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Low dose anandamide affects food intake, cognitive function, neurotransmitter and corticosterone levels in diet-restricted mice

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Abstract

This investigation reports the possible role of the endocannabinoid anandamide on modulating the behavioral and neurochemical consequences of semi-starvation. We studied the effect of very low dose anandamide (0.001 mg/kg) administration on food intake, cognitive function and catecholaminergic and serotonergic pathways in two murine brain areas concerned with appetite (hypothalamus) and learning (hippocampus), and the peripheral corticosterone response to the stress of 40% diet restriction. Anandamide-treated mice consumed 44% more food ($P < 0.05$) during 1 week of 2.5-h feeding each day. In the hypothalamus, there were significantly increased concentrations of norepinephrine ($P < 0.01$), dopamine ($P < 0.05$) and 5-hydroxytryptamine (5-HT) ($P < 0.001$). In the hippocampus, anandamide increased significantly norepinephrine and dopamine, but decreased 5-HT (all at $P < 0.001$). Diet restriction was accompanied in both areas by a significant decrease in all neurotransmitter concentrations that were partially restored by anandamide for dopamine and 5-HT, but not for norepinephrine. In animals on diet restriction, anandamide significantly improved impaired maze performance. Norepinephrine turnover and plasma corticosterone levels were also raised significantly by anandamide. The fact that low dose anandamide improved food intake, cognitive function and reversed some of the neurotransmitter changes caused by diet restriction, might have implications for the treatment of cachexia associated with acquired immunodeficiency syndrome (AIDS) and cancer, for mood changes sometimes associated with dieting, and in the extreme case, of patients with anorexia.

Keywords: Anandamide; Food intake; Diet restriction; Cognitive function; Hippocampus; Hypothalamus; Catecholamine, 5-hydroxytryptamine, serotonin (5-HT); Corticosterone

1. Introduction

Anandamide is an endogenous lipid substance isolated in 1992 from porcine brain and the first natural ligand binding to the endogenous cannabinoid receptor (Devane et al., 1992). It produces some effects similar to Δ^9-tetrahydrocannabinol, both in vivo and in vitro (Devane et al., 1992; Fride and Mechoulam, 1993; Onaivi et al., 1996). Both Δ^9-tetrahydrocannabinol and anandamide bind to the cannabinoid receptor, and Δ^9-tetrahydrocannabinol is used to increase appetite in acquired immunodeficiency syndrome (AIDS) patients (Mechoulam et al., 1998). Anandamide produces bi-phasic dose responses in both behavior and neuro-biochemistry (Fride, 1995; Fride and Mechoulam, 1993; Fride et al., 1995; Sulcova et al., 1998). Thus, at low doses, it stimulated ambulation and rearing, as well as gut motility in the open field situation, decreased the rate of immobility on ring standing, and analgesia on a hot plate; it also stimulated aggressive behavior in timid mice and phagocytosis by mouse leukocytes. At high doses, opposite effects of inhibition were observed (Sulcova et al., 1998).

The stimulating effect of a low dose of anandamide (0.001 mg/kg) on appetite and behavior might have possible therapeutic potential for dealing with mood changes sometimes experienced following dieting, and also in anorexic patients (Berry, 1999). We therefore evaluated its

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effects on food intake; and on cognitive function, brain neurotransmitters and plasma corticosterone concentration in a model of diet restriction in mice (Avraham et al., 1996).

2. Materials and methods

In all experiments, the principles of laboratory animal care were followed and the protocols were authorized by the local animal care facility board.

2.1. Choice of dose of anandamide used in the experiments

In order to find out the effect of anandamide on food intake of BALB/c mice, three different concentrations of anandamide were used: 0.001, 0.7 and 4 mg/kg. Only 0.001 mg/kg caused significant increase in food intake.

Previous studies by other workers have shown that the responses characterizing anandamide activity are extremely variable (Fride, 1995; Fride and Mechoulam, 1993; Sulcova et al., 1998). When injected in mice 20 min after the administration of 10 mg/kg Δ^9-tetrahydrocannabinol, anandamide (at doses of 0.1, 0.01 or 0.001 mg/kg), led to a partial or full reduction of the Δ^9-tetrahydrocannabinol-induced effects on ambulation in the open field test, immobility on ring positioning, analgesia on hot plate and body temperature. 0.001 mg/kg was the most effective dose in reversing Δ^9-tetrahydrocannabinol-induced actions on both hot plate analgesia and body temperature. 0.01 mg/kg was best for inhibiting Δ^9-tetrahydrocannabinol-induced reduction in activity in the open field test. The even lower dose of 0.0001 mg/kg blocked only hot plate analgesia (Fride et al., 1995). These findings supported the use of 0.001 mg/kg in the present study. Anandamide was dissolved in a vehicle which consisted of Emulphor 620, ethanol and saline, in a final formation of 1:1:18 (Fride, 1995; Fride and Mechoulam, 1993; Fride et al., 1995; Sulcova et al., 1998).

