Published in final edited form as:

Psychopharmacology (Berl). 2013 December ; 230(3): . doi:10.1007/s00213-013-3161-2.

The effects of anandamide signaling enhanced by the FAAH inhibitor URB597 on coping styles in rats

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Abstract

Rationale—Coping styles are fundamental characteristics of behavior that affect susceptibility to, and resilience during, mental and physical illness. Shifts from passive to active coping are considered therapeutic goals in many stress-related disorders, but the neural control of coping is poorly understood. Based on earlier findings we hypothesized that coping styles are influenced by endocannabinoids.

Objectives—Here we tested whether FAAH inhibition by URB597 affects behaviors aimed at controlling a critical situation and the degree to which environmental stimuli influence behavior i.e. we studied the impact of URB597 on the two main attributes of coping styles.

Methods—Rats were tested in the tail-pinch test of coping and in the elevated plus-maze test that was performed under highly divergent conditions.

Results—Under the effects of URB597, rats focused their behavior more on the discomfort-inducing clamp in the tail pinch test, i.e. they coped with the challenge more actively. In the elevated plus-maze, URB597-treated rats demonstrated an autonomous behavioral control by reducing both "wariness" induced by aversive conditions and "carelessness" resulting from favorable conditions.

Conclusions—URB597 treatment induced behavioral changes indicative of a shift towards active coping with challenges. This behavioral change appears compatible with the previously suggested role of endocannabinoids in emotional homeostasis. Albeit further studies are required to characterize the role of endocannabinoids in coping, these findings suggest that the enhancement of endocannabinoid signaling may become a therapeutic option in emotional disorders characterized by passive coping (e.g. anxiety and depression) and in physical diseases where active coping is therapeutically desirable.

Keywords

behavior; endocannabinoid; FAAH; URB597; context; coping; rats

Introduction

Since the endocannabinoid signaling was discovered, a large body of research focused on its role in emotions in general, and emotional disorders (e.g. anxiety and depression) in

Conflict of interest. The authors declare no conflicts of interest.

particular (Gorzalka and Hill 2011; Lutz 2009; Marco and Viveros 2009; Ruehle et al. 2012). Research involving transgenic animals provided clear evidence on the role cannabinoid system in anxiety and depression; however, studies with pharmacological agents that directly targeted the CB1 cannabinoid receptor often lead to conflicting findings (Zanettini et al. 2012). In addition, such agents proved to have significant side effects, which likely prevent their introduction into clinical practice (Moreira et al. 2009).

Therapeutic hopes were revived by the discovery of indirect endocannabinoid modulators like the FAAH inhibitor URB597, which blocks the degradation of lipid mediators e.g. anandamide, oleoylethanolamide, and palmitoylethanolamide (Fegley et al. 2005; Piomelli et al., 2006). Oleoylethanolamide and palmitoylethanolamide were recently shown to be ligands for the PPAR- receptors and to be involved in the control of memory (Mazzola et al., 2009). The anandamide-mediated effects of FAAH inhibition in contrast affect behavior in tests of anxiety and depression more reliably than agents that directly target the CB1 receptor (Piomelli et al. 2006; Zanettini et al. 2011). Discrepant findings were still obtained with this and similar compounds. Recently, Haller et al. (2009) suggested that success or failure in detecting anxiolytic effects with URB597 was largely explained by the degree of aversiveness of the testing environment in particular studies. These authors reported that URB597 did not decrease anxiety when the elevated plus-maze test was performed under mildly aversive conditions, but significantly ameliorated the anxiogenic effects of highly aversive conditions. It was also shown that anandamide transport blockade (the neurochemical consequences of which are similar to those of FAAH inhibition), affects learning in conjunction with the aversiveness of testing conditions (Campolongo et al. 2012). Based on such findings and on the neurochemical roles of endocannabinoids, Zanettini et al. (2011) hypothesized that the main role of cannabinoid signaling is to dampen excessive neuronal responses induced by aversive environments; consequently, the behavioral effects of endocannabinoid activation are situation-dependent. Indirectly, these and similar studies suggested that endocannabinoids affect the coping with environmental challenges.

