light.
Results Anandamide increased omission errors and decreased responding during inter-trial intervals. These effects were blocked by the TRPV1 antagonist capsazepine, but not by the cannabinoid-receptor antagonist rimonabant or the PPARα antagonist MK886. Testing with open-field activity and food-consumption procedures in the same rats suggested that the disruption of operant responding observed in the attention task was not due to motor depression, anxiety, decreased appetite, or an inability to find and consume food pellets.
Conclusions The vanilloid-dependent behavioral disruption induced by anandamide was specific to the operant attention task. These effects of anandamide resemble effects of systemically administered dopamine antagonists and might reflect changes in vanilloid-mediated dopamine transmission.

Keywords Endocannabinoid · 5-Choice serial reaction-time task (5-CSRRT) · Open-field activity · Transient receptor potential vanilloid 1 receptor (TRPV1) · Peroxisome proliferator-activated receptor (PPAR) · Anxiety · Feeding

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Introduction

Anandamide is an endogenous neurotransmitter that has primarily been studied as an endocannabinoid acting at the

CB_1 cannabinoid receptor (Devane et al. 1992; Palmer et al. 2002). However, anandamide is also a ligand for the transient receptor potential vanilloid 1 (TRPV1) receptor (Starowicz et al. 2007; Zygmunt et al. 1999) and has recently been reported to be a ligand for the peroxisome proliferator-activated receptor-α (PPARα; O'Sullivan 2007; Sun et al. 2006). The contributions of these cannabinoid and non-cannabinoid receptors to the behavioral effects of anandamide are just beginning to be studied. It has been shown that exogenous CB_1 receptor agonists can impair attention under certain conditions (Arguello and Jentsch 2004; Verrico et al. 2004), but the effects of anandamide on attention have not been studied. Therefore, in the present study, we used a five-choice serial reaction-time task (5-CSRTT; Robbins 2002) to investigate the attentional effects of anandamide in rats. After obtaining dose–effect functions for anandamide in the attention task, we determined whether the observed effects could be altered by blocking cannabinoid, vanilloid, or PPAR receptors. We also studied the effects of combining anandamide with URB597, a drug that inhibits fatty acid amide hydrolase (FAAH), the enzyme primarily responsible for metabolizing anandamide (Fegley et al. 2004). An open-field test was used to evaluate the possibility (Scherma et al. 2008) that the behavioral effects observed in the attention task could be attributed to anandamide altering locomotor activity or anxiety. Finally, under conditions similar to those of the attention task, a food-consumption test was used to determine whether the effects observed in the attention task could be attributed to anandamide altering appetite or the ability to find and consume food pellets.

Materials and methods

Subjects

Male Sprague–Dawley rats weighing 350–380 g (Charles River, Wilmington, MA, USA) were housed two per cage with water freely available. Food was restricted to approximately 15 g/day to maintain stable body weight. All rats were housed in temperature- and humidity-controlled rooms with a 12-h light/dark cycle (lights on from 6:45 A.M. to 6:45 P.M.). Experiments were conducted during the light phase. A single group of 32 rats was used throughout the study (including attention, open-field activity and food-consumption testing), except for the attention experiment involving THC (in which a separate group of 16 rats was used). In the open-field experiment, an additional group of 28 experimentally naive rats was also studied. The facilities were fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC), and all experiments were conducted in accordance with the guidelines of the Animal Care and Use Committee of the NIDA Intramural Research Program and the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research.

Drugs

Anandamide (given immediately before session), rimonabant (SR141716; CB_1 receptor antagonist; given 30 min before session), MK 886 (PPARα antagonist; given 60 min before session), capsazepine (TRPV1 antagonist; given 30 min before session), and THC (Δ^9-tethrahydrocannabinol; cannabinoid CB_1 receptor agonist; given 30 min before session) were prepared in a vehicle of 2% Tween80, 2% ethanol, and sterile water. URB597 (cyclohexyl carbamic acid 3′-carbamoyl-3-yl ester; FAAH inhibitor; given 40 min before session) was dissolved in 20% DMSO and sterile water. All drugs were injected intraperitoneally in a volume of 1 ml/kg. Anandamide was synthesized at the laboratory of Dr. Alexandros Makriyannis (University of Connecticut, Centre for Drug Discovery and Departments of Pharmaceutical Sciences and Molecular Cell Biology, Storrs, CT, USA and Northeastern University, Centre for Drug Discovery, Boston, MA, USA). URB597 was synthesized (Mor et al. 2004) at the Department of Pharmacology, University of California, Irvine, CA, USA. Rimonabant and THC were provided by NIDA/NIH (Baltimore, MD, USA). MK886 and capsazepine were purchased from Tocris Bioscience (Ellisville, MO, USA).

