

Central Endocannabinoid Signaling Regulates Hepatic Glucose Production and Systemic Lipolysis

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OBJECTIVE—The endocannabinoid (EC) system has been implicated as an important regulator of energy homeostasis. In obesity and type 2 diabetes, EC tone is elevated in peripheral tissues including liver, muscle, fat, and also centrally, particularly in the hypothalamus. Cannabinoid receptor type 1 (CB₁) blockade with the centrally and peripherally acting rimonabant induces weight loss and improves glucose homeostasis while also causing psychiatric adverse effects. The relative contributions of peripheral versus central EC signaling on glucose homeostasis remain to be elucidated. The aim of this study was to test whether the central EC system regulates systemic glucose fluxes.

RESEARCH DESIGN AND METHODS—We determined glucose and lipid fluxes in male Sprague-Dawley rats during intracerebroventricular infusions of either WIN55,212-2 (WIN) or arachidonoyl-2'-chloroethylamide (ACEA) while controlling circulating insulin and glucose levels through hyperinsulinemic, euglycemic clamp studies. Conversely, we fed rats a high-fat diet for 3 days and then blocked central EC signaling with an intracerebroventricular infusion of rimonabant while assessing glucose fluxes during a clamp.

RESULTS—Central CB₁ activation is sufficient to impair glucose homeostasis. Either WIN or ACEA infusions acutely impaired insulin action in both liver and adipose tissue. Conversely, in a model of overfeeding-induced insulin resistance, CB₁ antagonism restored hepatic insulin sensitivity.

CONCLUSIONS—Thus central EC tone plays an important role in regulating hepatic and adipose tissue insulin action. These results indicate that peripherally restricted CB₁ antagonists, which may lack psychiatric side effects, are also likely to be less effective than brain-permeable CB₁ antagonists in ameliorating insulin resistance. *Diabetes* 60:1055–1062, 2011

Obesity and type 2 diabetes continue to be growing health concerns of pandemic proportions in developed and developing nations. Insulin resistance is a hallmark of both of these conditions and leads to impaired glucose and lipid homeostasis. This often leads to multiple complications, including fatty liver, atherosclerosis, and cardiovascular disease, which reduce life expectancy (1). Insulin is the principal regulator of both glucose and lipid homeostasis. It lowers circulating glucose levels by decreasing hepatic glucose production (hGP) and increasing glucose uptake into

peripheral tissues like muscle and fat (2). Insulin suppresses hGP through direct effects via hepatic insulin receptors and indirect mechanisms, including the activation of neuronal insulin signaling (3,4) and the modulation of gluconeogenic substrate flux from adipose tissue by suppressing lipolysis (5,6). Hepatic insulin resistance is defined as the inability of systemic hyperinsulinemia to suppress hGP and contributes to both fasting and postprandial hyperglycemia seen in patients with obesity and type 2 diabetes (7). With regards to adipose tissue, insulin resistance is defined as the inability of hyperinsulinemia to suppress lipolysis (8). Unrestrained lipolysis is an important contributor to the pathogenesis of type 2 diabetes since elevated free fatty acids induce lipotoxicity and further worsen insulin resistance (9).

We have recently shown that hypothalamic insulin signaling is an important determinant of adipose tissue insulin action (10) and have confirmed the findings of others that it also regulates hGP (3). Insulin binds to receptors on hypothalamic neurons that eventually activate ATP-sensitive potassium channels, leading to the hyperpolarization of neurons (11). Through synaptic transmission, the resulting signal is then conveyed to second-order neurons (12), which regulate hepatic metabolism through what appear to be predominantly vagal efferents (11). Furthermore, we have found that insulin infused either into the third ventricle or, more specifically, into the mediobasal hypothalamus (MBH), restrains white adipose tissue (WAT) lipolysis by dampening sympathetic outflow to WAT. Moreover, insulin resistance caused by one day overfeeding is in part due to impaired hypothalamic insulin action (10,13), which is a failure of intracerebroventricular or MBH infused insulin to suppress hGP and adipose tissue lipolysis. However, the mechanism underlying this decreased hypothalamic insulin action remains unclear. Some publications suggest that decreased insulin signaling in the hypothalamus accounts for this impaired insulin action (13). On the other hand, it is possible that the insulin signaling cascade in the hypothalamus is intact but the synaptic transmission of this neuronal signal from the hypothalamus to the liver and WAT might be impaired.

