Interrelationship between CB1R and ObR pathways under ABA challenge

Interrelationship of CB1R and ObR pathways in regulation of metabolic, neuroendocrine and behavioral responses to food restriction and voluntary wheel running.

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Abstract

We hypothesized the CB1 receptor (CB1R) and leptin receptor (ObR) operate synergistically to modulate metabolic, neuro-endocrine and behavioral responses of animals exposed to a survival challenge (food restriction and wheel running). Obese-prone (OP) JCR:LA-cp rats, lacking functional leptin receptors (ObR), and Lean-prone (LP) JCR:LA-cp rats (intact ObR) were assigned to OP-C and LP-C (Control) or CB1R antagonized (SR141716, 10mg/kgBW in food) OP-A and LP-A groups. After 32 days, all rats were exposed to 1.5-h daily meals without the drug and 22.5-h voluntary wheel running, a survival challenge that normally culminates in activity-based anorexia (ABA). Rats were removed from the ABA protocol when body weight reached 75% of entry weight (starvation criterion) or after 14 days (survival criterion). LP-A rats starved faster (6.44 ± 0.24 days) than LP-C animals (8.00 ± 0.29 days); all OP rats survived the ABA challenge. LP-A rats lost weight faster than animals in all other groups (p < 0.001). Consistent with the starvation results, LP-A rats increased the rate of wheel running more rapidly than LP-C rats (p = 0.001) with no difference in hypothalamic serotonin levels. In contrast, OP-A rats showed suppression of wheel running compared to the OP-C group, (days 6-14 of ABA challenge, p < 0.001) and decreased hypothalamic serotonin levels (p < 0.01). Thus, there is an interrelationship between CB1R and ObR pathways in regulation of energy balance and physical activity. Effective clinical measures to prevent and treat a variety of disorders will require understanding of the mechanisms underlying these effects.

Keywords: SR141716, CB1R, JCR:LA-cp rats, ObR, wheel running, food restriction, activity-based anorexia.
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Introduction

The endocannabinoid system (ECS) plays an important role in the regulation of whole-body energy balance in both humans and rodents (29, 34, 38). The actions of endocannabinoids in appetite regulation and energy homeostasis are mediated primarily by G-protein coupled cannabinoid-1 receptors (CB1R), expressed densely in the central nervous system (2), including brain areas implicated in appetite regulation (hypothalamus) and movement (basal ganglia) (13, 22). Chronic stimulation of the CB1R promotes food intake leading to obesity and related metabolic abnormalities (46). Genetic animal models of obesity based on mutations affecting leptin signaling suggest that leptin exerts a negative feedback regulation on the ECS. Mutations, either at the peptide (ob/ob mouse) or receptor levels (db/db mouse, fa/fa and cp/cp rats), lead to long lasting over activation of ECS (12).

In humans, as well as in rodents, inhibition of CB1 signaling reduces body weight and food intake under free feeding conditions (7, 43, 48, 54, 55), indicating that the ECS participates in the regulation of energy balance when food is abundant. On a neural level, blockage of CB1R reduces extracellular dopamine release in the reward areas of the brain of rodents when food is ingested (36). Indeed, the CB1R inhibition may reduce motivation for food intake through this mechanism. In addition, it was recently reported that the suppressive effect on food intake and weight gain of CB1R inhibition is greater in ob/ob mice, lacking production of effective leptin, than in wild type mice with intact leptin-ObR interaction (17), suggesting a synergistic relationship between CB1R and ObR pathways. It is plausible that CB1R signaling could function to reinstate homeostasis when food is depleted and food-related behavior is required.
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(58). However, the role of the CB1R pathway in the physiological adaptation to an environmental challenge of food depletion and/or food-related travel is unknown.

A major problem for living organisms is to maintain energy balance when faced with environmental challenges, such as food depletion or famine (34). Such challenges activate a complex interplay of stress, metabolic and reproductive processes (1, 50). These processes, however, have limitations—a fact demonstrated when rodents are placed on time-limited daily meals (1.5 h) and provided with unlimited access to running wheels (22.5 h). Under these conditions, animals reduce food intake, lose body weight and escalate wheel running. The excessive physical activity further suppresses food consumption, body weight plummets, and animals die of this vicious feedback cycle (activity-based anorexia, ABA) (18, 43). Using the ABA model, we recently demonstrated (11) that obese-prone JCR-LA-cp rats survived longer than lean-prone rats with intact ObR; indicating that ObR deletion makes a critical difference to excessive food-related travel and survival during periods of food scarcity.

