Activation of the sympathetic nervous system mediates hypophagic and anxiety-like effects of CB1 receptor blockade

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Complex interactions between periphery and the brain regulate food intake in mammals. Cannabinoid type-1 (CB1) receptor antagonists are potent hypophagic agents, but the sites where this acute action is exerted and the underlying mechanisms are not fully elucidated. To dissect the mechanisms underlying the hypophagic effect of CB1 receptor blockade, we combined the acute injection of the CB1 receptor antagonist rimonabant with the use of conditional CB1-knockout mice, as well as with pharmacological modulation of different central and peripheral circuits. Fasting/refeeding experiments revealed that CB1 receptor signaling in many specific brain neurons is dispensable for the acute hypophagic effects of rimonabant. CB1 receptor antagonist-induced hypophagia was fully abolished by peripheral blockade of β-adrenergic transmission, suggesting that this effect is mediated by increased activity of the sympathetic nervous system. Consistently, we found that rimonabant increases gastrointestinal metabolism via increased peripheral β-adrenergic receptor signaling in peripheral organs, including the gastrointestinal tract. Blockade of both visceral afferents and glutamatergic transmission in the nucleus tractus solitarii abolished rimonabant-induced hypophagia. Importantly, these mechanisms were specifically triggered by lipid-deprivation, revealing a nutrient-specific component acutely regulated by CB1 receptor blockade. Finally, peripheral blockade of sympathetic neurotransmission also blunted central effects of CB1 receptor blockade, such as fear responses and anxiety-like behaviors. These data demonstrate that, independently of their site of origin, important effects of CB1 receptor blockade are expressed via activation of peripheral sympathetic activity. Thus, CB1 receptors modulate bidirectional circuits between the periphery and the brain to regulate feeding and other behaviors.

Appropriate feeding responses are determined by complex cross-talks between the central nervous system and peripheral organs (1). The endocannabinoid system (ECS) is an important modulator of central and peripheral regulation of energy metabolism (2, 3). In the brain, endogenous and exogenous cannabinoids oppositely regulate food intake, according to the neuronal population involved (4). On the other hand, the cannabinoid type-1 (CB1) receptor antagonist rimonabant (SR141716) acutely induces hypophagia (5), but the sites and the mechanisms involved are not well defined yet.

CB1 receptors control the activity of many neurotransmitter systems involved in central regulation of food intake (2). However, the ECS also regulates food intake and energy balance via peripheral mechanisms (2, 3). Few studies have suggested that CB1 receptor signaling could regulate food intake at peripheral sites (2, 6, 7). Fasting triggers the synthesis of gastrointestinal endocannabinoids, and endocannabinoids produced in the gastrointestinal tract may regulate food (mainly fat) intake (6). The peripheral sympathetic nervous system (SNS) is one of the main mechanisms engaged by CB1 receptor-dependent signaling for the modulation of energy balance (8) and genetic or pharmacological CB1 receptor blockade increases plasma levels of nor-epinephrine (8, 9). In turn, SNS activity regulates meal patterns (10) and is oppositely regulated by the organism’s energy status, being decreased by fasting and increased by feeding (11). Finally, visceral afferents also play an important role in transmitting nutrient-derived intestinal signals to the brainstem, where glutamate and NMDA receptors modulate visceral sensory signaling pathways, ultimately regulating food intake (12). Interestingly, the interplay between SNS and brainstem glutamatergic activity also regulates other brain functions, such as fear and anxiety responses (13), and alterations in these functions represent the main side-effects of rimonabant use in humans (14).

In this study, we systematically investigated the potential sites and the mechanisms of the acute and rapid hypophagic action of rimonabant under fasting/refeeding, as well as its central effects on fear- and anxiety-like behaviors.

Results

CB1 Receptors in Different Brain Neuron Types Are Dispensable for the Rapid Hypophagic Effect of Rimonabant. The acute hypophagic effect of rimonabant depends on CB1 receptors expressed in neurons (8). Thus, we tried to identify the exact neuronal population involved in this phenomenon. Rimonabant bore no effect on fasting-induced food intake in constitutive CB1-KO (5, 15) and in conditional mutant mice lacking CB1 receptor expression in forebrain and sympathetic neurons (8, 16) (CaMK-CB1-KO).