The role of endocannabinoids in coping was recently substantiated by several studies. E.g. it was shown that striatal anandamide levels participate in the emotional arousal resulting from a non-familiar social encounter, and are particularly important for coping responses to novel social contexts in terms of the time spent with social investigation (Marco et al. 2011). It was also shown that FAAH inhibition promotes active responses (e.g. swimming) and inhibits passive responses (e.g. immobility) in the forced swimming test, an effect that involved the prefrontal cortex and serotonergic neurotransmission (McLaughlin et al. 2012; Realini et al. 2011). Cognitive flexibility –an important characteristic of coping styles, see below– was markedly inhibited by URB597 in another study (Sokolic et al. 2011). Moreover, FAAH gene polymorphisms have been implicated in stress-coping in humans (Gunduz-Cinar et al. 2012). These findings substantiate the view that the endocannabinoid anandamide has an important role in coping with challenges. Particularly, anandamide signaling appears to promote active coping.

Active and passive coping styles are two distinct behavioral phenotypes, which differ in the way in which challenges are dealt with (Koolhaas et al. 1999; Koolhaas 2008). In novel situations, active copers base their behavior on routines that are weakly influenced by environmental stimuli (i.e. are cognitively less flexible) and attempt to control challenges when they occur. Thus, behavior is internally driven and problem-oriented in active copers. In contrast, passive copers are governed by environmental stimuli and tend to respond challenges by avoidant behavior. These temporally stable behavioral phenotypes have adaptive significance in animals, while in humans active (Type "A") and passive (particularly Type "C") coping styles influence disease susceptibility and resilience under

adverse conditions (Koolhaas 2008; Kessler et al. 1985; Temoshok 2000). Moreover, coping styles are believed to reliably predict disease-induced decreases in quality of life (Pucheu et al. 2004; Westerhuis et al. 2011). Consequently, interventions promoting active coping styles –which are associated more favorably with resilience–, have been proposed as therapeutic goals for a variety of physical diseases and mental disorders (Westerhuis et al. 2011; Cooke et al. 2007; Tiemensma et al. 2011). Thus, the putative effects of FAAH inhibition on coping styles are highly relevant from a therapeutic point of view. Despite a few promising studies, the effects of FAAH inhibition on coping styles are insufficiently characterized.

The aim of the present study was to investigate the effects of the FAAH inhibitor URB597 on coping responses in the tail-pinch test and the elevated plus-maze. In the tail-pinch test, a clamp is attached to tail of rats. This test was originally developed to study pain sensitivity (Gupta et al., 1975), but was later used to study the behavioral consequences of stressors (Mufson et al., 1976; Nobrega et al., 1989), and coping responses (Giorgi et al., 2003; Gomez et al., 2010). Attaching a clamp to the tail of rats is mildly stressful (Gibb et al., 2008; Kirby et al., 1997) and repeated exposures to the test (e.g. daily for 10 days) do not induce chronic stress responses (Sato et al., 2011). Physiological and behavioral responses to the test are highly stable over time. E.g. repeated exposures to the test (3–8 times) at variable intervals (5 min – 24 h) did not change nucleus accumbens dopamine release, heart rates, locomotion, clamp-directed behaviors (e.g. gnawing at the clamp), oral stereotypies, escape behaviors, and vocalizations induced by the procedure (Bespalov et al., 1998; Brake et al., 2000; El-Khodori and Boksa, 2000; Funk and Stewart, 1996; Hawkins et al., 1999). The repeatability of the test was exploited here with the aim of both reducing sample sizes and increasing the meaningfulness of findings. Behaviors indicative of coping styles have a biphasic distribution in the tail pinch test (Giorgi et al., 2003; Gomez et al., 2010) and more generally in tests of coping (Koolhaas et al. 1999; Koolhaas 2008). Consequently, "average" behaviors are less frequent than "extremes" (passive and active copers). As such, group averages do not describe behavior properly; in addition, the divergence of coping styles increases variability. Excessive sample sizes may have revealed general changes in coping styles if treatments were studied in different sets of rats; however, the overall change did not clarify which of the two styles were affected once the "original" coping style was unknown. To avoid such problems, we studied coping styles by testing subjects repeatedly under different treatment conditions that were balanced over time. The general design of tail-pinch studies was presented in Table 1.

The elevated plus-maze test was used here to investigate the impact of environmental conditions on anxiety-like behavior. As shown above, an important indicator of coping styles is the degree to which behaviors are autonomous. To reveal changes in behavioral autonomy, we studied the effects of URB597 under highly divergent conditions as described in Methods. Ultimately, the aim of these experiments was to study the impact of URB597 on two basic characteristics of coping styles: problem oriented behavior and behavioral autonomy.