Apparatus

Attention task Eight individually enclosed training chambers were used (model MED-NPW-9 L; MED Associates, St. Albans, VT, USA). The chambers had nine response holes, but four of these holes were blocked throughout the study. Thus, there were five holes on one side of the chamber, equidistant from a food tray on the opposite side, where 45-mg food pellets (type F0021; Bio-Serv, Frenchtown, NJ, USA) were dispensed. Each hole could be illuminated individually, and there was also a houselight in the roof of the chamber.

Open-field activity Open-field activity was measured in seven sound-attenuated chambers, with two fields in each chamber (Med Associates, East Fairfield, VT, USA). A light on the wall of the sound-attenuation chambers provided illumination of approximately 2.6 lux. The fields (41×41×32 cm) were composed of clear acrylic, and the floors were covered by sawdust bedding. Horizontal activity was measured with a 16×16 array of photobeams (lower beams), and vertical activity (rearing) was measured with 16 additional photobeams (upper beams), using Med Associates Open Field Activity Software.

Food consumption Testing was performed in clear plastic cages (19×19×30 cm) that were similar to the rats' home cages, except that no bedding was placed on the floor of the test cages. The cages were placed on a table in the same room where the open-field chambers were situated.

Procedure

Attention task The 5-CSRRT procedure was adapted from that used by Hahn et al. (2002). Sessions with this procedure lasted 30 min and were conducted Monday through Friday. After a pre-determined delay (ITI; average 10 s, range 6.5–14 s), a randomly chosen hole was illuminated for 2 s. If the rat responded in the illuminated hole while the light was on or within a 2-s period after it had gone out, a food pellet was delivered, and a correct response was counted. If the rat responded in a hole other than the one that had been illuminated, a commission error was counted. If the rat failed to respond within 2 s after the light was turned off, an omission error was counted. Either an incorrect response or an omission error resulted in a 5-s timeout during which the house light was extinguished and responding had no programmed effect. The next ITI began immediately after a correct response or after the end of a timeout; this ITI procedure differed slightly from that of Hahn et al. (2002), who measured the ITI starting from retrieval of the food pellet. Responses during the ITI were counted but had no programmed effect. Responses during timeout were not counted. The measures taken during the attention task were omission errors (percentage of trials on which no response was made), anticipatory responses (number of responses during the inter-trial interval), accuracy (number of trials with a correct response, as a percentage of all trials with a response), latency on correct trials (number of seconds to respond on trials with a correct response), and latency on incorrect trials (number of seconds to respond on trials with an incorrect response).

Training was continued until all rats responded correctly on at least 60% of trials with no more than 20% omission errors during the entire session for ten consecutive sessions. This required approximately 3 months of training. Once these training criteria were met, drug testing was begun. Drugs were given up to two times per week, with at least 70 h between tests. Normal daily training sessions were conducted between test sessions. For each drug or combination of drugs, each rat received all doses, with the order of doses counterbalanced between rats. First, the effects of anandamide alone (0, 1, 3, and 10 mg/kg) were determined. Then, to test whether anandamide's effects were altered by pretreatment with other drugs, anandamide (0 and 10 mg/kg) was given in factorial combination with URB597 (0, 0.1, and 0.3 mg/kg), with rimonabant (0 and 1 mg/kg), with MK886 (0 and 1 mg/kg), and with capsazepine (0 and 10 mg/kg); this testing was conducted in the stated order, except that MK886 and capsazepine were tested contemporaneously, in counterbalanced order.

Open-field activity Rats were injected with test drugs using the same treatment times as in the attention task, then placed in the open field (Prut and Belzung 2003; Scherma et al. 2008). Activity was monitored for 10 min, and the following measures were taken: distance traveled, number of ambulatory episodes, average velocity within ambulatory episodes, number of stereotypy counts, number of vertical counts (breaks of the upper beams), number of jump counts (number of incidents when the none of the lower horizontal beams were broken), number of entries into an unmarked center zone covering 1/9th of the field, and time spent within 5 cm of the walls of the field (thigmotaxis). Open-field testing was conducted after all attention testing was completed. The same rats used in the attention study were divided into four groups and tested with vehicle, anandamide (10 mg/kg, ip), capsazepine (10 mg/kg), or capsazepine and anandamide in combination. Each rat was tested only once in the open field. An additional group of 28 experimentally naive rats was also tested under the same open-field procedure.

Food consumption Rats from the attention study were divided into two groups and injected with anandamide (10 mg/kg) or vehicle 5 min before being placed into the test cage facing away from 25 food pellets (of the same type used in the attention task) that were on the floor in one corner of the cage. One experimenter was responsible for injecting the rat and placing it into the cage, and two observers, blind to treatment, recorded the latencies for the first pellet and the last pellet to be taken into the mouth (measures adapted from Wise and Raptis 1986). Food-consumption testing was conducted after all open-field testing was completed. Each rat was tested only once using the food-consumption procedure.