The endocannabinoid (EC) system plays an important role in the regulation of energy homeostasis (14), specifically of glucose and lipid homeostasis, and represents an important link between obesity and insulin resistance. The EC system is composed of the cannabinoid receptors (CB₁ [15,16] and CB₂ [15]), their endogenous ligands (the ECs), such as anandamide and 2-arachidonylglycerol, and the enzymes responsible for the synthesis and degradation of the ECs (17). CB₁ is present throughout the brain (16,17) and also in many peripheral tissues, including liver, muscle, and WAT (15). Several studies have demonstrated that in obese or diabetic rodents and humans, EC tone is elevated in adipose tissue, liver, and pancreas (18,19) but also in the brain, particularly the hypothalamus (20,21).

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Systemic CB₁ activation increases food intake, decreases energy expenditure, and disrupts glucose and lipid homeostasis by inducing insulin resistance (14,21,22). Furthermore, selective CB₁ blockade, through pharmacological means (23,24) or genetic knockout of CB₁ (25), decreases food intake and body weight and markedly improves insulin sensitivity, glucose, and lipid homeostasis in obesity (26). The relative contributions of peripheral versus central EC signaling to impairing glucose and lipid homeostasis are yet to be determined. ECs are thought to regulate appetite through central, hypothalamic effects (27), and peripheral metabolism through direct effects on target organs (28), such as the liver and WAT. The question of where excessive CB₁ signaling dysregulates glucose and lipid fluxes has clinical relevance. Although systemic CB₁ blockade in humans, through medications like rimonabant and taranabant (29), improves glucose and lipid homeostasis, it also significantly increases the risk of psychiatric adverse events, including depression and suicidal ideation (30). This led to the retraction of these otherwise successful antiobesity medications (31).

Prior studies have attempted to separate the brain effects of ECs from their systemic effects (32,33,34). These studies were inconclusive because they lacked a truly peripherally restricted CB₁ antagonist (32). A recent study demonstrated improvements greater than pair-fed vehicle but still less than rimonabant through peripheral CB₁ blockade (33). Another study found that rimonabant was ineffective in mice lacking CB₁ in neurons, highlighting the impact of neuronal CB₁ (34), but did not answer the question of whether this was through CB₁ in the central nervous system (CNS) or in peripheral sympathetic ganglions.

The aim of our study was to explore the role of central EC system activation on hepatic insulin sensitivity *in vivo*. Because ECs can act as retrograde, inhibitory messengers in the brain (17,35), we hypothesized that elevated brain EC signaling would inhibit the propagation of insulin-induced neuronal signals, resulting in decreased insulin action potentially in the absence of any hypothalamic insulin signaling defect. Therefore, the activation of CB₁ in the brain should dampen the ability of insulin to suppress hGP. Conversely, overfeeding-induced insulin resistance should be ameliorated by CB₁ blockade in the brain. Our results support this hypothesis, demonstrating that the activation of CB₁ in the brain impairs hepatic insulin sensitivity, whereas central CB₁ antagonism restores insulin sensitivity. Interestingly, we find that central CB₁ activation also impairs the ability of systemic insulin to suppress lipolysis.