Methods

Subjects

Subjects were 2–3 months old male Wistar rats purchased from Charles River Laboratories (Hungary). Their weight was approximately 250 g when purchased. Food and water were available ad libitum; temperature and relative humidity were kept at 22 ± 2 °C and $60 \pm 10\%$, respectively. Subjects were maintained in a normal light cycle of 12 h with lights off at 07:00 h. Acclimatization to local conditions lasted at least one week. Subjects were kept in groups of 4 in Macrolon cages $45 \times 35 \times 25$ cm. All subjects were experimentally naive and

had no drug history prior to the start of the studies. Different sets of rats were used for the tail-pinch and plus-maze tests. Behavioral studies were performed in the early hours of the light phase. Behavior was scored by an experimenter blind to treatments. All animals belonging to the same study were scored by the same observer. Intra-rater reliability was over 90%.

Experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and were reviewed and approved by the Animal Welfare Committee of our Institute.

Behavioral tests

In the *tail-pinch test*, a clamp is attached to the tail of rats and the behavioral response is recorded in a neutral arena (Giorgi et al., 2003). Trials of removing the clamp are considered active responses, while ignoring the clamp is considered a passive response. We used triangular clamps (width: 2 cm; side lengths 1, 0.7 and 1 cm), which were attached to the tail of rats such that the opening of the clamp was 6–8 mm. The pressure exerted by the clamp on the tail of rats was 1.47 ± 0.16 Pa as calculated from the force necessary to open the clamp to the width measured on the tail of rats and the contact surface. The latter was established in a separate investigation by applying the clamp on tails that were wet-painted. This pressure was not particularly painful as 30–40% of subjects engaged in exploring the novel environment without durable or intense attempts to remove the clamp. At the same time, the pressure was sufficient to prevent clamp removal; no rat succeeded in this respect.

The clamp was attached to the tail of rats and their behavior was recorded for 5 min in a neutral arena. The details of the procedure were described in the subsection Experimental design. To better characterize the behavior of rats, we performed a full analysis of the behaviors shown in this test. This analysis indicated that approximately 85% of time was devoted to two behaviors: gnawing at the clamp and clamp-independent exploration. In line with earlier studies, the former was considered a "problem-oriented" i.e. an active response, while the latter was considered a sign of "ignoring the problem" i.e. a passive response (Giorgi et al., 2003). Coping styles were characterized by the ratio of time spent with gnawing at the clamp and exploration.

The *elevated plus-maze test* was made of dark grey painted wood (arm length 50 cm, arm width 17 cm, wall height 30 cm and platform height 80 cm). Subjects were placed in the central area of the apparatus with head facing a closed arm. Exposure lasted 5 min. Closed-arm entries (counts/5 min) were considered indicators of locomotor activity, whereas open-arm exploration (% time spent in open arms and % open arm entries) were used as measures of anxiety. The test was performed under highly divergent conditions as described below.

Experimental design

Experiment 1 investigated the effects of URB597 in the tail-pinch test. Rats were treated with 0.0 (vehicle), 0.1 and 0.3 mg/kg URB597 in their maintenance room. 40 min later, rats were transferred to the test room; clamps were attached to their tail and were placed in Makrolon cages measuring $45 \times 35 \times 25$ cm and covered with a Plexiglas lid. The test lasted 5 min. Behavior was video recorded by a camera placed 2 m above the cage and scored by an experimenter blind to treatments. The test was performed under normal laboratory lighting (~300 lx). For the reasons clarified in Introduction, a repeated measures cross-over design was employed. Particularly, the test was performed three times in the same animals 3 days apart. Each rat received two of the treatments listed above but on different experimental days. The order of treatments was balanced over experimental days. E.g. half of the rats that were treated with 0.3 mg/kg URB597 received this treatment after the

experimental day when they received vehicle, while the other half received 0.3 mg/kg URB597 prior to the vehicle treatment day. The same design was employed for all treatments. The consistency of coping styles was evaluated by comparing the behavior of rats that was shown on two days when they were treated with vehicle. These two vehicle treatments were either performed on the 1st and the 2nd or on the 2nd and the 3rd testing day (see Table 1). The number of rats tested was 40.

Experiment 2 aimed at testing the involvement of the CB1 cannabinoid receptor in the behavioral changes seen in Experiment 1 by investigating the effects of the CB1 antagonist rimonabant alone or in combination with URB597. To this end, rats were treated with vehicle, 0.3 mg/kg URB597, 1 mg/kg rimonabant, and a mixture of 0.3 mg/kg URB597 and 1 mg/kg rimonabant. The experimental design was similar to that employed in Experiment 1. The number of rats tested was 50.