One way for animals to achieve energy balance in times of food depletion is to increase physical activity or food-related travel that allows for contacting a food supply and restoration of food consumption (18). Excessive physical activity during food restriction is rewarding (42), and may even be addictive, by causing release of endogenous opioid peptides in the brain (23, 26). The hypophagic effect of the CB1R antagonism also involves the modulation of feeding-related reward processes (28, 52). Furthermore, pharmacological studies implicate dopamine and serotonin in brain-reward pathways related to the reinforcing properties of running (8, 24). Importantly, there is evidence that the ECS interacts with dopamine-serotonin pathways within the brain (8, 24). When food is freely available, both CB1R antagonism and CB1 deletion in rodents with intact ObR consistently reduce reward-driven behaviors such as wheel running and
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Increase hippocampal neurogenesis (14, 33); the role of ECS for survival when food is depleted as not yet been investigated and is a primary focus of current study.

Leptin and the ECS also are implicated in animals’ response to the stress of ABA (6, 16, 44). The stress response involves activation of the hypothalamic-pituitary-adrenal (HPA) axis and corticosterone hypersecretion (4, 11). Corticosterone stimulates dopamine and serotonin synthesis in the brain (5, 57). Zucker (fa/fa) obese-prone rats with a partial ObR defect display hypersensitivity to stress (16) and hyperactivity of the ECS (12), indicating the involvement of ObR signaling in both ECS and HPA-axis actions on energy balance regulation. Currently, there is limited research on the separate and combined effects of both leptin and cannabinoid pathways for the survival of animals exposed to the ABA challenge. Previous research has shown that genetic differences based on ObR genes control running wheel, energy homeostasis (11, 32) and sensitivity to reward (21) in ABA. In the current study, we asked whether lean-prone (ObR-present) and obese-prone (ObR-absent) JCR:LA-cp rats exposed to the ABA challenge would show different metabolic, neuro-endocrine and behavioral responses when the CB1R is functional (CB1R-Control) or dysfunctional (CB1R-antagonized by SR141716).

MATERIALS AND METHODS

Apparatus and materials for ABA challenge. Twelve Wahmann running wheels (1.1m circumference) with metal side cages (25 cm x 15.5 cm x 12.5 cm) were used. A computer recorded the number of wheel turns in 1 min intervals as previously described (37). Feeding was conducted either in home cages or in feeding cages with the same materials and dimensions as the home cages but without bedding. Animals were fed standard laboratory chow (LabDiet™ 5010 Rodent Diet, PMI Nutrition International Inc., Brentwood, MO, USA).
An electronic scale (Sartorius™, Model TE4101, Sartorius AG, Goettingen, Germany) was used to measure food and body weight to the nearest gram. Rats were given a fixed amount of food (30 g) at the beginning of the feeding period and the amount of chow remaining after the meal was subtracted from this fixed amount to provide a measure of food intake. Subsequently, food intake was converted to calories (food (g) x 3.42 kcal/g), yielding a measure of daily caloric intake.

**Animals.** Male JCR:LA-cp rats that incorporate the autosomal recessive cp gene, a nonsense Tyr763Stop mutation in the Ob-R gene (56) were used. Thus, rats that are homozygous for the cp trait (cp/cp) are obese-prone (OP) and those that are heterozygous for the cp trait (+/cp) or wild type (+/+ ) are lean-prone (LP). As the wild type (+/+) and the heterozygous (cp/+) are both lean-prone and physically indistinguishable they are represented as (+/?). The "+/?" means a 2:1 mix of cp/+ (heterozygote) and +/+ (homozygous wild type). These animals were obtained from the established breeding colony at the University of Alberta (48) and were housed individually in clear polycarbonate cages (47cm X 27cm X 20cm) with sterile wood chip bedding in a temperature (22 ± 2 °C) and humidity controlled environment with free access to chow (LabDiet™ 5010, PMI Nutrition International Inc., Brentwood, MO, USA) and water. Rats were maintained on a 12 h light/dark cycle (lights off 7:00–19:00 h) with food and body weight measured daily. Throughout the experiment, animals had free access to water and were fed as outlined in the feeding protocol for each experimental group. The care and use of animals were in accordance with Guidelines the Canadian Council of Animal Care and subject to prior review and approval by the Animal Care and Use Committee: Health Sciences of the University of Alberta.