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To test the role of CB₁ receptors in VMH neurons, we crossed CB₁-floxed mice with SF1-Cre mice, characterized by the expression of the recombinase under the regulatory sequences of the steroidogenic factor-1 (SF1), leading to recombination in the VMH, pituitary gland, and gonads (21). SF1-CB₁-KO mice displayed ~80% CB₁ receptor deletion in the VMH (Fig. S1E) and showed a reduced fasting-induced hyperphagia (Fig. 1E). However, the hypophagic effect of rimonabant was still present (Fig. 1E). Thus, VMH CB₁ receptors are required for the endogenous control of fasting-induced food intake, but they are dispensable for the hypophagic effect of rimonabant.

Blockade of Peripheral β-Adrenergic Receptors Prevents Rimonabant-Induced Hypophagia in Fasting and Lipoprivic Conditions. The SNS plays an important role in the regulation of food intake, particularly through the activation of peripheral β-adrenergic receptors (22, 23), and CB₁ receptors can inhibit central and peripheral norepinephrine (NE) release (24, 25). The systemic injection of the generic β-blocker propranolol induced a slight and nondose-dependent reduction of food intake in fasted mice (Fig. 2A and Fig. S2A). Interestingly, however, propranolol (10 mg/kg) fully blocked the hypophagic effect of rimonabant (Fig. 2A), suggesting that activation of β-adrenergic receptors is necessary for the acute anorectic effect of rimonabant. To distinguish between central and peripheral effects of β-adrenergic receptors blockade, we systematically pretreated C57BL/6J mice with the peripherally restricted β-blocker sotalol (Fig. S2B) (26). Sotalol pretreatment (3 mg/kg) fully prevented the hypophagic effect of 3 and 10 mg/kg rimonabant (Fig. 2A). The central administration of sotalol (2 μg, intracerebroventricularly) did not affect rimonabant-induced hypophagia (Fig. 2B), confirming that the modulation of peripheral, but not central, β-adrenergic neurotransmission is involved in the hypophagic effect of rimonabant. Pretreatment with the selective β-adrenoreceptor antagonist SR59230A (1 mg/kg) (27) or the ganglionic blocker hexamethonium (28) both abolished rimonabant-induced hypophagia (Fig. 2C and D), suggesting that the activation of sympathetic ganglia and of β-adrenoreceptors is necessary for rimonabant-induced hypophagia.

SNS activity affects the use of substrates and the metabolic pathways engaged in signaling nutrient availability (11). To investigate whether the SNS-dependent effect of rimonabant might modulate specific food intake induced by acute glucose or lipid-deprivation, we administered rimonabant together with 2-deoxy-glucose (2-DG), an inhibitor of glucose utilization (29), or mercaptoacetate (MA), an inhibitor of fatty acid oxidation (30). Rimonabant partially reversed 2-DG–induced hyperphagia (Fig. 2E) but it fully prevented MA-triggered feeding (Fig. 2F). Moreover, sotalol did not alter the effect of rimonabant on 2-DG-induced hyperphagia (Fig. 2E), but it did block the effect of rimonabant on MA-induced feeding (Fig. 2F). Thus, the activation of peripheral β-adrenergic receptors specifically mediates the CB₁ receptor-dependent inhibition of hyperphagia induced by lipoprivation, but not that elicited by glucoprivation. According to this hypothesis, we measured the levels of plasma free fatty acids, which is a well-established marker of SNS-driven lipolysis (31). As observed in obese rats by others (9), rimonabant injection markedly increased this parameter only in conditions of food availability, such as free-feeding and refeeding (Fig. S2C).
substrates of PKA showed that rimonabant administration increases PKA activity (Fig. S34). Despite the heterogeneity of the bands, semiquantitative evaluation revealed that rimonabant increased overall PKA-dependent phosphorylation by ~70%, 120%, and 1,000% in fed, fasted, and refed conditions, respectively (Fig. S34). These results were in agreement with increased cAMP levels in the same samples, as measured by ELISA (Fig. S35). Quantitative RT-PCR analysis revealed that the expression levels of β1- and β2-adrenoceptor subtypes were not altered by rimonabant treatment, whereas CB1 receptor blockade specifically reduced β3-adrenoceptor mRNA expression in the duodenum (Fig. S3C). In line with several reports showing that increased sympathetic activity is able to rapidly and strongly decrease β3-adrenoceptor mRNAs both in vitro and in vivo (33–35), this effect has been proposed as a mechanism of receptor desensitization and is mediated by a CAMP-dependent process of transcriptional repression (36). Thus, increased β3-adrenergic signaling in the duodenum seems to be the main event responsible for the β3-adrenoceptor-mediated hypophagic effect of rimonabant. To further confirm the activation of peripheral SNS under our study conditions, we analyzed different biochemical parameters also in the brown adipose tissue (BAT), an organ whose functions are critically under the control of the SNS (37). As in the gastrointestinal tract, rimonabant administration was able to increase glucose uptake and metabolic activity also in the BAT (Fig. S3D). Rimonabant action is most likely mediated by β3-adrenergic receptor activation, as it was blocked by sotalol cotreatment (Fig. S3D). Furthermore, rimonabant increased PKA activity in this tissue when fasted animals were re-exposed to food (Fig. S3E). Direct measurement of NE uptake by PET imaging of [13C]-methylhydroxyephedrine, a NE analog (38), revealed that rimonabant increased tracer uptake in the BAT of both free-fed and fasted animals (Fig. S3F). Taken together, these data show that rimonabant increases noradrenergic tone in peripheral tissues.