In *Experiment 3*, we studied the effects of the benzodiazepine anxiolytic chlordiazepoxide to see whether the URB597-induced changes in coping style are mere consequences of anxiolysis. The design was similar to that described for Experiment 1. Rats were treated with 0 (vehicle), 5, and 10 mg/kg chlordiazepoxide. The number of rats tested was 40.

Experiment 4 aimed at clarifying whether the behaviors seen in Experiment 1 and 2 were secondary to pharmacological treatment-induced or coping style-related changes in pain sensitivity. Pain sensitivity was investigated by means of the hot-plate test. To study the impact of URB597, rats were treated with vehicle or 0.3 mg/kg URB597 in their maintenance room (N=10 per group), and were exposed to the hot-plate test 40 min later. Animals were transferred to the test room immediately prior to testing. Each animal was placed alone on the hot plate surrounded with a Plexiglas box (IITC Life Science, Woodland Hills, CA, USA), and covered with a transparent lid. After 3 min habituation, the heating was started. The basal temperature of the plate was 30 °C, the maximum temperature was 55 °C, and the heating speed was 6 °C/min. Exposure lasted until the animal licked one of its hind paws; when this happened, heating was stopped and time and temperature were recorded. The interaction between coping styles and pain perception was investigated in a separate experiment involving 40 rats. Coping styles were established as described above. The hot plate test was performed 3 days later as shown above.

Experiment 5 tested the effects of 0.0 (vehicle), 0.1, and 0.3 mg/kg URB597 in the elevated plus-maze under three sets of conditions. At one extreme, we studied rats under non-aversive conditions i.e. under low light and in a habituated testing environment. At the other extreme, we studied rats under aversive conditions: high light in an unfamiliar environment. The third condition involved an intermediate level of aversiveness: a habituated environment but with high lighting. For habituation, cage racks were transferred from the housing room to the testing room for 2h prior to testing. The high and low light conditions involved 4*40 w white (>300 lx) and one red neon lamp (<5 lx), respectively. Behavior was video recorded by a camera placed 1.8m above the elevated plus-maze. These experimental conditions elicit differential HPA-axis activation consistent with the intended level of aversiveness (Haller et al. 2009).

Drugs and doses

URB597 was dissolved in 0.2 ml dimethylsulfoxide (DMSO), which was diluted to the final volume with saline that contained 0.4% methylcellulose, a biologically neutral solvent. Rimonabant was also dissolved in 0.2 ml DMSO diluted to the final volume with 0.4% methylcellulose in saline. We observed earlier that two consecutive injections slightly alter behavior compared to when one injection is given; therefore, rimonabant and URB597 were administered *via* the same injection when given as a combined treatment. The injected

volume was similar to that of single treatments. Chlordiazepoxide was dissolved in 0.4% methylcellulose in saline. All drugs were obtained from Sigma (Budapest, Hungary), and were injected intraperitoneally in a volume of 1 ml/kg. Doses were selected based on earlier experience with these compounds (Haller et al. 2007, 2009).

Statistics

One-way or two-way analysis of variance (ANOVA) was used to estimate main effects; ANOVA for repeated measures was used for time series. Post-hoc Tukey tests were performed only for significant main effects. Behavioral data were square root-transformed to ensure compliance with ANOVA assumptions. Significance level was set at $P < 0.05$. P values for multiple comparisons underwent Bonferroni correction (Holm's procedure). Correlations were calculated by means of the Spearman test.

Results

Experiment 1: The effects of URB597 in the tail-pinch test

We identified 8 different behaviors in this test as follows: *digging* (digging in the sawdust), *exploration/locomotion* (ambulation or sniffing at the floor, cage walls or air); *gnawing* (biting or nibbling the clamp); *grooming* (washing with fore paws or scratching with hind paws), *resting* (no obvious movements; small postural changes allowed); *jumping* (escape attempts); *pulling the clamp* (pulling the clamp with teeth); *rotating* ("chasing" the clamp by turning around). Only exploration/locomotion, gnawing and resting were consistently shown; the rest of behaviors occurred sporadically (Table 2). Taken together, gnawing and exploration accounted for about 85% of total test time, and these behaviors were mutually exclusive as shown by the strong negative correlation between these two behaviors (*gnawing*exploration vehicle day 1*: Spearman $R = -0.890$; $p < 0.0001$; *gnawing*exploration vehicle day 2*: $R = -0.886$; $p < 0.0001$). No similar correlations were found for resting that accounted for about 10% of total time. Based on these findings, we concluded that the main behavioral responses in the tail pinch test are gnawing at the clamp and environment-oriented exploration that were mutually exclusive.