RESEARCH DESIGN AND METHODS

Animals. Animal protocols were approved by the Mount Sinai School of Medicine Institutional Animal Care and Use Committee. Male Sprague-Dawley (SD) rats (8 weeks old) (Charles River Breeding Laboratories, Wilmington, MA) were fed a standard rodent diet (Rodent Diet 5001; LabDiet, St. Louis, MO) and housed in a temperature- and light-controlled (21°C, 12-h light-dark cycle) facility. Rats were stereotaxically fitted with 22-gauge intracerebroventricular guide cannulae (PlasticsOne, Roanoke, VA) targeting the third ventricle, 2.5 mm posterior from bregma, on the midline 9 mm below the cortical surface, 2 weeks before the clamp study. One week later carotid arterial and jugular venous catheters were implanted for blood sampling and infusion, respectively. Rats recovered for at least 4 days and returned to within 10% of their presurgical body weight before being studied. After recovering from surgery, rats participating in the rimonabant study were given highly palatable 10% lard diet (HFD) (Modified Laboratory w/ 10% Lard 57IR, Lab-Diet) for 3 days before the clamp while food intake was monitored.

Pancreatic hyperinsulinemic-euglycemic clamp studies. The rat clamp studies have been described previously (10) and are depicted schematically in Fig. 1A. In brief, intracerebroventricular infusion cannulae were inserted and a 5 μL/h intracerebroventricular infusion was initiated ($t = -120$ min) and

maintained throughout the study. Treatments dissolved in artificial cerebrospinal fluid (aCSF) (Harvard Apparatus, Holliston, MA) contained 0.25 μg/μL WIN55,212-2 (WIN) (Sigma-Aldrich, St. Louis, MO; soluble with 5% DMSO, 0.5% Tween-80), 36.5 ng/h arachidonoyl-2'-chloroethylamide (ACEA) (with 0.036% DMSO), 0.2 μg/μL rimonabant (with 0.35% DMSO, 0.02% Tween-80) (both Cayman Chemical, Ann Arbor, MI), or the appropriate vehicle composed of aCSF, DMSO, and Tween 80 as needed for treatment solubility.

At $t = 0$ min, we started a tracer equilibration period consisting of primed-continuous infusions of [³H-3]glucose (radiochemical concentration >97%; PerkinElmer, Waltham, MA) and [³H-5]glycerol (98 atom percent excess from Isotec [Miamisburg, OH]) with boluses of 20 μCi and 13.3 μmol followed by continuous infusions of 0.5 μCi/min and 0.334 μmol/min, respectively. At $t = 90$ min, blood samples were taken every 10 min to determine basal hGP and R_a glycerol. At $t = 120$ min, an insulin clamp began with primed-continuous infusions of human insulin (Humulin R, Lilly; Indianapolis, IN) and somatostatin (Bachem Biosciences; Torrance, CA) with boluses of 53.2 mU · kg⁻¹ and 53.2 μg · kg⁻¹ followed by infusions at 3 mU · kg⁻¹ · min⁻¹ and 3 μg · kg⁻¹ · min⁻¹, respectively, while continuing the tracer infusions at the aforementioned rates. Euglycemia was maintained by measuring blood glucose and infusing 25% glucose as necessary. Blood samples were obtained every 10 min for the last hour of the experiment to calculate hGP, R_a of glucose, and R_a glycerol under clamped conditions.

Analytic procedures. At the end of the clamp, rats were anesthetized and killed and tissue samples were freeze-clamped in liquid nitrogen and stored at -80°C. Blood was collected in EDTA tubes. Blood glucose was measured by the GM9-D glucose analyzer (Analog Instruments, London, U.K.). Plasma insulin was analyzed by a rat insulin ELISA from Mercodia (Uppsala, Sweden). Plasma nonesterified fatty acid (NEFA) and triglycerides were measured by kits from Wako Diagnostics (Richmond, VA) and Sigma-Aldrich.

Glucose fluxes. Plasma samples were deproteinized using barium hydroxide and zinc sulfate, centrifuged, and the supernatant was dried overnight to eliminate tritiated water. Glucose was redissolved in water and counted using Ultima Gold in a MicroBeta TriLux (both PerkinElmer) liquid scintillation counter. Under preclamp steady-state conditions, hGP equals the glucose turnover rate, which was determined from the ratio of the [³H-3]glucose tracer infusion rate and the specific activity of plasma glucose. During the clamp, hGP was calculated by subtracting the exogenous glucose infusion rate (GIR) from the glucose turnover rate, which in a steady state equals the R_a .