Data analysis

Since anandamide is a short-acting drug and all drug effects occurred in the first 10 min, only data from this period are included in the figures and analyses. Data were analyzed using Proc Mixed (SAS Institute, Cary, NC, USA). Each attention-task measure was analyzed with anandamide dose (for the anandamide dose–effect functions) or anandamide dose and pretreatment drug dose (for the blockade tests) as within-subject factors. Open-field test data were analyzed as an independent-groups ANOVA with the two test drugs as factors. Post hoc paired comparisons were conducted

using the Tukey procedure, maintaining an experiment-wise significance level of 0.05. Food-consumption test data were analyzed as independent-groups Student's *t* tests.

Results

Attention task

When anandamide (0, 1, 3, and 10 mg/kg) was tested with the attention task, the two highest doses significantly increased omission errors [Fig. 1a; $F(3,91)=81.65$, $p<0.0001$]. Anticipatory responses (Fig. 1b) were reduced at the same doses [$F(3,91)=32.81$, $p<0.0001$]. However, the accuracy of responding (Fig. 1c) was not significantly affected by anandamide. The latency to respond on correct trials (Fig. 1d) was decreased at the two highest doses [$F(3,80)=7.19$, $p<0.0002$], and the latency to respond on incorrect trials (Fig. 1e) was decreased at the highest dose [$F(3,67)=13.79$, $p<0.0001$].

After dose–effect functions for anandamide were determined (as shown in Fig. 1), four treatments were given alone and in combination with anandamide (10 mg/kg) to determine whether they would alter anandamide's behavioral effects. The FAAH inhibitor URB597 (0.1 and 0.3 mg/kg), the CB_1 receptor antagonist rimonabant (1 mg/kg), and the PPARα antagonist MK866 (1 mg/kg) each failed to block the effects of anandamide (compare bars in Fig. 2c to those in Fig. 2d). None of these treatments had significant effects when given alone (compare bars in Fig. 2a to those in Fig. 2b). During testing of these treatments, anandamide continued to have effects comparable to those seen in Fig. 1, whether it was combined with an additional vehicle injection (Fig. 2c) or an injection of one of the treatment compounds (Fig. 2d). Consistent with the failure of the cannabinoid CB_1 receptor antagonist rimonabant to alter the effects of anandamide in this attention task, the cannabinoid agonist THC (0, 1, 3, 5.6, and 10 mg/kg) had no significant effect on any measure under the attention task when given alone (Fig. 2e).

In contrast with the failure of these other treatments to alter the effects of anandamide, the vanilloid receptor antagonist capsazepine (10 mg/kg) successfully blocked all of anandamide's effects in the attention task (Fig. 3). Capsazepine alone did not significantly affect any measure under the attention task, nor did capsazepine's vehicle alter the effects of anandamide. However, capsazepine significantly blocked each of the significant effects of anandamide,