2.2. Food intake study

Inbred female BALB/c mice were placed two in a cage and randomly assigned to vehicle or anandamide treatments. Food cakes were used to measure the amount of food consumed. They were made from a mixture of 700 g of purina chow and 1 l 3% agar, frozen and stored at 4°C. The cakes were weighed before and after the feeding periods, and any spillage on the floor was collected. Mice were fed 7 days for 2.5 h per day between 9:00 a.m. and 12:00 p.m. Ten minutes before feeding or maze testing, 0.001, 0.7 and 4 mg/kg anandamide in vehicle or vehicle alone, was injected intraperitoneally (i.p) in a volume of 0.1 ml/10 g mouse body weight (Fride and Mechoulam, 1993; Fride et al., 1995).

2.3. Diet restriction experiment

Mice were placed in cages containing wood-chip bedding in a temperature-controlled room at 22°C on a 12-h light/dark cycle (lights on at 7:00 a.m.). They had access to water ad libitum. The food provided was purina chow and was given at 9:00 a.m. except on the days of maze testing when they were fed afterwards. The control (100%) group received a diet of 95 kcal/week/mouse (3.6 g/day/mouse) as suggested by Ingram et al. (1987) being sufficient for weight maintenance. The 40% diet restriction group received 1.44 g/day/mouse. Diet restriction was continued until either weight plateaued (control group) or reached 15 g or less (Avraham et al., 1996).

2.4. Eight-arm spatial maze

The radial eight-arm spatial maze was a scaled-down version for mice (Yanai and Pick, 1987) of the maze developed for rats by Olton and Samuelson (1976). Mice were randomized into each treatment group and were put in the maze between 9:00 and 12:00 a.m. each day. Observations were recorded until entries were made into all eight arms or until 16 entries were completed. Food was put as a reward at the end of each arm.

2.5. Measurement of catecholamines and 5-hydroxytryptamine, serotonin (5-HT)

Fasting mice were sacrificed by decapitation between 9:00 and 12:00 a.m., 10 min after vehicle or anandamide treatment. Trunk blood was collected. Brain areas from the hippocampus and hypothalamus were immediately dissected out and kept at −70°C. Assays for norepinephrine, methoxyhydroxyphenylglycol (MHPG), dopamine, 3,4 dihydroxyphenylacetic acid (DOPAC), 5-HT and 5-hydroxyindoleacetic acid (HIAA) were performed by HPLC/ECD (high performance liquid chromatography/electrochemical detector) separation and detection using dehydroxybenzylamine (DHBA) as an internal standard (Keller et al., 1976; Yanai and Pick, 1987; Avraham et al., 1996). The detection limit was 50 pg /ml. Protein was determined using a commercial protein assay kit, based on the method of Bradford (Sigma). Turnover was defined as the ratios of the breakdown products MHPG, DOPAC and 5-HIAA divided by their respective pre-cursors — norepinephrine, dopamine and 5-HT.

2.6. Corticosterone assay

Blood was centrifuged at 3000 rpm at 4°C for 10 min. Plasma was separated and samples were stored at −70°C until assayed for corticosterone. This was performed using a dextran-coated charcoal radioimmunoassay for rabbit

anti-corticosterone 21 thyroglobulin serum (Bio-Yeda, Rehovot) and the tracer was [1,2,6,7-^{3}H] corticosterone (NEN, Boston). Corticosterone was extracted with ethyl acetate. Incubation with antibody at room temperature (0.5 h) was followed by incubation with tracer at 37°C (1 h). A charcoal step achieved separation of bound and free fractions. The sensitivity limit of the assay was 0.5 μg/100 ml, and the intra- and inter-assay coefficients of variation were 6.3% and 7%, respectively (Weidenfeld et al., 1994a).

2.7. Data analysis

Homogeneity of variances was assessed by Bartlett's test. Post-hoc testing was only performed if the overall P value was less than 0.05. Differences between two groups were analysed using the Student's t-test. Analysis of variance (ANOVA), Tukey–Kramer multiple comparisons were used to evaluate the differences among three groups or above. Two-way ANOVA with repeated measures was used to analyze the results of maze performance over time.

3. Results

3.1. Food intake (Fig. 1A)

During the daily feeding time period 0.001 mg/kg anandamide-treated mice consumed significantly more food over a week than did the vehicle treated mice (6.4 g vs. 4.6 g ($P < 0.05$)) (data not shown) while 0.7 and 4 mg/kg anandamide treated mice did not show any significant change (Fig. 1).

3.2. Maze performance (Fig. 1B)

Maze performance was evaluated by the number of entries used to complete the eight arms of the maze, such that the less the number of entries the better the performance. Bars in the figure represent the S.E.M of daily trials. Two-way ANOVA with repeated measures showed that both diet restriction ($F(1,55) = 12.26,\ P = 0.001$) and anandamide ($F(1,55) = 4.72,\ P = 0.034$) exhibit significant effects on maze performance. Anandamide had no effect on the maze performance of the control animals. Diet restriction animals showed impaired learning ability over 5 days, while anandamide treatment restored it to the level of the controls.

3.3. Effect of diet restriction on catecholamines and 5-HT

40% diet restriction decreased the levels of norepinephrine and dopamine in both hippocampus (white columns, Fig. 2A and C) and hypothalamus (Fig. 3A and C). The effects of diet restriction on turnover in these two brain areas were different. It decreased it in the hippocampus (Fig. 2B and D) but increased it in the hypothalamus (Fig. 3B and D). 40% diet restriction decreased the concentration of 5-HT (Fig. 4A and C) and increased its turnover in both brain areas (Fig. 4B and D).