**Experimental procedure.** The design included four experimental groups (n = 9 per
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Group) based on genotype (cp/cp vs. +/-?) as well as on the functionality of CB1R. At 28-days of age, rats were matched for body weights within genotypes (OP or LP) and randomly assigned to CB1R-antagonized (OP-A and LP-A) and CB1R-Control (OP-C and LP-C) groups as outlined below. The OP-A and LP-A groups were maintained on the CB1R antagonist SR141716 while controls did not receive the drug; all rats were transferred to the experimental laboratory at 50 days of age.

The CB1R was antagonized using SR141617 (Rimonabant®; Sanofi-Synthelabo Recherche, Ruel Malmaison, France) incorporated in the animals’ food from 28 to 60 days of age. Rimonabant, a drug without any known effects on food palatability (47; 3), was blended in powdered rat chow at concentrations based on body weight and daily food consumption of the rats, to maintain a dose of 10 mg·kg$^{-1}$.day$^{-1}$, as recommended by Sanofi-Synthelabo Recherche. The food was moistened, extruded as pellets through a die, and air-dried as previously described (48). Reagents and chemicals were obtained from Sigma Chemical (Oakville, ON, Canada). After 29 days of drug treatment (56 days old), rats were fasted overnight and blood was taken by tail bleed to provide baseline biochemical parameters. Subsequently, animals were given three days to recover, with rats in treatment groups continued on the drug. Following the recovery period, rats (after 32 days of chronic treatment with rimonabant) were weighed to establish entry weight for ABA and transferred daily to feeding cages (home cages without bedding) at 7:00 h with 1.5 h free access to regular chow (LabDiet™ 5010, without drug) followed by 22.5 h of access to running wheels (ABA protocol). Rats were removed from the protocol when body weight reached 75% of entry weight (starvation criterion) or after 14 days of the ABA challenge (survival criterion). During the ABA challenge, body weight, food intake and wheel turns were measured daily. Also
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during the ABA protocol the drug was no longer added to the food, allowing for the
assessment of prior CB1R antagonism on survival of the ABA challenge. Previous research
has shown slow pharmacokinetic properties and long half-life of rimonabant (25, 30, 53).
Based on this evidence, the antagonistic effect of rimonabant on the CB1R should not
dissipate during the 14 days of ABA protocol, especially after a prolonged 32-day period of
treatment.

Post-mortem analyses. When animals met the criterion for either starvation or survival
of the ABA challenge, they were removed from their home cages, and anaesthetized with
isoflorane. Blood was taken by cardiac puncture. The blood was collected in 10 ml
polyethylene tubes containing EDTA, and stored on ice until centrifugation. Plasma samples
were stored at -80°C until analysis. The rats were immediately perfused intracardially with
ice-cold isotonic saline: the brain was removed and immediately flash frozen using liquid
nitrogen and then stored at -80°C until being processed. The brains were halved and the
biogenic amines and their metabolites were assessed for either the left or right hemispheres on
a random basis; the assessment included the hypothalamus and the primary neural-reward
areas including the nucleus accumbens and the stratum as previously described by Parent et
al.(39).

Plasma biochemical analysis. Total plasma triglyceride concentrations were measured
using direct colorimetric enzymatic reactions (Wako Chemicals USA, Inc., Richmond, VA).
Plasma glucose, insulin, leptin, GLP1, GLP2 and PYY were measured using specific Enzyme-
Linked Immunosorbtent (ELISA, Wako) kits for rats. Plasma corticosterone was measured
using Milliplex Map (Millipore Corporation, Billerica, MA). Biogenic amines (dopamine,
noradrenaline, 5-hydroxy tryptophane) and their metabolites (homovanillic acid or HVA, 3,4-
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dihydroxyphenylacetic acid or DOPAC and 5-hydroxyindole-3-acetic acid or 5-HIAA) in the brain hemisphere were measured using HPLC–ECD. For the separation and analysis the biogenic amines and their metabolites, the procedure of Parent et al. (39) was used. To aliquots of tissue in 5 volumes of water, 1/10th the volume of ice-cold 1 N HClO₄ containing ascorbic acid (500 μM) and EDTA (100 mg%) was added. These homogenates were vortexed, centrifuged at 10,000g for 4 min and the supernatant was removed for analysis by HPLC. The applied potential for electrochemical detection was 0.65 V. Calibration curves were constructed for each HPLC run.