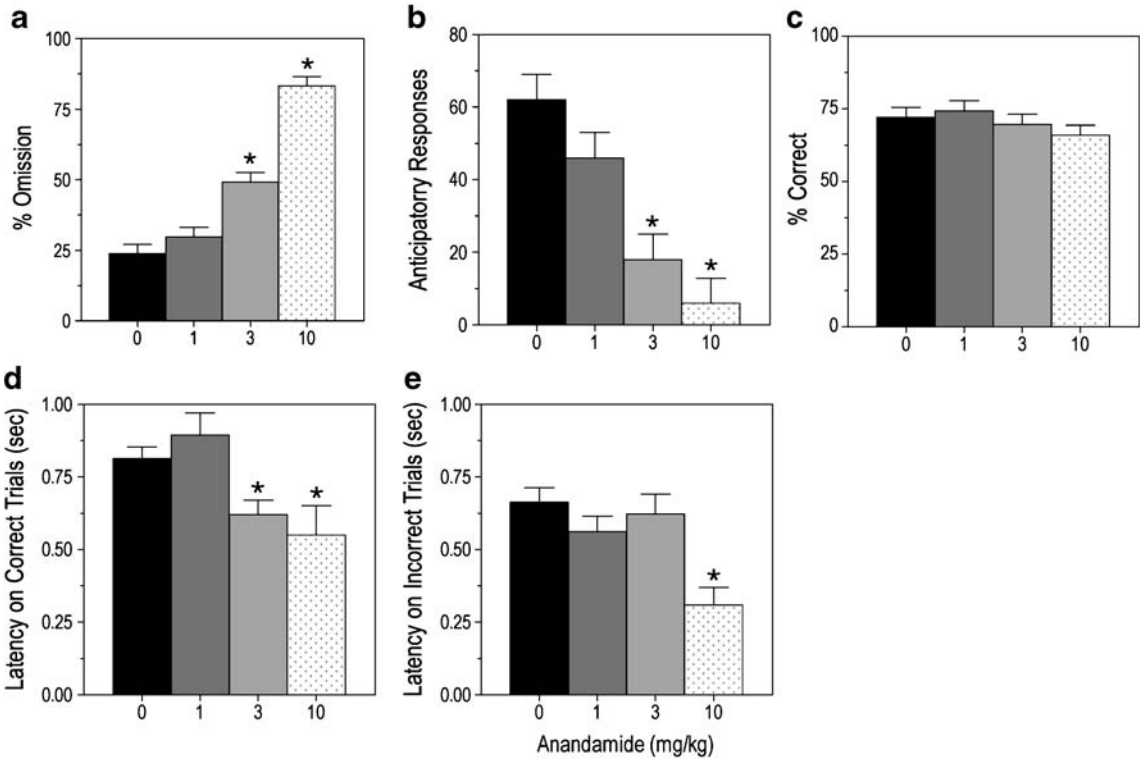

Fig. 1 Effects of anandamide (0, 1, 3, and 10 mg/kg, ip) on behavior in the attention task. **a** Omission errors (percentage of trials on which no response was made). **b** Anticipatory responses (number of responses during the inter-trial interval). **c** Accuracy (number of trials with a correct response, as a percentage of all trials with a response). **d** Latency on correct trials (number of seconds to respond on trials with a correct response). **e** Latency on incorrect trials (number of seconds to respond on trials with an incorrect response). *Asterisks* indicate bars that differ significantly ($p<0.05$) from vehicle (0 mg/kg anandamide). Error bars in all figures indicate SEM

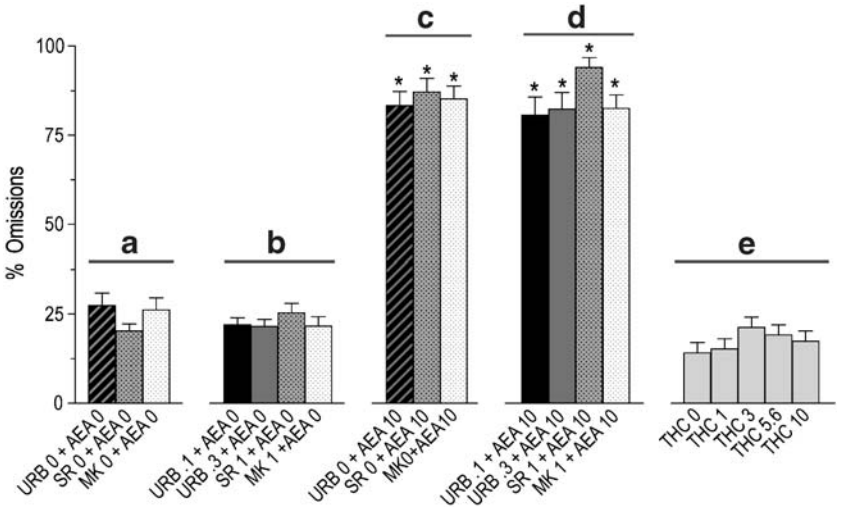

Fig. 2 Effects of treatments that failed to alter anandamide's effects on behavior in the attention task. Results are shown only for omission errors since the treatments also failed to alter the effects of anandamide on the other measures shown in Fig. 1. *a* Effects of vehicle-only injections. *b* Effects of treatment drugs (i.e., treatment drug plus the vehicle for anandamide). *c* Effects of anandamide (i.e., vehicle for the treatment drug plus 10 mg/kg anandamide). *d* Effects of treatment drugs plus anandamide. *e* Effects of THC alone. *AEA* anandamide, *URB* URB597 (FAAH inhibitor), *SR* SR141716 (rimonabant, cannabinoid-receptor antagonist), *MK* MK886 (PPARα antagonist). *Number* after drug abbreviation = dose (milligrams per kilogram), with *0* dose indicating vehicle. *Asterisks* indicate bars that differ significantly ($p<0.05$) from appropriate vehicle-only condition (0 mg/kg anandamide plus 0 mg/kg treatment)

which during this phase of testing, were comparable to those seen during the original anandamide dose–effect determination (Fig. 1). The interaction of capsazepine and anandamide was significant for omission errors [$F(1,28)=41.23$, $p<0.0001$], anticipatory responding [$F(1,28)=10.51$, $p<0.003$), and latency on incorrect trials [$F(1,7)=7.77$, $p<0.03$]; this interaction was marginally significant for latencies on correct trials [$F(1,20)=4.05$, $p<0.057$]. As in earlier testing (Fig. 1), anandamide did not affect the accuracy of responding. In subsequent testing (not shown), the blockade