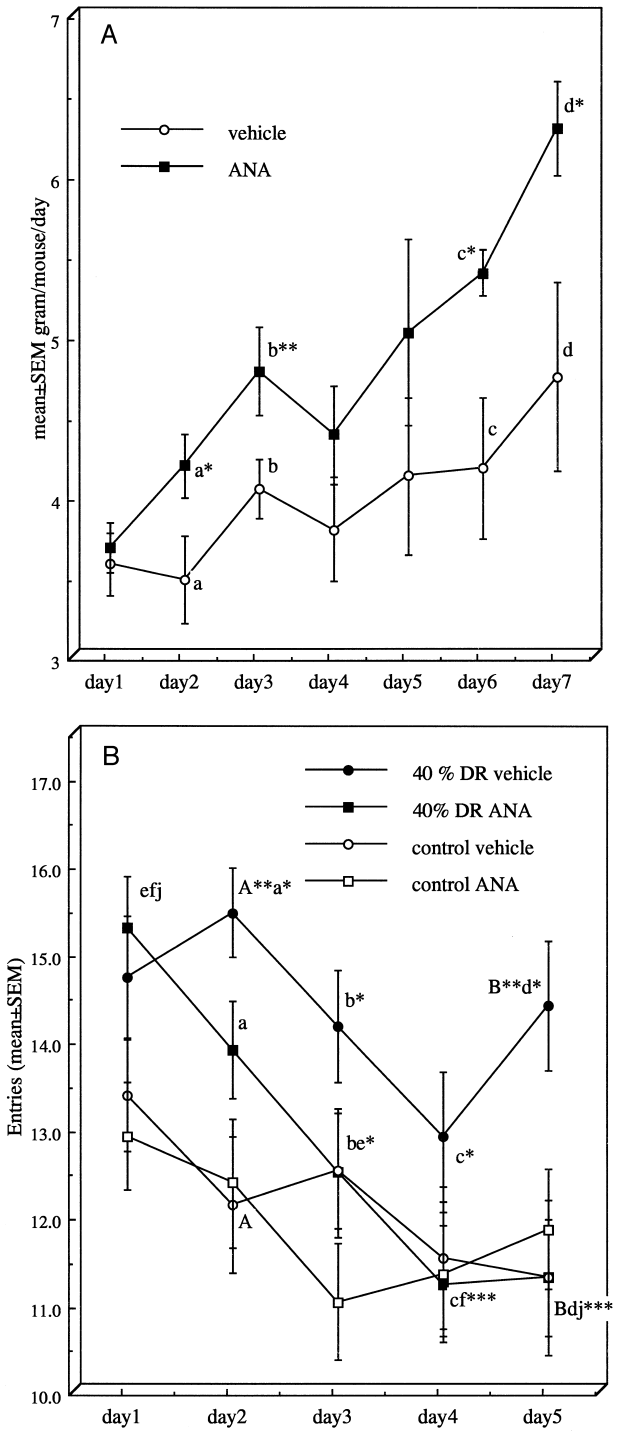

Fig. 1. (A) Effect of anandamide on food intake. $^*P < 0.05$, $^{**}P < 0.01$. Letters represent pairs which are statistically different; 16 mice in each group. (B) Effects of diet restriction and anandamide on maze performance. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Letters represent pairs that are statistically different; 14–15 mice in each group.