Statistical analyses. Data are presented as mean ± S.E.M. Food intake, body weight, wheel turns, and days lasted in the ABA challenge were analysed by mixed-effects ANOVA with genotype (OP vs. LP) and CB1R pathway (control vs. antagonized) as between-subjects, factors and days, with either CB1R antagonism period or ABA challenge, as the within-subjects term. We used one-way ANOVA to further elucidate the within-subject and mixed effects. The interaction of genotype and treatment was explored by one-way ANOVA on the four experimental groups followed by post hoc comparisons of the means using a Tukey procedure. One-way ANOVAs were conducted on the four treatment groups to analyse plasma biochemical measures followed by pairwise contrasts and multiple comparisons of means. For interactions by days, Greenhouse-Geisser correction was used if Mauchly’s test of sphericity was significant (27). We conducted a survival analysis (Kaplan-Meier) and used the standard log-rank test to assess the effect of groups on survival. For the bioamines, we conducted statistical comparisons within each genotype. That is, we compared the means for control vs. treated groups (Control vs. CBR1 antagonized) for only lean-prone rats and then conducted a separate comparison of means for obese-prone animals. Alpha was set at .05 for all analyses.
For all pairwise contrasts and multiple comparisons of means tests, the least significance alpha level for the several contrasts is given in the text. Figures were prepared using SigmaPlot 12.5 (Systat Software, Inc., San Jose, CA).

**RESULTS**

*Body Weight and Survival.* Body weight over the two phases of the study (CB1R antagonism and ABA protocol) is shown in Fig. 1, together with body weight as a percentage of weight on entry to ABA challenge (Fig 2). OP-C rats had greater body weight than LP-C rats throughout the study period and greater rate of increase in body weight by days during the CB1R Inhibition phase (linear trend, p < 0.001). Similar differences were shown by OP-A rats compared to LP-A rats. Throughout the period from Day 50, only LP-A rats had significantly lower body weight than its respective control animals, LP-C (p < 0.001). OP-A and OP-C did not significantly differ in body weight.

Over the first 5 days of the ABA challenge, when all rats remained in protocol, LP-C rats lost weight faster than OP-C animals (p < 0.001) (Fig 2). Body weight of OP-A rats decreased more rapidly than that of OP-C rats and LP-A rats lost weight more rapidly than LP-C rats (p < 0.001). In the ABA challenge, CR1R antagonized rats lost relative body weight (%) faster than the respective CB1R control groups (p < 0.001). Also, OP rats showed a lower rate of weight loss than LP animals (p < 0.001).

Multiple regression analysis was performed using entry body weight for ABA (last day of CB1R antagonism period), and CB1R function to predict survival in the ABA challenge. Both predictors had significant effects on survival, p < 0.001 with an $R^2 = 0.68$. Higher entry body weight and intact CB1R function (Control state) were correlated with increased survival.
All OP rats (both OP-C and OP-A) survived to day 14 in the ABA challenge, and also survived significantly longer than LP rats (Fig. 3, p < 0.001). LP-A rats survived fewer days than LP-C animals (6.44 ± 0.24 vs. 8.00 ± 0.29 days; p < 0.05), under the ABA challenge. Kaplan Meier survival analysis for the LP groups (Control vs. Antagonized), omitting the OP groups as all rats were censored (no variability to estimate error), indicated distinct survival functions over days of the ABA challenge (Fig. 3). Log-rank test showed a significant difference between LP-C and LP-A groups in survival ($\chi^2 = 11.89$, df = 1, p ≤ 0.001).

**Caloric intake.** Over the last 10 days of the CB1R antagonism phase (50-59 days of age, Fig. 4), OP-C rats ate more (103.24 ± 0.23 kcal/d) than LP-C animals (89.79 ± 3.33 kcal/d) (p < 0.001). OP-A rats did not differ in caloric intake from OP-C rats, while LP-A animals (86.05 ± 4.17 kcal/d) consumed less food than LP-C rats (p < 0.001). OP-A rats had lower caloric intake than OP-C animals, only during the first two weeks of the treatment (p < 0.03; data not shown in Fig. 4).