In order to investigate the temporal stability of behaviors that are believed to characterize coping styles, we studied the inter-trial correlation of gnawing and exploration in vehicle trials. The two vehicle trials were performed in the same animals 3 days apart. Correlations were highly significant for both behaviors (*gnawing vehicle 1* vehicle 2*: Spearman $R = 0.565$, $p < 0.005$; *exploration vehicle 1* vehicle 2*: $R = 0.602$, $p < 0.001$) (Fig.1). For brevity, Fig. 1 depicts the correlations for the other two studies as well. Behaviors correlated significantly in these studies as well (*gnawing URB+SR study*: $R = 0.917$, $p < 0.0001$; *gnawing CDP study*: $R = 0.606$, $p < 0.0001$; *exploration URB+SR study*: $R = 0.850$, $p < 0.0001$; *exploration CDP study*: $R = 0.401$, $p < 0.04$). Thus, the behavioral profile of rats was stable over time.

The gnawing /exploration ratio differentiated three subgroups of rats on the first vehicle day. In one subgroup, more than 60% of total time was spent with gnawing at the clamp, and less than 40% was spent with exploration. Thus, the gnawing/exploration ratio was over 1.5 in this subgroup, which was considered to employ an active coping style. By contrast, less than 40% of total time was spent with gnawing at the clamp, and more than 60% was spent with exploration in the other subgroup. The gnawing/exploration ratio was less than 0.66 in this subgroup, which was considered to employ a passive coping style. Taken together, more than 75% of rats showed a clear predilection to gnawing (active copers) or to exploration (passive copers). In the rest of rats, the gnawing/exploration ratio was between 0.66 and 1.5. Such rats were considered mixed copers. Beyond a significant correlation between behaviors

in vehicle trials the stability of coping styles was also demonstrated by the stability of gnawing/exploration ratios (see below).

In Experiment 1, there was a significant interaction between coping style and treatment ($F_{\text{coping style} \times \text{treatment}}(2,70) = 8.00$; $p < 0.0001$). When rats were repeatedly treated with vehicle, the behavioral profile of individuals was consistent over time (Fig. 2a). In contrast to vehicle treatments, URB597 dose-dependently altered coping styles, such that behavioral differences between active, passive and mixed copers disappeared (Fig. 2a). Generally, URB597 shifted the passive and mixed copers towards a more active style, while the active copers remained active although the gnawing/exploration ratio slightly but not significantly decreased.

Experiment 2: The impact of the CB1 blocker rimonabant

The cannabinoid CB1 receptor antagonist/inverse agonist rimonabant at a dose that had no effect when given alone abolished the effects of URB597 on coping ($F_{\text{coping style} \times \text{treatment}}(2,68) = 5.68$; $p < 0.01$) (Fig 2b). Again, the second vehicle treatment did not alter coping styles seen after the first one, while 0.3 mg/kg URB597 promoted active coping in animals that showed passive coping or a mixed style after vehicle injections. The decrease in the gnawing/exploration ratio in active copers was somewhat stronger than in Experiment 1, but on average, active copers still maintained a predilection towards active coping. Rimonabant per se had no significant effects on coping, but abolished the effects of URB597. Although a slight shift towards active coping did occur in both rimonabant-treated groups, the differences in coping styles remained significant.

The frequency distribution of coping styles in Experiments 1 and 2 revealed a significant shift towards active coping after 0.3 mg/kg URB597 treatment (Chi-Square= 11.16; $p = 0.048$) (Fig. 2c).

Experiment 3–4: The impact of anxiolysis and pain perception

The benzodiazepine anxiolytic chlordiazepoxide did not affect coping styles significantly ($F_{\text{treatment}}(2,71) = 0.24$; $p = 0.78$; $F_{\text{coping style} \times \text{treatment}}(4,71) = 0.41$; $p = 0.78$), and differences in coping styles remained consistent throughout ($F_{\text{coping style}}(2,71) = 55.94$; $p = 0.00001$) (Fig. 3a). The behavioral changes seen in Experiments 1 and 2 were not secondary to altered sensitivity to clamp-induced discomfort, as neither coping style nor URB597 affected pain perception ($F(2,37) = 0.92$; $p > 0.4$ and $F(1,18) = 0.26$; $p > 0.6$, respectively) (Fig. 3b).