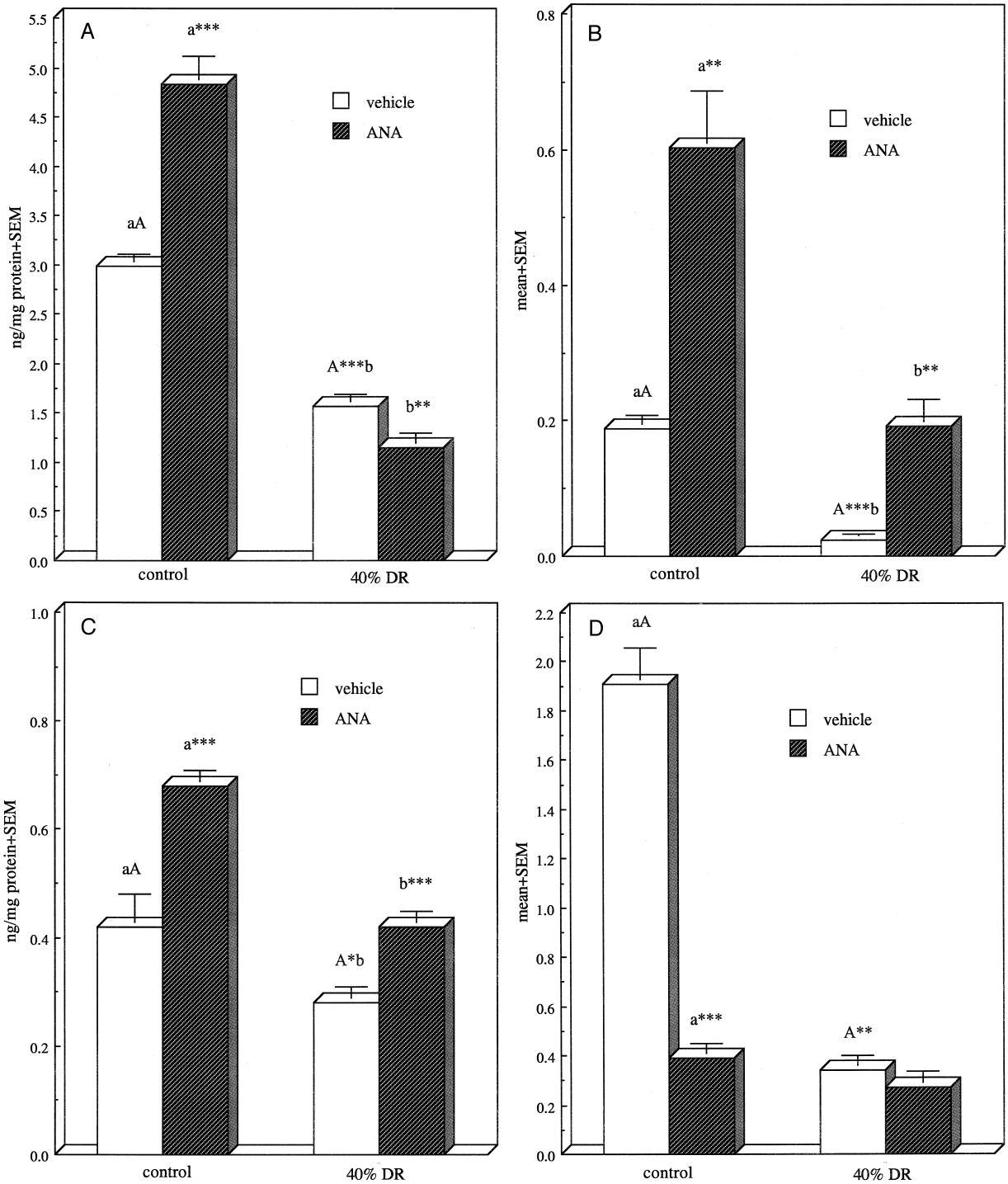

Fig. 2. (A) The level of norepinephrine in the hippocampus. $^{**}P < 0.01$, $^{***}P < 0.001$. Letters represent pairs that are statistically different; 8–10 mice in each group. (B) The level of norepinephrine turnover in the hippocampus. $^{**}P < 0.01$, $^{***}P < 0.001$. Letters represent pairs that are statistically different; 6–8 mice in each group. (C) The level of dopamine in the hippocampus. $^{**}P < 0.05$, $^{***}P < 0.001$. Letters represent pairs that are statistically different; 8–10 mice in each group. (D) The level of dopamine turnover in the hippocampus. $^{**}P < 0.01$, $^{***}P < 0.001$. Letters represent pairs that are statistically different; 6–8 mice in each group.

3.4. Effect of anandamide on catecholamines

In the control (100%) group, anandamide administration increased significantly both norepinephrine and dopamine in both brain areas (hatched columns, Figs. 2A,C, 3A,C). The effect on turnover was different in that it increased that of norepinephrine but decreased that of dopamine in the hippocampus (Fig. 2B and D), while no effect was observed in the hypothalamus (Fig. 3B and D). Anandamide treatment in the hippocampus reversed the effect