Glycerol fluxes. R_a glycerol (in μmol · kg⁻¹ · min⁻¹) was calculated by the equation:

$$R_a = \left(\frac{ENR_{inf}}{ENR_{pl}} - 1 \right) \cdot R$$

where ENR_{inf} is the fractional isotopic enrichment of the infused glycerol in atom percent excess and ENR_{pl} is the enrichment in the plasma sample. R is the rate of isotope infusion in μmol · kg⁻¹ · min⁻¹ (36). The ³H-labeling of plasma glycerol was determined as follows: 20 μL of plasma was deproteinized with 200 μL methanol by centrifugation. The fluid fraction was dried and reacted with 50 μL bis(trimethylsilyl)trifluoroacetamide plus 10% trimethylchlorosilane for 20 min at 75°C. Isotope enrichment was determined by gas chromatography-mass spectrometry. Ions of 205–208 mass-to-charge ratios were monitored.

Western blot analyses. Western blots were performed as in Ref. 10. In brief, proteins were extracted from tissue, separated on 4–12% NuPAGE gels (Invitrogen, Carlsbad, CA), blotted onto Immobilon-FL PVDF (Millipore, Billerica, MA) membranes, blocked, and incubated with primary antibodies. Antibodies used were: phospho-Akt473, GSK3-α/β, phospho-hormone-sensitive lipase (HSL) (Ser563 and Ser660), adipose triglyceride lipase (ATGL), PKA Substrate (all Cell Signaling Technology, Beverly, MA), β-actin, total HSL, and GAPDH (all Abcam, Cambridge, MA). Perilipin and CGI-58 were gifts from Drs. Andrew Greenberg (37) and Dawn Brasaemle (38), respectively. Phosphoperilipin was assessed by the PKA Substrate motif antibody. Blots were washed, incubated with Dylight conjugated secondary antibodies (Thermo Fisher Scientific, Waltham, MA) against rabbit and mouse IgG, and then washed before being scanned with the LI-COR Odyssey (LI-COR, Lincoln, NE) and quantified with Odyssey 3.0 software on the basis of direct fluorescence measurement.

Statistics. Values are presented as means ± SE. Comparisons between groups were made using unpaired, two-tailed Student t tests. Differences were considered statistically significant at $P < 0.05$.

RESULTS

Central EC system activation induces hepatic insulin resistance. To assess the effects of central CB₁ activation on systemic insulin action, the authors performed hyperinsulinemic (3 mU · kg⁻¹ · min⁻¹), euglycemic clamp