During the first 5 days of the ABA challenge, OP-C rats ate significantly more during the 1.5 h meal (32.31 ± 0.65 kcal/d) than LP-C animals (19.82 ± 0.77 kcal/d, p < 0.001). OP-C and OP-A rats did not significantly differ in daily caloric intake from day 6 to day 14 of ABA. However, for the overall period, OP-A rats ate less (26.34 ± 0.64 kcal/d) than OP-C animals (31.97 ± 1.32 kcal/d) (p = 0.001). In addition, over the first 5 days of the ABA challenge, LP-A rats ate less (24.43 ± 1.67 kcal/d) during the 1.5 h meal than LP-C (27.96 ± 1.61 kcal/d, p < 0.001), (Fig 4). Notably, total caloric intake under the ABA challenge was positively correlated (r = 0.93) with survival (p < 0.001). Animals that consumed more, during the 1.5 h access to food, survived longer.
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For the first five days of the ABA challenge, when all rats remained in the protocol, there were significant days, and days by genotype effects on daily rate of wheel running (p < 0.001). Fig. 5 shows that over the first five days of the ABA challenge LP-A rats ran significantly more than LP-C and both OP-C and OP-A animals (p<0.001); OP-C and OP-A groups, however, did not significantly differ in daily rate of wheel turns over the same 5-day period. We also found a significant interaction of ObR and CB1R pathways (p = 0.002) for total distance over the first 5 days of ABA. LP-A rats ran more total distance (7,220 ± 608 m/d) than LP-C animals (4,771 ± 366 m/d), p < 0.01; but OP-A rats (3,489 ± 416 m/d) did not significantly differ from OP-C (4,528 ± 778 m/d).

For days 6 to 14 of the ABA phase, when only the OP rats were still in the protocol, there was a significant CB1R function effect on rate of running (p < 0.001). OP-C rats increased the rate of running more rapidly than OP-A animals, the differences in means reaching statistical significance for days 9 to 14 (p = 0.012). OP-A rats continued to run at the same lower pace until day 14 (survival criterion). Overall, antagonism of CB1R increased daily rate of wheel turns in LP (+/?) rats, but suppressed the running rate in OP (cp/cp) rats.

Pearson correlation analysis revealed that total distance run negatively correlated with both percentage body weight on day 5 (r = -0.61; p<0.001) and days of survival in the ABA challenge (r = -0.55; p < 0.001).

**Plasma biochemical parameters and hypothalamic biogenic amines.** Biochemical parameters are shown in Table 1. At the end of the treatment phase (before ABA challenge), plasma glucose, insulin, triglycerides, total cholesterol and leptin levels were significantly higher in OP rats compared to their LP counterparts (p < 0.05). Plasma TG levels were
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significantly lower in OP-A rats compared to OP-C rats (p < 0.001); while plasma insulin
levels were significantly increased (p = 0.04). CB1R antagonism significantly increased
plasma total cholesterol only in LP-A animals compared to LP-C (p < 0.05, Table1).

On removal from the ABA challenge, plasma glucose, TG, total cholesterol and leptin
levels all remained higher in OP rats (all survived) compared to LP animals (all starved), (p <
0.05). Post ABA challenge, plasma GLP-2 levels were higher in LP-A than LP-C rats (p <
0.001). Corticosterone levels, in contrast, were lower in LP-A and OP-A than in the control
LP-C and OP-C rats (p < 0.001). Plasma TG and glucose levels were significantly lower in
OP-A than OP-C rats (p < 0.03), while there were no significant differences between LP-C
and LP-A rats.

On removal from the ABA challenge, brain levels of serotonin and 5HIAA were
significantly lower in OP-A than in OP-C rats (Table 2, p < 0.001). There were no other
significant differences in biogenic amine levels between control and CB1R antagonized
groups within the same genotype (lean-prone or obese-prone).