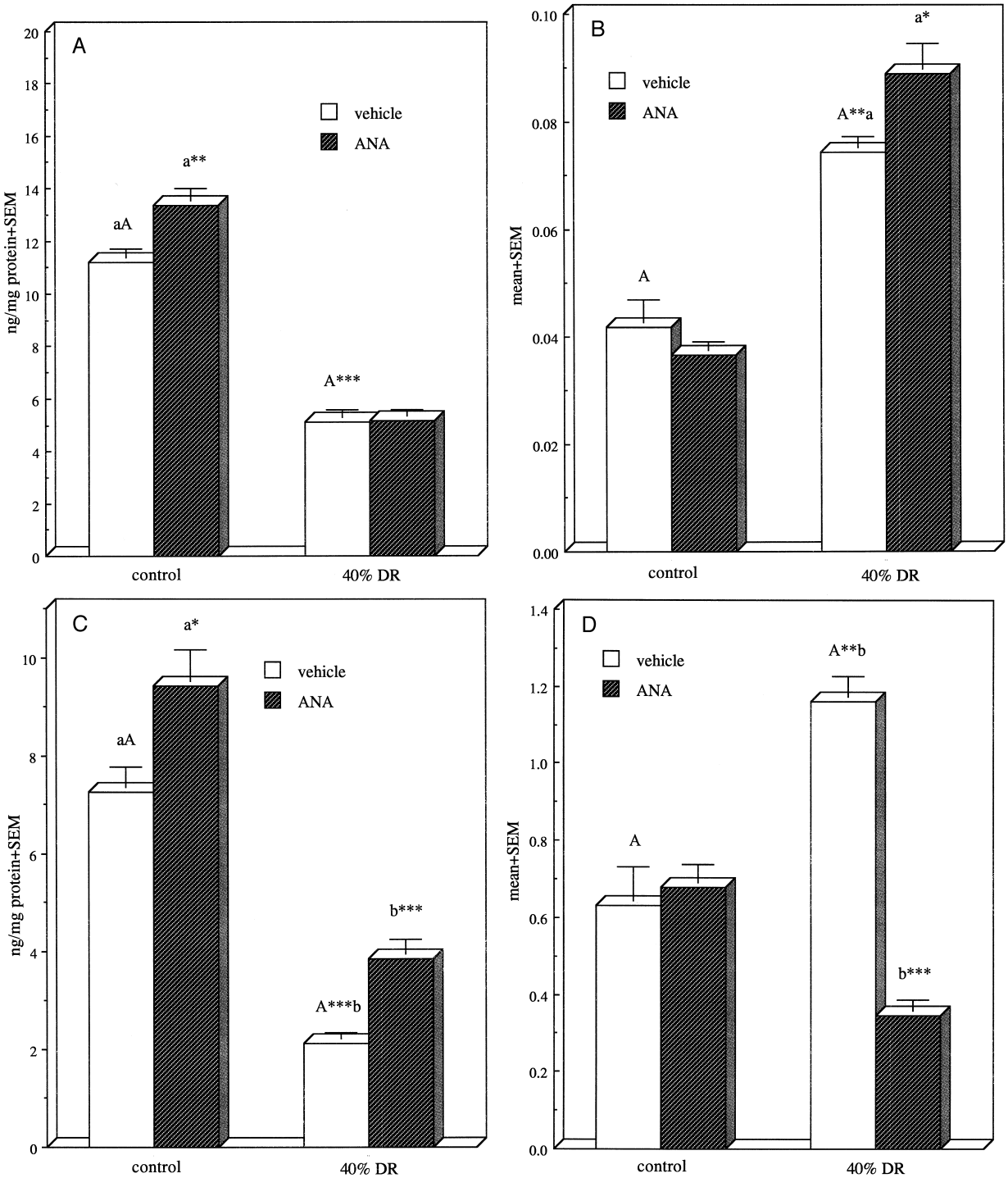

Fig. 3. (A) The level of norepinephrine in the hypothalamus. $^{**}P < 0.01$, $^{***}P < 0.001$. Letters represent pairs that are statistically different; 8–10 mice in each group. (B) The level of norepinephrine turnover in the hypothalamus, $^{*}P < 0.05$, $^{**}P < 0.01$. Letters represent pairs that are statistically different; 9–12 mice in each group. (C) The level of dopamine in the hypothalamus. $^{*}P < 0.05$, $^{***}P < 0.001$. Letters represent pairs that are statistically different; 9–12 mice in each group. (D) The level of dopamine turnover in the hypothalamus. $^{**}P < 0.01$, $^{***}P < 0.001$. Letters represent pairs that are statistically different; 8–10 mice in each group.

of diet restriction on dopamine concentrations and norepinephrine turnover to the control values (Fig. 2C and B), and also partially reversed the effect of diet restriction on dopamine and its turnover in the hypothalamus (Fig. 3C and D).

3.5. Effect of anandamide on 5-HT and intermediates

In the control (100%) group, anandamide decreased significantly the level of 5-HT in the hippocampus (Fig. 4A) yet increased it significantly in the hypothalamus