Experiment 5: The effects URB597 in the elevated plus-maze

In the elevated plus-maze, closed arm entries were significantly affected by experimental conditions only ($F_{\text{condition}}(2,110) = 12.95$; $P < 0.0001$), while the interaction between factors was significant for both the duration, and the relative frequency of open arm visits ($F_{\text{treatment} \times \text{condition}}(4,110) = 3.71$ ($P < 0.01$) and 3.59 ($P < 0.01$), respectively) (Fig. 4a–c). In line with earlier observations, URB did not affect anxiety under the intermediate, mildly aversive condition (habituated environment, high light) but decreased anxiety under the aversive condition. Surprisingly, however, URB597 increased anxiety-like behavior when given under the least aversive conditions (habituation, low light). However, an alternative analysis showed that the anxiety-like behavior of the URB597-treated rats was about the same under all three conditions, while the vehicle-treated rats showed marked anxiety-like behaviors in the intermediate and aversive conditions (Fig 4d–f). In contrast to anxiety-like behaviors, the effects of testing conditions on closed arm entries were similar in the three groups.

Discussion

Main findings

Active copers attempt to control challenges when these occur, and their behavior is autonomous compared to passive copers which are governed by environmental stimuli (Koolhaas et al. 1999; Koolhaas 2008). Our findings suggest that FAAH inhibition by URB597 induces behavioral changes that are compatible with these two criteria of active coping. When FAAH was inhibited, rats focused their behavior on the clamp in the tail pinch test, i.e. they coped with the challenge in a more active manner. The interaction between testing conditions and behavioral effects show that FAAH inhibition altered coping styles also in the elevated plus-maze. While the behavior of vehicle-treated rats depended on environmental conditions, rats treated with URB597 behaved in the elevated plus-maze in the same manner across all testing conditions, suggesting an autonomous behavioral control.

Control over challenges

In line with earlier findings (see Introduction), behavior was stable over time in the tail-pinch test as shown by the comparison of vehicle trials. Behaviors not only correlated in the two vehicle trials performed in the same animals, but coping styles were also stable over time as far as vehicle trials are compared. Although some variation did emerge, these were minor. In Experiment 1, passive and mixed copers appeared to shift towards mixed and active styles, respectively. However, the opposite happened in the rimonabant and chlordiazepoxide studies, where mixed copers shifted towards passive coping in the second vehicle trial. We consider such small shifts in coping styles as consequences of between-trial variations of behavior. In support of this assumption, differences between coping styles were significant in all vehicle trials.

The effects of URB597 were particularly strong in passive and mixed copers. Rats that showed passive coping in vehicle trials, displayed a mixed style under the effect of URB597. Similarly, rats showing a mixed coping style in vehicle trials shifted towards active coping when treated with the FAAH inhibitor. Somewhat surprisingly, the gnawing/exploration ratio slightly decreased in active copers, an effect that was more marked in the URB-rimonabant study than in the URB-alone study. Despite this shift, rats showing active coping in vehicle trials remained active copers after URB597. The promotion of active coping by FAAH inhibition is best shown by the differences in the frequency distribution of coping styles after vehicle and URB597 treatments. The share of active copers increased from about 30% –seen during vehicle trials– to about 60% after treatment with 0.3 mg/kg URB597. Such changes were not due to altered pain perception or anxiolysis, as neither URB597 treatment nor coping affected pain perception, whereas the benzodiazepine anxiolytic chlordiazepoxide did not alter coping styles. Importantly, the FAAH inhibition-induced changes in coping styles appeared reversible, as vehicle and URB597 were tested in the same rats.

Behavioral autonomy

At the first sight, the effects of URB597 in the elevated plus-maze surprisingly suggested that FAAH inhibition can have either anxiogenic or anxiolytic effects depending on environmental conditions. There was, however, a marked difference in the behavior of vehicle and URB597-treated rats. While anxiety increased in parallel with the aversiveness of the testing environment in vehicle treated rats, the behavior of URB-treated rats appeared more autonomous and was weakly influenced by the aversiveness of the environment. Yet, URB597-treated rats did not seem to be disrupted from environmental information as condition-induced changes in locomotion were similar in the three groups. Based on these considerations we suggest that the strange effects of URB597 in the elevated plus-maze

should be interpreted in terms of coping styles: the behavioral consistency of URB597-treated rats suggests that their behavior was internally rather than externally driven, which is one of the main attributes of active coping (Koolhaas et al. 1999; Koolhaas, 2008).