Discussion

The results of this study show that adult obese-prone JCR rats lacking the ObR gain a
survival advantage over lean-prone animals under conditions of food restriction and food
related travel (wheel running), supporting, at a basic level, the thrifty gene hypothesis of
obesity (44). All obese-prone (OP) rats survived the 14 d ABA challenge while none of the
lean-prone (LP) rats lasted the 14 days, replicating our recent findings for juvenile JCR rats
(11). A new finding is that survival in the ABA challenge is influenced by the function of both
the CB1R and ObR pathways. In LP rats, with intact ObR, prolonged blocking the CB1R prior
to ABA reduced survival in the challenge. Analysis showed that the low survival of LP-A rats relates to the excessive physical activity (wheel running), low caloric intake and rapid weight loss of these animals—a pronounced activity-anorexia cycle (18). In contrast, antagonism of CB1R had no effect on survival of OP rats in the 14-day ABA challenge.

Rats, left in the ABA protocol on a 1.5 h daily access to food, will normally all reach a threshold beyond which survival is not possible; this being a critical body weight of 65% of entry weight (18). The present study was restricted by the Animal Care and Use Committee to 75% body weight as starvation and to 14 days as survival criteria. Most (83%) adolescent obese-prone rats continue to run and survive the ABA challenge at 14 days based on high energy reserves (fat mass) and energy efficiency (11, 41). The data in Figs 4 and 5, showing markedly greater and increasing running by OP-C rats than OP-A rats coupled with similar caloric intakes, suggest that beyond day 14, OP-C rats would have ultimately attained the 75% body weight criterion, and not survived. Plasma TG levels (a marker of available energy reserve) were higher in OP-C rats than OP-A animals. However, the OP-A rats ran approximately 30% of the distance run by OP-C rats on day 14, expending markedly less energy. The higher TG levels probably reflect increased metabolism in the OP-C rats and not a long lasting survival advantage based on energy reserves.

Previous research shows that CB1R blockade decreases wheel running activity in non-deprived rodents (14, 58), a finding replicated with our OP-A rats, but under the starvation conditions of the ABA challenge. Thus, under conditions of severe food depletion, OP-A rats display low levels of physical activity, similar to non-deprived rodents, when the CB1R is blocked. In contrast, OP-C with functional CB1 receptors, show wheel running that increased quasi linearly from days 2-14. Thus, for obese-prone rats, the blocking of the CB1R pathway
suppresses the excessive physical activity of the ABA challenge. In contrast to what has been observed previously for intact-ObR rodents under free-feeding conditions (14, 58), LP-A rats increased running and starved faster than LP-C animals under the ABA challenge, suggesting a functional ObR system increases physical activity and reduces survival when CB1R control is absent and food is restricted. That is, CB1R regulation provides a survival advantage for lean-prone animals faced with food depletion. Taken together, our observations for OP and LP rats imply a synergistic interrelationship between CB1R and ObR pathways in the regulation of food-related travel and survival under famine-like conditions.

Voluntary wheel running often is considered a self-reinforcing behaviour (31, 55), and CB1R signalling is well-known to influence central reward networks (33). Indeed, both CB1R blockade and CB1R deletion reduce reward-driven behaviours, including wheel running (14, 33). For our model, results for the monoamines and their metabolites showed no difference in dopamine concentrations between groups. However, decreases in brain serotonin and its metabolite (5-HIAA) in the OP-A rats provide tentative support for the neural-reward hypothesis of wheel running, as these monoamines were assessed in the brain extract that contained the reward areas (49). Serotonin is linked to the rewarding effects of cocaine (19) and wheel running is known to immediately and effectively reduce cocaine–seeking behaviour (59)—suggesting that cocaine and wheel running are substitutes or partial substitutes (9). One implication is that serotonin modulates the rewarding effects of wheel running in much the same way as serotonin regulates the rewarding effects of cocaine. Our data show antagonizing the CB1R decreased serotonin and running, in OP rats, under ABA challenge. In the LP-A rats serotonin levels were statistically unchanged, but, running was significantly increased and survival reduced relative to LP-C animals. Thus, the rewarding effect of wheel running for
animals with functional ObR pathway (LP rats) is increased when CB1R pathway is non-functional. When the ObR pathway is non-functional (OP rats), antagonism of the CB1R pathway was associated with a decrease in serotonin, which paradoxically did not result in an increase in the rewarding effects of wheel running, but rather a decrease in running. These findings indicate that both the CB1R and ObR pathways exert an interactive effect on wheel running as a rewarding behaviour under conditions of food deprivation.