Capsaicin-sensitive fibers of the abdominal vagus nerve are known to contribute to both satiety and hyperphagia, particularly when the latter is induced by administration of fatty acid oxidation inhibitors, such as MA (30, 39, 40). To investigate whether vagal sensitive fibers may contribute to the anorectic effect of CB1 receptor blockade, we pretreated mice with a low dose of capsaicin (5 mg/kg) 1 wk before the experiment. This dose of capsaicin fully blocked cholecystokinin (CCK)-induced hypophagia, but did not alter eye wiping (Fig. S4A and B) (41, 42). Thus, this treatment effectively disrupted vagal signaling, but spared primary sensory reflexes (41, 42). Interestingly, as previously shown with higher doses (7), capsaicin treatment blunted the hypophagia induced by rimonabant (Fig. 3C), suggesting a primary role of vagal transmission in the hypophagic effect of CB1 receptor antagonism.

Glutamatergic Transmission in the Nucleus Tractus Solitarii Mediates Rimonabant-Induced Hypophagia. Glutamatergic transmission in the caudal brainstem is an important mechanism engaged by satiety signals derived from visceral afferents (12) and plays a specific role in feeding induced by lipopropion (43, 44). Thus,
Peripheral β-Adrenergic Signaling Mediates the Effects of Rimonabant on Conditioned Freezing and Anxiety-Like Behaviors. After determining that peripheral β-adrenergic signaling mediates the acute hypophagic effect of rimonabant, we wondered whether such a mechanism might also underpin other behavioral effects of CB1 receptor antagonism. Indeed, brain functions, like fear and anxiety responses, are regulated by interactions between the SNS and brainstem glutamatergic activity (13), and alterations in these functions represent the main side-effects of rimonabant use in humans (14). For example, the increase in SNS activity induced by rimonabant may be partly responsible of the drug’s effect on conditioned freezing in fear-conditioning experiments (15).

Rimonabant increased freezing of conditioned C57BL/6-N mice (Fig. 4A), and this effect was significantly impaired by sotalol pretreatment (Fig. 4B), suggesting that sotalol β-adrenergic signaling is involved in the effects of CB1 receptor blockade on fear-induced freezing responses. Given the proposed role of noradrenergic transmission in anxiety disorders (45), we wondered if the anxiogenic effect of rimonabant (46) also depends on SNS activity. Systemic sotalol pretreatment prevented the anxiogenic effect of both systemic (Fig. 4C) and central, (Fig. 4D) CB1 receptor blockade in the elevated-plus-maze test, without altering locomotor activity (Fig. S5).

Discussion

An incessant cross-talk between periphery and brain guarantees proper control of energy balance. Our study reveals that the acute hypophagic effect of the CB1 receptor antagonist rimonabant in fasting-refeeding experiments does not primarily depend on CB1 receptors expressed in a wide range of brain neuron types. Conversely, modulation of SNS activity, activation of the gastrointestinal tract, and afferent stimulation of glutamatergic transmission in the NTS appear to be necessary mechanisms for the suppression of fasting- or lipoprivic-induced food intake caused by rimonabant. Furthermore, increase in peripheral β-adrenergic transmission seems to account for other behavioral effects of systemic and central pharmacological CB1 receptor blockade, such as increased fear-induced responses and anxiety-like behaviors.