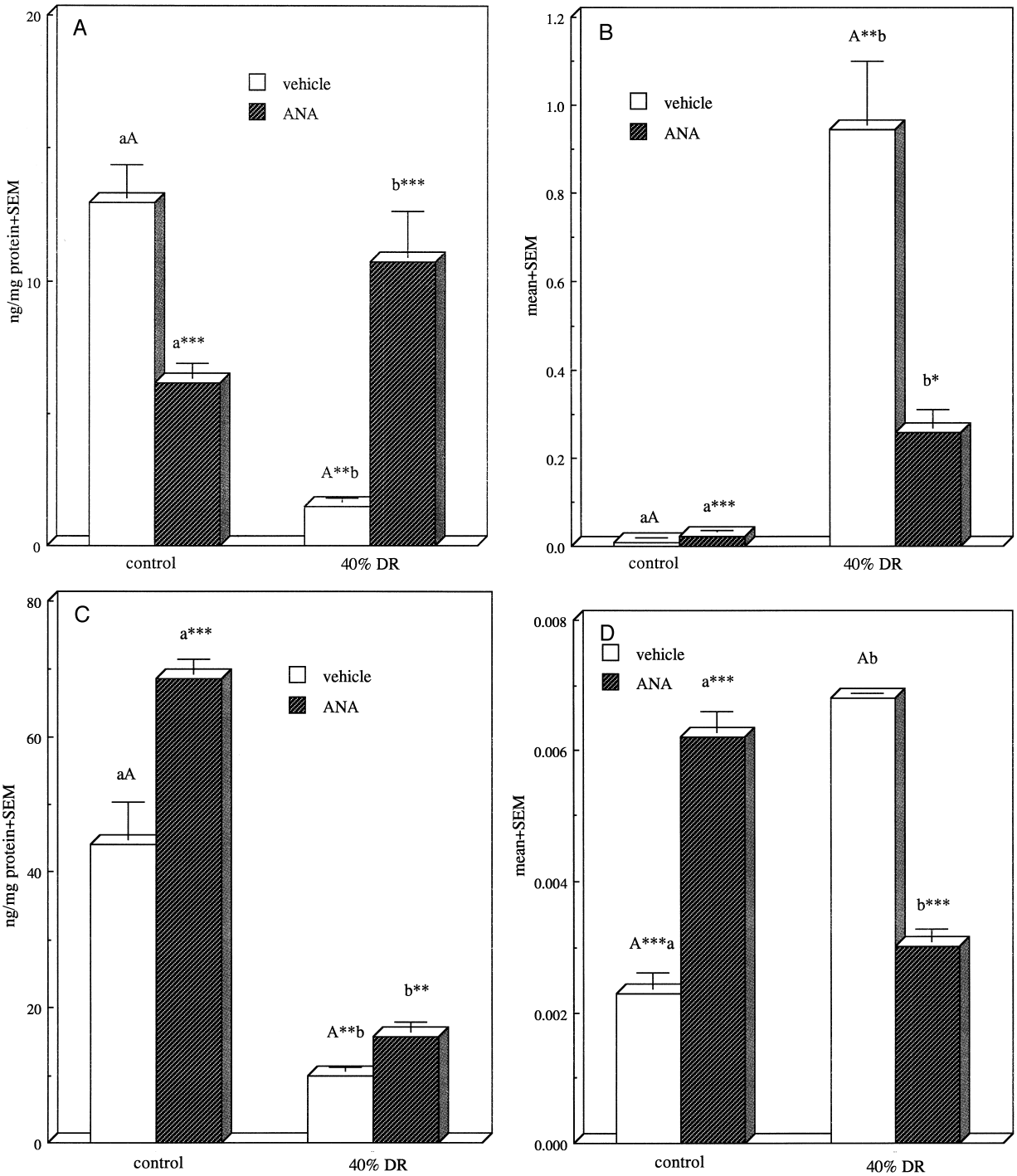

Fig. 4. (A) The level of 5-HT in the hippocampus. $^{**}P < 0.01$, $^{***}P < 0.001$. Letters represent pairs that are statistically different; 5–9 mice in each group. (B) The level of 5-HT turnover in the hippocampus. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Letters represent pairs that are statistically different; 5–9 mice in each group. (C) The level of 5-HT in the hypothalamus. $^{**}P < 0.01$, $^{***}P < 0.001$. Letters represent pairs that are statistically different; 5–12 mice in each group. (D) The level of 5-HT turnover in the hypothalamus. $^{***}P < 0.001$. Letters represent pairs that are statistically different; 5–8 mice in each group.

(Fig. 4C). It increased significantly its turnover in both areas (Fig. 4B and D). Anandamide administration to the 40% diet restriction group reversed or partially reversed both 5-HT and its turnover to control levels in both the hippocampus (Fig. 4A and B) and hypothalamus (Fig. 4C and D).

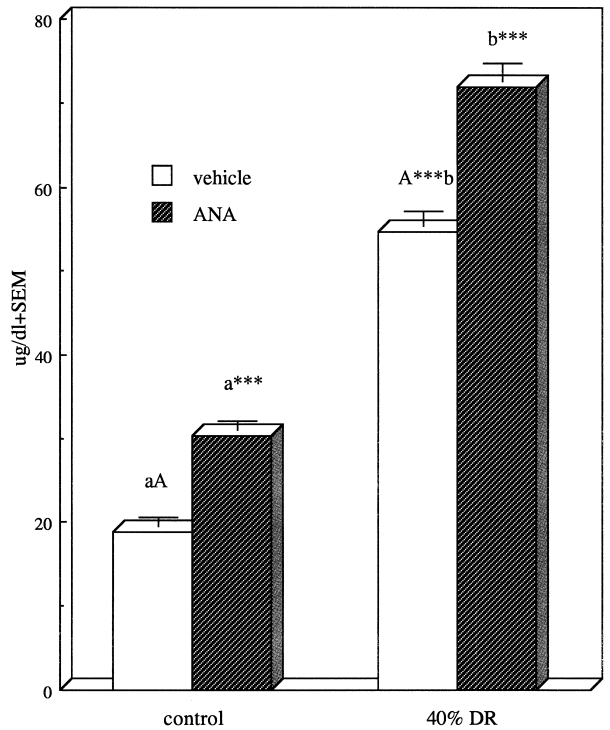

Fig. 5. Effect of diet restriction and anandamide on plasma corticosterone. *** $P < 0.001$. Letters represent pairs that are statistically different; 18–20 mice in each group.

3.6. Corticosterone

40% diet restriction was associated with significantly increased corticosterone concentrations. Anandamide additionally increased significantly corticosterone in both control (100%) and diet restriction mice (Fig. 5).

4. Discussion

The principal findings of this study were that a very low dose of anandamide significantly increased food intake, improved cognitive function and reversed most of the effects of severe food restriction on catecholamine and 5-HT concentrations in the hypothalamus and hippocampus, as well as increasing the concentrations of corticosterone. This pluripotency of action requires further investigation and confirmation, but could have obvious therapeutic potential.