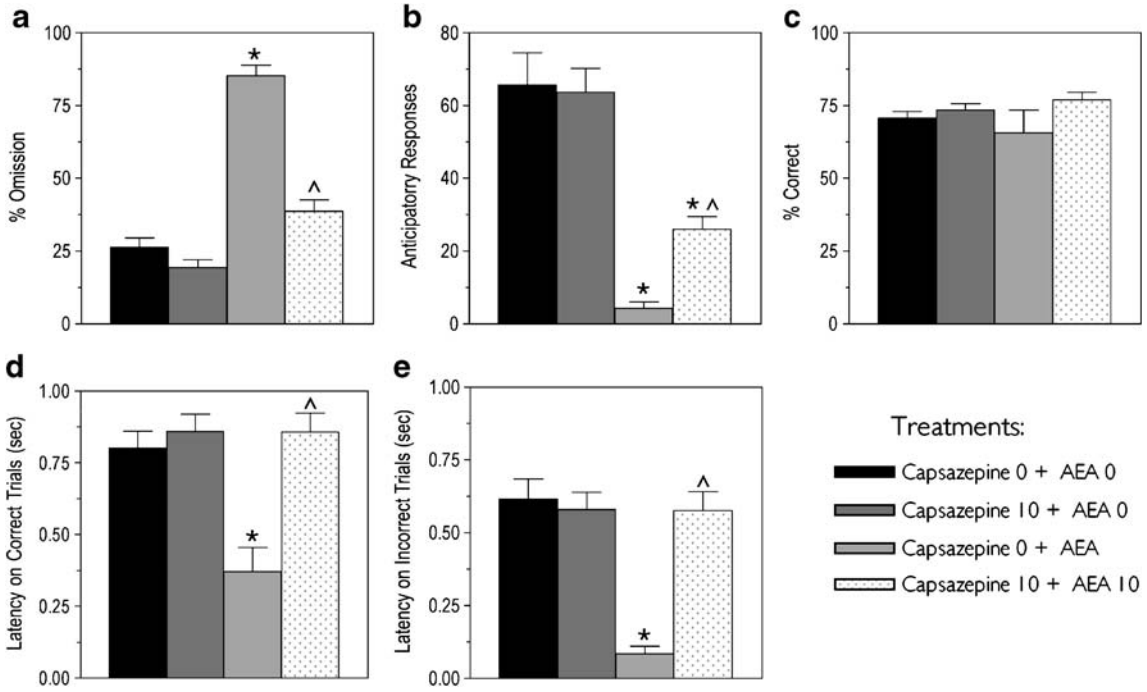

Fig. 3 Reversal by capsazepine (10 mg/kg) of the effects of anandamide (10 mg/kg) in the attention task. **a** Omission errors. **b** Anticipatory responses. **c** Accuracy. **d** Latency on correct trials. **e** Latency on incorrect trials. *AEA* anandamide, *0* vehicle, *10* 10 mg/kg dose. *Asterisks* indicate bars that differ significantly ($p<0.05$) from anandamide vehicle plus capsazepine vehicle condition. *Carets* indicate bars that differ from anandamide plus capsazepine vehicle

of anandamide's effects in the attention task by capsazepine (10 mg/kg) was replicated even when anandamide (10 mg/kg) was combined with URB597 (0.1 mg/kg), which prevents the degradation of anandamide by FAAH; these results closely matched the blockade effects shown in Fig. 3, with URB597 having no observable effect on either anandamide-induced omissions or the blockade of anandamide-induced omissions by capsazepine.

Open-field behavior

When the rats that had already been tested in the attention task were subsequently tested in the open field (Fig. 4), anandamide (10 mg/kg) was found to have little or no effect on any of the measures of general locomotor activity. However, anandamide produced a significant decrease in center-zone entries [main effect of anandamide: $F(1,6)=27.74$, $p<0.002$] and a significant increase in thigmotaxis [main effect of anandamide: $F(1,6)=6.76$, $p<0.05$], suggesting that anandamide had an anxiogenic effect. These anxiety-related effects of anandamide were not altered by capsazepine (10 mg/kg). Capsazepine alone did not affect any of the open-field measures.

Given the results of earlier locomotion studies (e.g., Di Marzo et al. 2001; de Lago et al. 2004), the fact that anandamide (10 mg/kg) failed to decrease general activity in these rats was surprising. To determine whether this lack of effect was due to the training history of these rats or to insensitivity of the procedure, a group of experimentally naive rats was tested using the same open-field test and the same doses of anandamide and capsazepine (Fig. 5). In the naive rats, general locomotor activity was significantly decreased when anandamide was given alone [main effects of anandamide on distance traveled: $F(1,24)=17.26$, $p<0.0004$; stereotypy counts: $F(1,24)=14.16$, $p<0.001$; and ambulatory episodes: $F(1,24)=18.07$, $p<0.0003$], but not when anandamide was given with capsazepine. Thus, capsazepine attenuated the locomotor-depressant effects of anandamide in naive rats. Anandamide slightly decreased center-zone entries in naive rats, but not significantly; this decrease may have resulted from the general depression of activity, and it was not accompanied by an increase in thigmotaxis. The baseline level of locomotor activity (i.e., the level when only vehicle was injected) was higher in the naive rats (Fig. 5) than in the rats that had previously undergone training and testing in the attention task (Fig. 4), with naive rats exhibiting a longer distance traveled and more ambulatory episodes, but about the same velocity of travel within episodes. With regards to the two anxiety-related measures (center-zone entries and thigmotaxis), the experienced rats appeared to have lower baseline levels of anxiety than the naive rats.