Deletion of CB1 receptor in GABAAergic or cortical glutamatergic neurons strongly affects the (endo)cannabinoid control of stimulated food intake (4). However, acute rimonabant injection maintained its effect in these two mutant lines, suggesting that CB1 receptor-dependent control of glutamatergic or GABAAergic transmission is not involved in the hypophagia caused by pharmacological CB1 receptor blockade. Furthermore, although CB1 receptors are present in serotonergic neurons (47, 48), their expression is not required for the hypophagic effect of rimonabant in line with the additive, but not synergistic effects of CB1 receptor blockade and serotonin reuptake inhibition (2, 49). The hypothalamus is a key structure for endocannabinoid-mediated control of energy balance and food intake (2). CB1 receptors are expressed in several hypothalamic nuclei where they can regulate both glutamatergic and GABAergic transmission (50), and expression and action of both orexigenic and anorexigenic hypothalamic neuropeptides (2, 51).

Among the hypothalamic nuclei, the VMH and the PVN are generally considered to exert food intake suppressor functions (1). The well-known inhibitory role of the ECS on neurotransmission would, therefore, find a logical mechanism of action in regulating the activity of PVN and VMH neurons to eventually increase food intake. Our data suggest that this finding holds true for VMH neurons, but not for PVN ones. Mice lacking CB1 receptors in the VMH displayed a reduced fasting-induced food intake, whereas mice lacking CB1 receptors in PVN neurons failed to show any phenotype. Nevertheless, CB1 receptor deletion in these nuclei did not alter rimonabant-induced acute hypophagia. The phenotype of SF1-CB1-KO mice is very similar to the one of Glu-CB1-KO one (4), as both these mutant mouse lines display reduced stimulated food intake, but normal response to rimonabant. We recently showed that virally induced partial deletion of the CB1 gene in the hypothalamus did not alter the rapid hypophagic effect of rimonabant 1 h after refeeding in fasted mice, although the drug did not decrease 24-h food intake (52). Thus, hypothalamic mechanisms may be involved in long-term effects of rimonabant but not in the rapid hypophagic properties of acute rimonabant administration. A key difference between CaMK-CB1-KO mice (not responding to rimonabant) and all of the other mouse lines used in this study is the deletion of CB1 expression in a subset of peripheral sympathetic neurons (8). CB1 receptor activation inhibits peripheral noradrenergic transmission (24) and genetic deletion and pharmacological blockade of CB1 receptors increase the levels of circulating noradrenaline (8, 9). In turn, SNS activity inhibits food intake (22, 23), and specific blockade of peripheral, but not central, β-adrenoceptors blunted rimonabant-induced hypophagia. Thus, we propose that rimonabant-induced reduction of food intake is because of an increase in SNS activity. At this stage, however, we cannot conclude whether this SNS activation is a result of direct blockade of CB1 receptors at SNS terminals or because of simultaneous upstream effects of the drug.

Activation of β-adrenergic receptors abolishes lipoprivic-induced hyperphagia (53) and enterocytes might specifically translate fatty-acid oxidation into a vagal afferent signal to control food intake (30). Here, we demonstrate that rimonabant blocks fasting- and lipoprivic-induced food intake by engaging sympathetic transmission in peripheral tissues, including the gastrointestinal tract.