In the present study, rimonabant abolished the effects of FAAH inhibition in the tail pinch test, while AM-251 –another CB1 blocker– abolished the effects of FAAH inhibition on plus-maze behavior in an earlier study (Haller et al., 2009). This suggests that the effects reported here were mediated by the CB1 receptor. Yet, it was recently shown that some of the behavioral effects of URB597 can be also mediated by oleoylethanolamide activation of PPAR-alpha nuclear receptors (Mazzola et al., 2009). The role of this and similar mechanisms in coping may need further studies.

Theoretical considerations

Recent studies suggested that endocannabinoids exert their effects in tight conjunction with the emotional condition of subjects in tests of all three depression, anxiety, and learning (Abush and Akirav, 2010; Campolongo et al., 2012, 2013; Haller et al., 2009; Naidu et al., 2007; Zanettini et al., 2012). Based on these and similar findings, it was hypothesized that "normative" endocannabinoid functioning maintains emotional homeostasis and ensures appropriate reactions to stressful events (Marco and Viveros 2009; Ruehle et al. 2012; Zanettini et al., 2012). This hypothesis indirectly involves that endocannabinoid signaling – in addition to controlling particular behaviors– has a general effect on emotions that is consequential for behavior in a variety of contexts. In other terms, the behavioral effects of endocannabinoids result from an interaction between environmental aversiveness, the impact of endocannabinoids on emotional homeostasis, and their more specific role in behavioral control.

The present findings support this inference. Albeit it is not easy to properly define the phrase "*appropriate* reaction to stressful events" (Ruehle et al. 2012), problem-oriented behavior (e.g. attempts to remove the clamp in the tail pinch test) may fall into this category – not lastly because proactive approaches was shown in humans to favorably affect resilience under adverse conditions (Koolhaas 2008; Kessler et al. 1985; Temoshok 2000). At the first sight, the incorporation of plus-maze findings into this theoretical framework is more difficult; the findings ostensibly suggest that URB597-treated rats were not able to adjust their behavior to environmental conditions. However, the phrase "homeostasis" covers processes that keep the body's internal environment stable. From this point of view, the behavior of URB597-treated rats can be interpreted in terms of emotional homeostasis, as the compound reduced both "wariness" induced by aversive conditions and "carelessness" resulting from more favorable conditions. As such, FAAH inhibition may have contributed to emotional homeostasis by promoting an active coping style that increased behavioral autonomy and by this, kept emotional responses stable.

There are several earlier observations suggesting that endocannabinoids promote an active coping style. The effects of endocannabinoids in non-familiar social encounters, in the forced swimming test and their effects on cognitive flexibility all point to this possibility; moreover, genetic studies demonstrated a link between FAAH gene polymorphisms and coping in humans (Marco et al. 2011; McLaughlin et al. 2012; Realini et al. 2011; also see Introduction).

Based on these information, we propose that enhanced anandamide signaling by FAAH inhibition promotes an active coping style in the meaning of the criteria outlined by Koolhaas and coworkers in a series of publications (see Koolhaas et al. 1999; Koolhaas 2008 for reviews). This effect is consistent with the role of endocannabinoids in emotional homeostasis that was proposed earlier (Marco and Viveros 2009; Ruehle et al. 2012;

Zanettini et al., 2012). The impact of endocannabinoids on coping may shed new light on the clinical prospects for agents that indirectly increase endocannabinoid signaling, such as FAAH inhibitors. Basic alternative strategies by which individuals respond to environmental challenges (i.e. coping styles) have wide-ranging health implications from immunity to psychopathology (Koolhaas 2008; Kessler et al. 1985; Pucheu et al. 2004; Temoshok 2000; Westerhuis et al. 2011). In fact, promoting active coping styles has been indicated as a therapeutic goal for psychological interventions in various disorders (Westerhuis et al. 2011; Cooke et al. 2007; Tiemensma et al. 2011). As such, enhancement of endocannabinoid signaling may become a therapeutic option not only in emotional disorders characterized by passive coping (e.g. anxiety and depression) but also in physical diseases where active coping is therapeutically desirable. Naturally, more data in diverse tests performed under different conditions are necessary to further clarify the specific situations under which inhibition of endocannabinoid metabolism may have a beneficial effect on coping with stress and adversity.

Acknowledgments

This research was made possible by grants from OTKA (Hungarian Scientific Research Fund) grant No. K101645 to JH and was also supported by the Intramural Research Program of the National Institute on Drug Abuse, National Institutes of Health, Department of Health and Human Services to SRG and LVP.