Experiment 7: food consumption

Food-consumption testing was conducted to determine whether the effects of anandamide in the attention task might be due to an interference with appetite or the ability

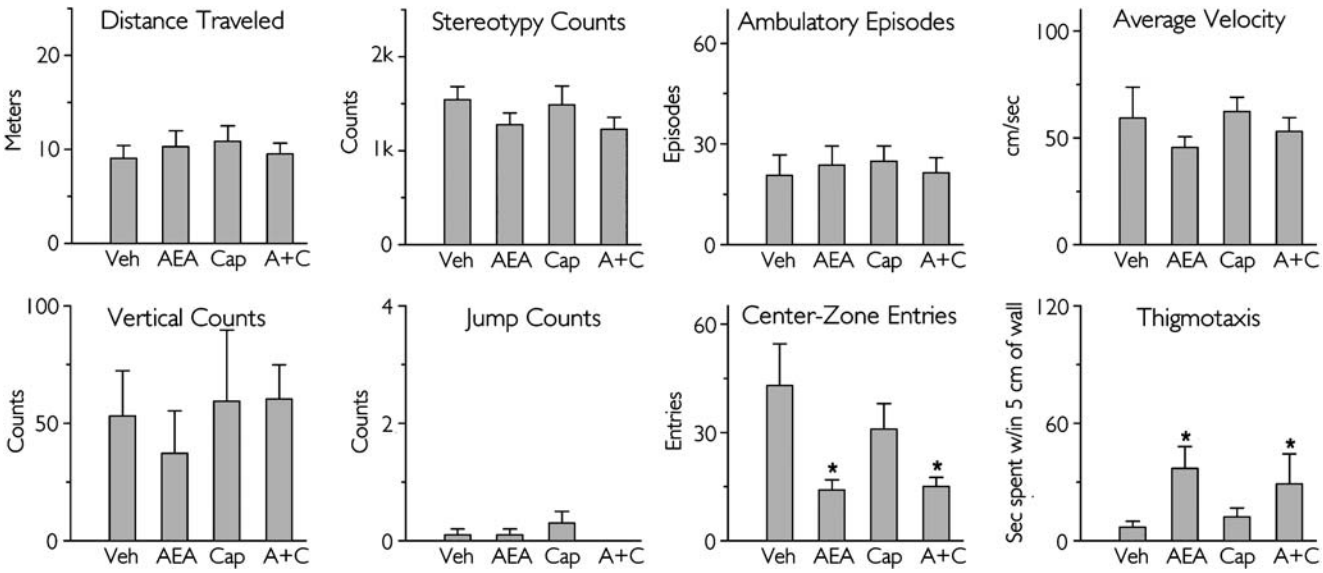

Fig. 4 Effects of anandamide (10 mg/kg) and capsazepine (10 mg/kg), alone and in combination, on open-field behavior in the same rats tested previously in the attention task. Measures of general activity: distance traveled, stereotypy counts, ambulatory episodes, average velocity, vertical counts (rearing), jump counts. Anxiety-related measures: center-zone entries, thigmotaxis (time spent within 5 cm of a wall). *Veh* anandamide vehicle plus capsazepine vehicle, *AEA* anandamide 10 mg/kg plus capsazepine vehicle, *Cap* capsazepine 10 mg/kg plus anandamide vehicle, *A+C* anandamide 10 mg/kg plus capsazepine 10 mg/kg. *Asterisks* indicate significant ($p<0.05$) difference from anandamide vehicle plus capsazepine vehicle condition