4.1. Food intake

The study of the effect of anandamide on food intake of BALB/c mice started with the use of different doses of anandamide: 0.001, 0.7, and 4 mg/kg. 0.001 mg/kg anandamide significantly increased food intake while 0.7 and 4 mg/kg did not cause any significant change.

Williams and Kirkham (1999) have recently reported that anandamide (0.5, 1, 5, 10 mg/kg) increased food intake during a 3-h feeding period and this was blocked by the cannabinoid receptor antagonist, SR 141716. Williams and Kirkham (1999) did not use concentrations as low as 0.001 mg/ kg, and their experimental protocol was completely different to ours. They used male rats while we used female mice under conditions of diet restriction; the food was supplied 2 h before injection and 3 h afterwards, while in our case, food was given 10 min after injection; they injected the anandamide subcutaneously while we injected it i. p.; they measured the food during 3 h at hourly intervals, while we measured it after 2.5 h; they measured it over 1 day while we measured it over a week. The effects of anandamide (0.7 mg/kg) in our experiment are comparable to those of anandamide in Kirkham's work (0.5 mg/kg). Neither dose caused any significant change in food consumption during the first 2 h. However, Kirkham observed a significant increase after 3 h. A dose of 4 mg/kg in our experiment did not show any significant change while 5 mg/kg in Kirkham's produced a significant increase only after 1 h and not later. Because of the completely different paradigms, we cannot compare these experiments.

The effect of anandamide on appetite may be variable (even absent) depending on dose and experimental circumstances (Crawley et al., 1993; Williams and Kirkham, 1999). Doses from 0.03 to 30 mg/kg anandamide had no significant effect on chow consumption (Crawley et al., 1993).

As was mentioned in the Introduction, anandamide produces a bi-phasic dose response in both behavior and neuro-biochemistry (Fride, 1995; Fride and Mechoulam, 1993; Fride et al., 1995; Sulcova et al., 1998). Thus, at low doses, it stimulated ambulation and rearing, as well as gut motility in the open field situation, decreased the rate of immobility on ring standing, and analgesia on a hot plate; it also stimulated aggressive behavior in timid mice and phagocytosis by mouse leukocytes. At high doses, opposite effects of inhibition were observed (Sulcova et al., 1998).

In an early review on the effects of Cannabis, Paton and Pertwee (1973) noted the following: ''Nor does one readily find another substance so contradictory, capable of taming yet producing aggressiveness of both enhancing and depressing spontaneous activity, of being anticonvulsant yet generating epileptiform cortical discharges''. This review points out that cannabis has always been regarded as a material that has biphasic effects. Another early example of such an effect is from the work of McLaughlin et al. (1979). They showed that Δ^9-tetrahydrocannabinol when administered to sheep, initially increased food intake but after 24 h Δ^9-tetrahydrocannabinol decreased this behaviour.

In view of such biphasic effects comparison of experiments is well nigh impossible except in those cases where such experiments are done under similar conditions.

Because of the completely different paradigms, both experiments are not comparable. Anandamide binds to

both cannabinoid CB_1 (central) and CB_2 (peripheral) receptors. SR 141716 decreases appetite and body weight in obese and non-obese rodents by antagonism of a putative endogenous cannabinoid tone that normally stimulates feeding (Arnone et al., 1997; Chaperon et al., 1998; Colombo et al., 1998). These results suggest a possible neuromodulatory role of brain cannabinoid receptors in the control of energy balance, possibly through neuropeptide Y (Arnone et al., 1997; Di Marzo et al., 1998). Cannabinoids stimulate appetite and increase body weight in human subjects and patients with AIDS (Mechoulam et al., 1998). Alternative mechanisms might involve the balance between norepinephrine (stimulatory), dopamine and 5-HT (inhibitory) neurotransmitters involved in feeding behaviour (Leibowitz and Brown, 1980; Toornvliet et al., 1996), and whose changes are described in Section 4.3. However, the effect of anandamide on appetite may be variable (even absent) depending on dose and experimental circumstances (Crawley et al., 1993; Williams and Kirkham, 1999).

4.2. Cognitive function

Anandamide administration reversed impaired performance of 40% diet restriction mice to that of the controls. Several pathways affect learning and memory capabilities and may be affected by nutritional status (Wurtman et al., 1981; Schweiger et al., 1985b; Philipp and Pirke, 1987; Collier et al., 1988; Haleem and Haider, 1996). The interactions between them in the hippocampus are complex (White and Viaud, 1991; Imperato et al., 1993; Hersi et al., 1995; Myslivecek, 1997; review McEntee and Crook, 1991; Cassel and Jeltsch, 1995). Since low dose anandamide administration to the 40% diet restriction group normalized dopamine and 5-HT but not norepinephrine turnover, the latter may be less associated with improved cognitive function. Depletion of 5-HT during synaptogenesis impaired adult rat performance in tests including the eight-arm maze (Mazer et al., 1997), while in adult rats, such depletion impaired learning in the Morris water maze (Richter-Levin and Segal, 1991). Conversely, 5-HT uptake antagonists improve task performance (review McEntee and Crook, 1991).