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Figure 1.

Correlation plots of behaviors consistently shown during the tail-pinch test. Note that a subgroup of rats was treated with vehicle twice either on the first and second or on the second and third experimental day (vehicle treatment 1 and vehicle treatment 2, respectively). Data obtained in the three experiments were shown on the same graphs to spare space. For clarity, linear trend lines were fitted for each experiment. *URB*, URB597; *Rim*, rimonabant; *CDP*, chlordiazepoxide. For statistical details see Results.

Figure 2.

FAAH inhibition promotes active coping in the rat tail-pinch test. **a**, The effects 0.0 (vehicle), 0.1 and 0.3 mg/kg of URB597 on the gnawing at clamp / clamp-independent exploration ratio in rats showing active, mixed and passive coping styles; **b**, the effects of URB597, the CB1 antagonist rimonabant and their combination on coping styles; **c**, the distribution of strategies after vehicle and URB597 3 mg/kg treatment. *Dotted lines*, gnawing/exploration ratios that delimited active, mixed, and passive coping styles; *Veh*, vehicle; *Rim*, rimonabant; ‡, all three coping styles differ significantly ($P < 0.05$ at least); †,

active and passive copers show significant differences; ns, no significant differences between coping styles; *, significant differences in frequency distribution.

Figure 3.

Studies controlling for pain perception and anxiolysis. **a**, the impact of coping styles and URB597 on pain thresholds in the hot plate test; **b**, the effects of the benzodiazepine anxiolytic chlordiazepoxide. *Dotted lines*, gnawing/exploration ratios that delimited active, mixed, and passive coping styles; *Veh*, vehicle; *CDP*, chlordiazepoxide; ‡, all three coping styles differ significantly ($P < 0.05$ at least); †, active and passive copers show significant differences.

Figure 4.

FAAH inhibition by URB597 eliminates the impact of light and habituation on plus-maze behavior in rats. **a–c**, closed arm entries, relative open arm entries (open/ total arm entries*100) and time spent in open arms, respectively, in treatment groups exposed to the elevated plus-maze under different conditions **d–e**, Changes from the low light/habituation (L-h) condition that visualize the dependence of behavior from testing conditions. Grey horizontal bands in **d–e**, s.e.m. range under L-h condition; * and #, significant effect of URB597 and condition, respectively ($P < 0.05$). Statistics was made on raw data; figures **d–e** were composed for clarity.

Table 1

The general design of tail-pinch tests.

Treatment schedule	Trial number		
	1 (day 1 of the study)	2 (day 4 of the study)	3 (day 7 of the study)
<i>A</i>	Vehicle	Vehicle	Study compound
<i>B</i>	Study compound	Vehicle	Vehicle

We employed a repeated measure design balanced over treatment order. Rats were assigned either to treatment schedule "A" or to treatment schedule "B". Thus, each rat was tested 3 times at 3-day intervals.

The repeatability of the test was checked by comparing the behavior of rats on the two vehicle trials (1/2 for schedule "A" and 2/3 for schedule "B").

Behavior averaged for the two vehicle trials served to identify "baseline" coping strategies, while study compound-induced changes in coping styles were identified based on behavior shown on study compound trials (trials 1 or 3, depending on treatment schedule). This general design allowed for both checking the repeatability of the test and establishing *changes* in coping styles (as opposed to *differences* that were detected if vehicle and study compounds were administered to different sets of rats). Further reasons for employing a repeated-measures design were explained in Introduction.

Table 2

The frequency and duration of behaviors shown in the tail-pinch test under vehicle conditions in Experiment 1.

Behavior	Frequency$\pm$SEM (counts/5 min)	Duration$\pm$SEM (% of test time)
Gnawing the clamp	19.28 $\pm$ 1.41	52.22 $\pm$ 3.02
Exploration/locomotion	25.40 $\pm$ 1.41	32.19 $\pm$ 2.63
Resting	12.78 $\pm$ 0.93	11.97 $\pm$ 1.02
Digging	3.38 $\pm$ 0.57	0.82 $\pm$ 0.23
Jumping	1.28 $\pm$ 1.08	0.19 $\pm$ 0.15
Pulling the clamp	3.45 $\pm$ 1.25	0.16 $\pm$ 0.06
Rotating	0.83 $\pm$ 0.18	0.07 $\pm$ 0.02
Grooming	0.03 $\pm$ 0.03	0.01 $\pm$ 0.01

Data were shown as mean $\pm$ standard error of the mean.