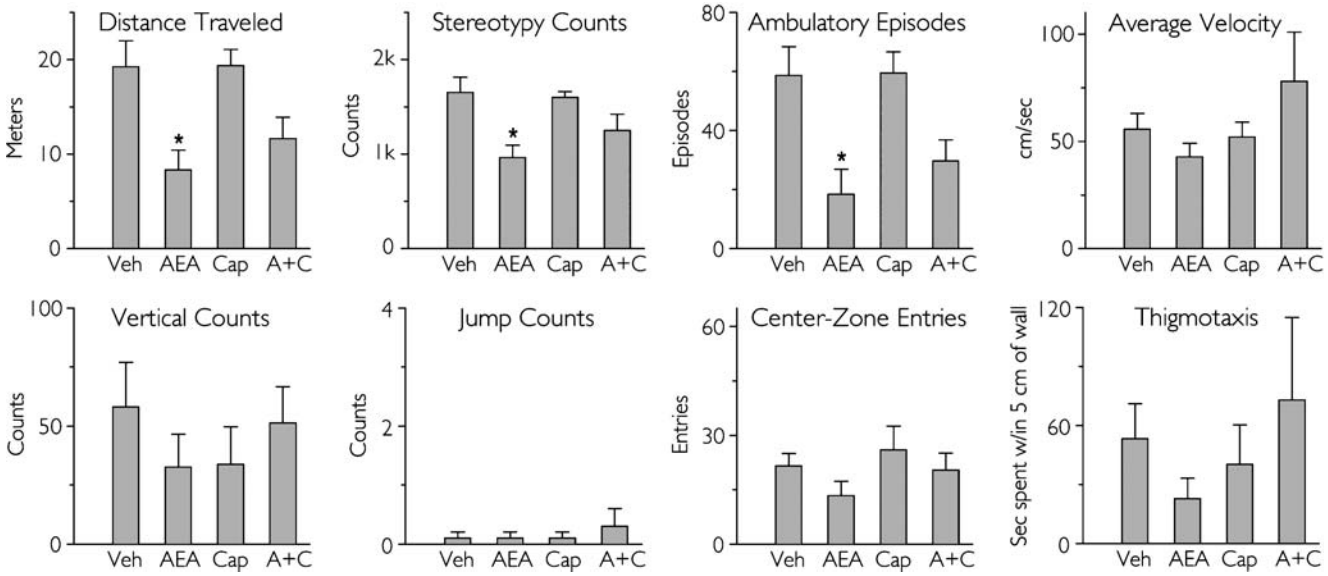

Fig. 5 Effects of anandamide (10 mg/kg) and capsazepine (10 mg/kg), alone and in combination, on open-field behavior in experimentally naive rats. Details are the same as in Fig. 4

to find and consume the food pellets (Fig. 6). Anandamide (10 mg/kg) had no significant effects on the amount of time to eat the first pellet, the amount of time between eating the first and last pellet, or the sum of these two measures (i.e., total time to find and consume all pellets).

Discussion

The primary behavioral effects of anandamide in the attention task were to increase the number of trials in which no response was emitted (omission errors) and to decrease responding during the inter-trial interval (anticipatory responding). Anandamide's direct pharmacological actions

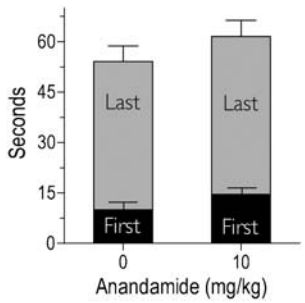

Fig. 6 Consumption of food pellets after treatment with 0 or 10 mg/kg anandamide in the same rats tested previously in the attention task. *Solid portion of bar* represents number of seconds before the first pellet was picked up. *Gray portion of bar* represents number of seconds between picking up the first pellet and last pellet. *Sum of black and gray portions stacked together* represents total number of seconds to pick up all pellets. There were no significant differences between the conditions

involve cannabinoid, PPAR, and vanilloid receptors, but only the vanilloid receptor antagonist capsazepine prevented anandamide-induced behavioral disruption. Therefore, behavioral disruption was most likely due to anandamide's actions at the vanilloid receptor, TRPV1. Further confirmation of this mechanism will require testing with more selective TRPV1 antagonists, since capsazepine may also block voltage-gated calcium channels, hyperpolarization-activated cyclic nucleotide-gated channels, and cholinergic receptors (Valenzano and Sun 2004).

Anandamide's actions at cannabinoid receptors were not responsible for its disruptive effects since none of these effects were altered by the cannabinoid CB_1 receptor antagonist rimonabant, and the cannabinoid agonist THC did not produce effects like those of anandamide. In the previous study showing effects of THC on accuracy of responding under a similar attention task, THC was given subchronically prior to acquisition of the task (Verrico et al. 2004). In contrast, the rats in the present study were well trained before testing with any drug. In the previous study showing attentional effects of the cannabinoid CB_1 receptor and PPAR agonist WIN 55,212-2 (Arguello and Jentsch 2004), rats were trained prior to drug testing; however, WIN 55,212-2 was found to affect behavior (decreasing accuracy and increasing omissions) only when stimulus durations were short (0.5 and 1 s), not when they were as long as in the present study (2 s). In a related procedure, THC only affected visual signal detection in rats when stimulus durations were 100 ms, not when they were 300 or 1,000 ms (Presburger and Robinson 1999). Results such as these suggest that if different parameters (e.g., shorter stimulus durations) were to be used in the 5-CSRTT task,

THC-induced effects or cannabinoid- or PPAR-related effects of anandamide might be revealed. Be that as it may, the attention task used here was sensitive to behavioral effects of anandamide, and these effects were clearly blocked by the vanilloid receptor antagonist capsazepine.

The FAAH inhibitor URB597, which blocks the primary mechanism by which anandamide is degraded, has been found to enhance certain behavioral effects of anandamide, but it did not enhance the effects of anandamide in the present study, nor did it alter behavior when given alone. However, it should be noted that FAAH inhibition would not prevent the production of anandamide's lipoxygenase metabolites, such as 12- and 15-HPETE [12- and 15-(*S*)-hydroperoxyeicosatetraenoic acid], which have much higher efficacies as TRPV1 ligands than does anandamide itself (Hwang et al. 2000; Piomelli 2001; Veldhuis et al. 2003). Thus, it is possible that the net effect of URB597 on anandamide's direct and indirect TRPV1 actions may have been small.