Serotonin, however, not only mediates the rewarding effects of wheel running; this neurotransmitter also is involved in the regulation of the HPA-axis response to deprivation-stress induced by the ABA challenge (46), which subsequently feeds back on the ECS (20, 40). The ECS modulates the sensitivity and activation of the HPA axis (49) and is implicated in the wheel activity of food-deprived, and ABA challenged rats (15). Disruption of ECS signalling increases the HPA-axis activity of non-deprived rodents, raising the level of plasma corticosterone (10). But, under the starvation conditions of ABA, our results show that JCR drug-treated rats, both OP and LP, had lower plasma corticosterone levels than untreated controls. The lower plasma corticosterone levels were associated with increased wheel running and early starvation in the ObR present (LP-A) rats. In contrast, decreases in these stress markers did not correlate with wheel running and survival in OP-A rats (ObR absent). These findings suggest that the link between the stress system and wheel running is dependent on the ObR.

**Summary**

The regulation of food intake and body weight is complex and involves interrelated pathways, from search responses to deprivation to hormonal signal transduction and metabolic
adaption. If any of these behavioural and physiological mechanisms change, beyond the range of control, dysfunction results in states such as obesity or activity-based anorexia, both with potentially severe consequences. Absence of the leptin-ObR pathway blunts this response and inhibition of the CB1R response further reduces the life threatening ABA cycle. In the presence of intact leptin-ObR response, CB1R activity is partially protective and inhibition of CB1R exacerbates the ABA cycle. Thus, there is an interrelationship between CB1R and ObR pathways in regulation of energy balance and physical activity. Effective clinical measures to prevent and treat a variety of disorders will require understanding of the mechanisms underlying these effects.

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GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: A.D., W.D.P., and S.D.P. participated in research design; A.D. and W.D.P. conducted experiments; A.D., W.D.P. and J.C.R. performed data analysis and interpreted results; J.C.R. prepared figures; A.D., D.F.V, J.C.R., C.D.H., W.D.P and S.D.P. wrote or contributed to the writing of the manuscript.
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Figure Legends

Figure 1. Absolute body weight of LP-C, LP-A OP-C and OP-A rats under CB1R antagonism and ABA protocol. Data are mean ± s.e.m. *p < 0.05 indicates a genotype effect (lean vs. obese); #, indicates a CB1R pathway effect (Control vs. Antagonized), p < 0.05, only in LP animals. ABA, activity-based anorexia.

Figure 2. Retained body weight (as a percentage of initial weight) of LP-C, LP-A OP-C and OP-A rats under CB1R antagonism and ABA protocol. Data are mean ± s.e.m. *, indicates a genotype effect (lean vs. obese), p < 0.05; #, indicates a CB1R pathway effect (Control vs. Antagonized), p < 0.05 in either LP or OP animals. ABA, activity-based anorexia.

Figure 3. Survival plot of rats under the ABA challenge during the ABA protocol. All rats of obese prone (OP-A and OP-C) groups survived the ABA challenge. Due to lack of variability in days lasted in the ABA protocol for the obese-prone group (all animals survived 14 days), we could only test the differences in survival between lean-prone groups. Thus the Kaplan Meier survival analysis was conducted only for the lean-prone groups (Control vs. Antagonized) using the Log-rank test. The Log-rank test showed a significant difference between LP-C and LP-A groups in survival ($\chi^2 = 11.89, \text{df} = 1, p \leq 0.001$).

Figure 4. Caloric intake during the two phases of the study (CB1R antagonism and ABA protocol). Data are mean ± s.e.m. *, indicates a genotype effect (lean prone vs. obese prone), p < 0.05, #, indicates a CB1R pathway effect (Control vs. Antagonized), p < 0.05 in either LP or OP animals.

Figure 5. Running rate of LP-A, LP-C, OP-A and OP-C rats under ABA challenge. Data are mean ± s.e.m. *, indicates a genotype effect (lean prone vs. obese prone), p < 0.05; #, indicates a CB1R pathway effect (Control vs. Antagonized), p < 0.05. ABA, activity-based anorexia.
## Interrelationship between CB1R and ObR pathways under ABA challenge