Cannabinoids may also influence memory either directly (Lichtman et al., 1995) or as a neuromodulator (Kimura et al., 1998), and the hippocampus is a brain area with very high concentrations of cannabinoid receptors (Di Marzo et al., 1998). However, under some circumstances, cannabinoid CB_1-receptor agonists may *inhibit* neurotransmitter release and thus may impair memory (Di Marzo et al., 1998). Anandamide (10 and 30 mg/kg) has no or negative effects on learning of rats in the eight-arm maze (Lichtman et al., 1995). The facilitatory effects of anandamide in our experiments may be related to the low dosage used and could be explained by the bi-phasic effect of anandamide action (Di Marzo et al., 1998; Sulcova et al., 1998).

4.3. Neurotransmitters and their metabolites

Diet restriction was accompanied in every case by a decrease in neurotransmitter concentrations that were partially restored by anandamide for dopamine and 5-HT, but not for norepinephrine. The neurochemical results reveal at least six patterns of relationships between neurotransmitter and its turn-over in response to diet restriction and anandamide treatment.

(a) Both neurotransmitter and turnover increase (two examples).
(b) Neurotransmitter level increase and turnover decrease (four examples).
(c) Increase in synthesis of neurotransmitter with no change in turnover (three examples).
(d) Decrease in both neurotransmitter and its turnover (two examples).
(e) Decrease in neurotransmitter in response to an increase in turnover (six examples).
(f) No change in neurotransmitter, despite an increase in turnover (one example).

The point is that interpretation of such results may be as complicated as the interactions of the different neurotransmitters within, and between, the different brain areas. Mechanistic explanations depend on the balance between synthesis and utilization/degradation of the specific neurotransmitter. These issues await clarification from studies using biochemical, pharmaceutical, histochemical and imaging techniques. However, despite these reservations, there are certain predominant patterns of response which appear to be consistent for either anandamide treatment (a,b,c) or diet restriction (d,e). Some biochemical support for these types of responses may be found in the literature for catecholamines (Pirke and Spyra, 1982; Schweiger et al., 1985a,b; Avraham et al., 1996) and for 5-HT (Knott and Curzon, 1974; Kantak et al., 1978; Broocks et al., 1991; Haleem and Haider, 1996). One could arrange similarly the response patterns (a–f) by individual neurotransmitter, but, in order to avoid over-interpretation, this has not been attempted here. Yet such analyses may provide directions for future research on the effects of interactions between energy balance, anandamide and neurotransmitter concentrations and function.

Although the manner by which anandamide may affect the concentrations of the neurotransmitters is not known, the treatment did appear to reverse many of the changes of diet restriction. Published papers provide some support. For example, Romero et al. (1995a,b) have shown that 3 and 10 mg/kg anandamide over a 60-min time-course, caused a bi-phasic response (decrease, then increase) in the activity of tyrosine hydroxylase and in the concentrations of dopamine and DOPAC in the striatum. They explained this phenomenon as a compensatory effect following initial inhibition. A recent in vitro study showed that anandamide

inhibited [^{3}H] 5-HT and [^{3}H] ketanserin binding in bovine cortices (Kimura et al., 1998), but is difficult to compare these findings with our in vivo study in different brain areas.

4.4. Corticosterone

Diet restriction is a stress that affects the secretion of corticosterone (Doerr et al., 1980; Pirke and Spyra, 1982). Corticosterone has a modulating effect on brain neurotransmitter concentrations. Thus, on one hand it may decrease tyrosine (and probably norepinephrine) availability through enhanced brain tyrosine aminotransferase activity (Badawy et al., 1982), while on the other, it may induce tryptophan hydroxylase activity, the rate-limiting enzyme for 5-HT synthesis (Neckers and Sez, 1975). Such steroid-sensitive pathways are another means by which anandamide may affect neurotransmitter function. This leads to a further unanswered question as to how anandamide increases plasma steroid levels, whether by central or peripheral routes (Ganong, 1980; Weidenfeld et al., 1994b). Measurements of adrenocorticotrophic hormone (ACTH) and corticotrophin releasing factor (CRF) will help clarify this point.

4.5. Possible therapeutic implications

Patients with anorexia nervosa refuse to eat and show decreased concentrations of tyrosine, norepinephrine metabolites in the cerebrospinal fluid (Gross et al., 1979; Riederer et al., 1982; Kaye et al., 1984; Philipp and Pirke, 1987) and a variety of endocrine abnormalities (Rock and Curran-Celentaro, 1994). Some patients have memory and learning deficits (Gelder and Gath, 1996). Two brain areas are prominent in these processes — the hippocampus, which is involved in aspects of cognitive function (Low et al., 1982; Avraham et al., 1996) and the hypothalamus, the center for the regulation of systemic energy balance (Leibowitz and Brown, 1980). In this study, anandamide administration was able to reverse many of the neurochemical and behavioral deficits following semi-starvation, even before weight gain. Such properties could have therapeutic potential in the treatment of cachexia associated with cancer and AIDS, the side effects of weight loss and in the maintenance of a reduced body weight (Berry, 1999) and also in the extreme case of AN. However, much further work is required in studying the effects of chronic ANA treatment on these functions in animals at different levels of systemic energy balance.

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