Even as anandamide increased omission errors, it decreased latencies to respond on trials in which a response did occur, without decreasing accuracy. These results might indicate that the rats only responded when they were relatively close to the hole when the stimulus was presented. In the 5-CSRTT procedure, changes in accuracy are generally considered the hallmark of an effect on attention, but such changes did not occur under the parameters used in the present study. The surprising fact that there was little effect on accuracy even when omissions were increased suggests that anandamide did not produce cognitive impairment, but perhaps motor, emotional, or motivational impairment.

However, subsequent testing of open-field behavior in the same rats revealed that the anandamide-induced disruption of operant responding in the attention task cannot easily be attributed to a general depression of locomotor activity. Earlier studies have shown that anandamide can decrease locomotor activity in experimentally naive rats (Di Marzo et al. 2001) by a vanilloid-dependent mechanism (de Lago et al. 2004; Lee et al. 2006; Tzavara et al. 2006). Testing with naive rats in the present study confirmed that our open-field procedure was capable of detecting this effect of anandamide and its reversal by the vanilloid receptor antagonist, capsazepine. However, anandamide did not alter the level of locomotor activity in the rats that had previously been trained and tested in the attention task. Even though there are limits to the extent to which locomotor activity can be used to represent all forms of motor function or dysfunction, it is clear that these rats were not incapacitated by treatment with anandamide. The precise reason for this resistance to the locomotor-depressant effects of anandamide is unclear, but tolerance was not a factor since the effects of anandamide were consistent over time (as seen in Figs. 1, 2, and 3).

Open-field testing also revealed that these experienced rats showed evidence of an anxiogenic reaction (see Prut and Belzung 2003) to anandamide. There is recent evidence that high doses of intracranially administered anandamide produce anxiogenic effects that are TRPV1-mediated (Rubino et al. 2008). However, the anandamide-induced anxiogenic-like effects observed in the open field in the present study were not blocked by capsazepine, indicating that anxiogenic effects were probably not responsible for the vanilloid-dependent behavioral disruption observed in the attention task.

The results of the food-consumption test in these experienced rats indicate that anandamide-induced omission errors in the attention task also cannot be attributed to effects on feeding or appetite. When treated with anandamide, these rats were fully capable of finding and consuming food pellets under conditions similar to those of the attention test (i.e., with a limited amount of food made available within a short period of time). Thus, anandamide produced a selective reduction of food-reinforced operant responding but did not reduce the consumption of freely available food. This profile of effects resembles that of dopamine antagonists, which have been described as selectively decreasing operant behavior that requires effort, without altering the consumption of free food or the performance of simple operant tasks (see review by Salamone and Correa 2002; see also Arizzi et al. 2004; Berridge 1996).

The behavioral effects of anandamide in the attention task also closely resembled the effects of dopamine antagonists in a number of other studies using the 5-CSRRT attention task (e.g., Hahn et al. 2002; Harrison et al. 1997; Koskinen and Sirviö 2001; Passetti et al. 2003). Anticipatory responding in the 5-CSRRT procedure is considered a model of behavioral inhibition related to impulsivity, and this behavior is clearly sensitive to dopamine manipulations (Harrison et al. 1997; van Gaalen et al. 2006; Pattij et al. 2007; Pattij and Vanderschuren 2008). In the present study, anandamide decreased not only anticipatory responding but responding during trials and therefore cannot be described as selectively altering impulsivity. However, the fact that anandamide reduced operant responding without affecting general locomotor activity or feeding suggests that the behavioral disruption induced by anandamide might involve modulation of dopaminergic neurotransmission. This possibility would be consistent with recent evidence that anandamide binds to vanilloid receptors on nigrostriatal dopaminergic neurons, thereby decreasing the activity of these neurons (de Lago et al. 2004; see also Tzavara et al. 2006; Lee et al. 2006).

By acting at cannabinoid, vanilloid, and PPAR receptors, anandamide can clearly affect a wide range of brain systems, physiological processes, and behaviors. Anandamide is metabolically related to other fatty acid ethanolamides, such

as the satiety factor oleoylethanolamide and the anti-inflammatory factor palmitoylethanolamide, both of which are PPARα ligands, and with the endocannabinoid 2-arachidonoylglycerol (2-AG; see Di Marzo and Maccarrone 2008), all of which are degraded by the enzyme FAAH. Recent work has indicated that anandamide modulates levels of 2-AG through a vanilloid-dependent mechanism and that anandamide is "critically involved in the control of excitability of striatal neurons" (Maccarrone et al. 2008).

To increase our understanding of these complex systems, it will continue to be important to study them at multiple levels, including the level of behavior. The present study indicates that anandamide's actions at vanilloid receptors produce selective decreases in operant responding that cannot be attributed to motor depression, anxiety, or decreased appetite. The similarity between these effects and the effects of dopamine antagonists suggests that they might be due to anandamide's modulation of dopaminergic neurotransmission.

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