### Table 1: Biochemical parameters before and after the ABA challenge (n = 9/group)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>LP-C</th>
<th>LP-A</th>
<th>OP-C</th>
<th>OP-A</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Initial (baseline)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>91.1 ± 2.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>104.2 ± 7.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>110.8 ± 8.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>119.9 ± 9.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Insulin (mU/L)</td>
<td>3.5 ± 1.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.7 ± 2.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.5 ± 1.9&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>14.8 ± 6.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>21.3 ± 1.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.9 ± 2.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>182.4 ± 17.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>98.8 ± 13.3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Leptin (ng/dl)</td>
<td>1.19 ± 0.17&lt;sup&gt;a&lt;/sup&gt;*</td>
<td>1.30 ± 0.36&lt;sup&gt;a&lt;/sup&gt;*</td>
<td>123.7 ± 5.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>118.2 ± 5.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>59.9 ± 1.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>71.7 ± 4.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>102.8 ± 6.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>95.7 ± 5.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Final (removal from Protocol)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>100 ± 6.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>86.5 ± 6.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>125.2 ± 3.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>109 ± 10.8&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Insulin (mU/L)</td>
<td>0.74 ± 0.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.88 ± 0.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.57 ± 1.74&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.75 ± 1.20&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>7.06 ± 1.55&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.48 ± 0.72&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47.7 ± 4.48&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35.4 ± 4.29&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Leptin (ng/dl)</td>
<td>0.04 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.04 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>94.8 ± 3.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>112.1 ± 22.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>35.3 ± 10.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.0 ± 6.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>77.4 ± 8.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>83.3 ± 9.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>GLP-1 (ng/ml)</td>
<td>3.30 ± 0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.19 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.07 ± 0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.79 ± 0.20&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>GLP-2 (ng/ml)</td>
<td>1.20 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.11 ± 0.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.09 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.18 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>PYY (ng/ml)</td>
<td>1.69 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.97 ± 0.09&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.04 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.93 ± 0.12b</td>
</tr>
<tr>
<td>Corticosterone (pg/ml)</td>
<td>997 ± 49&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>512 ± 47&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1087 ± 17&lt;sup&gt;c&lt;/sup&gt;</td>
<td>609 ± 83&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. The superscripts a, b, c and d are used to denote statistical differences among groups. Within the same row, means with different superscripts letters are significantly different (p < 0.05). That is, for any row, if cell means do not share the same superscript letter there is a statistically reliable difference between them. The symbol * is used to denote statistically significant difference between the baseline mean (initial) for a given biochemical parameter and the mean at removal from the ABA protocol (final), using a paired t-test. One rat for the glucose assay and 2 rats for the leptin assay were removed from the statistical analysis as they were outliers (more than 2 standard deviations from the mean).
## Interrelationship between CB1R and ObR pathways under ABA challenge

Table 2: Hypothalamic biogenic amines at removal from the ABA challenge (n = 9/group)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Lean-prone rats</th>
<th>Obese-prone rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LP-C</td>
<td>LP-A</td>
</tr>
<tr>
<td>Noradrenaline (mg/g tissue)</td>
<td>514 ± 10.5(^1)</td>
<td>534 ± 16.0(^1)</td>
</tr>
<tr>
<td>Dopamine (mg/g tissue)</td>
<td>993 ± 25.9(^1)</td>
<td>990 ± 27.9(^1)</td>
</tr>
<tr>
<td>Serotonin (mg/g tissue)</td>
<td>385 ± 7.8(^1)</td>
<td>367 ± 16.8(^1)</td>
</tr>
<tr>
<td>Tryptophane (ug/g tissue)</td>
<td>1.95 ± 0.26(^1)</td>
<td>1.89 ± 0.15(^1)</td>
</tr>
<tr>
<td>Dopac (mg/g tissue)</td>
<td>127.8 ± 2.84(^1)</td>
<td>122.8 ± 4.25(^1)</td>
</tr>
<tr>
<td>5-HIAA (mg/g tissue)</td>
<td>267.7 ± 17.2(^1)</td>
<td>237.5 ± 9.8(^1)</td>
</tr>
<tr>
<td>HVA (mg/g tissue)</td>
<td>85.2 ± 1.52(^1)</td>
<td>79.1 ± 3.14(^1)</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. The superscripts numeros 1 and 2 are used to denote statistical differences between the lean-prone (LP-C vs. LP-A) or obese-prone (OP-C vs. OP-A) groups. That is, within the same genotype, any two means with different numeric superscripts are significantly different (pairwise tests; p < 0.05). HVA, homovanillic acid; 5HIAA, 5-Hydroxyindoleacetic acid; Dopac, 3,4-Dihydroxyphenylacetic acid.