Deafferentiation of vagal afferents by capsaicin pretreatment abolishes satiety signals (refs. 12 and 42, and present results), likely through increase of NMDA signaling in the NTS (12). Similar
mechanisms are likely involved in rimonabant-induced hypophagia, which requires intact abdominal capsaicin-sensitive fibers (ref. 7 and present results) and NMDA receptor activation in the NTS (present results). Vagal afferents contain CB1 receptors (54). However, it is unlikely that rimonabant directly acts on vagal terminals in the NTS to reduce fasting-induced food intake. First, local injection of rimonabant into the NTS and the fourth ventricle did not acutely alter food intake. Second, CaMK-CB1-KO mice, where rimonabant effects are abolished, still contain normal levels of CB1 receptors in the vagal nodose ganglion (8). Conversely, our data suggest that the increased NMDA receptor signaling in the NTS is indirectly mediated by the rimonabant-induced increase of peripheral sympathetic adrenergic transmission. Interestingly, sural nerve chemical lesions of vagal afferents, and lesions of sensory terminals in the NTS specifically abolish lipoprivic-induced feeding and Fos expression in the NTS (40, 43, 55). Thus, rimonabant likely exerts its hypophagic action by specifically interfering with a periphery-to-brain mechanism engaged to encode signals related to the use of fatty-acid substrates and availability. This conclusion is in agreement with recent evidence suggesting that gut-derived endocannabinoids exert a critical control over fat intake (6).

Other data suggest that systemic pharmacological CB1 receptor blockade under fasting or lipoprivic conditions (i) directly or indirectly increases SNS activity (particularly in the gastrointestinal tract), eventually leading to (ii) activation ofafferent fibers sending glutamatergic projections to the NTS, where (iii) increased NMDA neurotransmission triggers the decrease of food intake (27, 56). At this stage, we cannot determine which downstream events follow the rimonabant-induced activation of NTS neurons to decrease food intake. However, NTS signaling is known to engage several brain regions to modulate food intake, including hypothalamic circuits and dopaminergic transmission in limbic systems controlling food-related reward (2, 57–59).

Apart from the rapid modulation of food intake, rimonabant administration induces many additional behavioral and neuro-psychiatric effects in animals and humans (14, 26). In particular, rimonabant increases freezing responses to conditioned cued fear stimuli in rodents (15). Interestingly, conditioned freezing shares similar β-adrenergic–dependent mechanisms (13). Our findings surprisingly suggest that rimonabant-induced freezing requires increased peripheral SNS activation. Interestingly, blockade of peripheral β-adrenergic transmission is also able to prevent the anxiogenic effect of systemic rimonabant injections. In addition, when rimonabant treatment is restricted to the central nervous system (intracerebroventricularly), its anxiogenic action still requires active peripheral β-adrenergic transmission. These data indicate that CB1 receptor blockade is able to act centrally to increase peripheral noradrenaline release, and this may in turn lead to increased freezing and anxiety. This theory can be explained by two main sets of observations: (i) increased SNS activity is positively correlated with an increased anxiety status in both humans and laboratory animals (45); and (ii) the nutritional status strongly influences mood and behavior, in particular anxiety and fear. For example, the vagal satiety mediator CCK is also able to increase anxiety via vagal signaling (60). This result is likely because of the widespread expression of CCK receptors in the central nervous system (61), as well as to an increase in noradrenergic-projection signaling, after vagal activation, from the NTS to key brain regions involved in the control of emotional behaviors, such as the PVN, the bed nucleus of stria terminalis, and the basolateral amygdala (62). Hence, rimonabant action in the central nervous system may be a means of increasing peripheral noradrenergic tone and of CCK-mediated vagal transmission (63), thus changing the perception of the nutritional status. The synergistic actions of these two mediators likely result in increased fear and anxiety behavior, as observed after acute rimonabant administration in our experimental setting. Thus, this finding leads us to conclude that the behavioral and hypophagic actions of rimonabant described herein share strong functional connections.

These data reveal a specific CB1 receptor-dependent circuit linking peripheral lipid-sensing mechanisms to brain activity to modulate food intake in mammals through the SNS. Other behavioral effects of rimonabant, such as those on fear and anxiety, share similar neurobiological substrates. Taken together, the present findings pinpoint a unique CB1 receptor-dependent bidirectional cross-talk between brain and periphery for the regulation of feeding and anxiety-related behaviors.

Experimental Procedures

Experimental procedures are described in SI Experimental Procedures. Procedures discussed are the conditional CB1R mutant mice used, behavioral procedures, local and systemic pharmacological injections, biochemical experiments, and data analysis (for statistics, see Table S1). All animal procedures were conducted in accordance with standard ethical guidelines (European Communities Directive 86/609-EEC) and were approved by the Committee on Animal Health and Care of Institut National de la Santé et de la Recherche Médicale and French Ministry of Agriculture and Forestry (authorization A3310035).

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