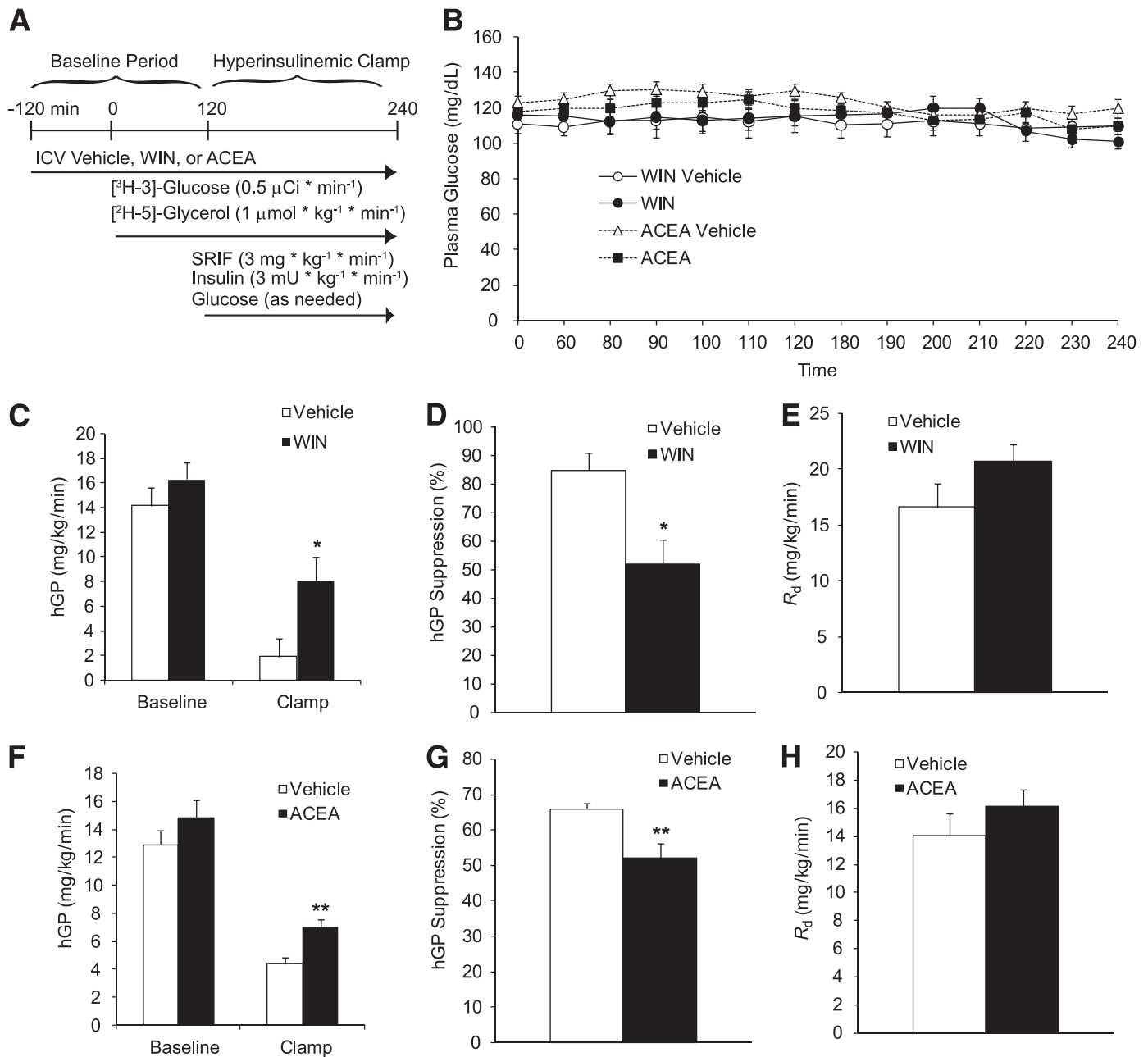


FIG. 1. Intracerebroventricular CB_1 activation impairs hepatic insulin action. **A:** Scheme depicting the clamp protocol. **B:** Blood glucose throughout the experiments ($n \geq 6$). **C–E:** Intracerebroventricular WIN infusion induces hepatic insulin resistance ($n \geq 8$). **C:** Hepatic glucose production (hGP) before and during the clamp. **D:** Percent suppression of hGP. **E:** Peripheral glucose utilization (R_d) during the clamp. **F–H:** Intracerebroventricular ACEA infusion impairs hepatic insulin sensitivity ($n \geq 6$). **F:** hGP before and during the clamp. **G:** Percent suppression of hGP. **H:** R_d during the clamp. See also Supplementary Fig. 1 and Supplementary Table 1. Data are presented as means \pm SE. * $P < 0.05$, ** $P < 0.01$.

studies in SD rats while infusing the CB_1 agonist WIN (protocol in Fig. 1A). This protocol consisted of a 4-h baseline period followed by a 2-h hyperinsulinemic clamp. Glucose levels were controlled, and insulin concentrations were equal between groups before and during the clamp (Fig. 1B and Supplementary Fig. 1A). We used tracer dilution methodology to assess glucose and lipid fluxes simultaneously through the utilization of ^3H -3]glucose and ^2H -5]glycerol, respectively.

During baseline, the intracerebroventricular infusion of WIN tended to elevate hGP compared with vehicle, although this did not reach statistical significance. During the clamp, whereas systemic hyperinsulinemia lowered

hGP in the vehicle infused rats, intracerebroventricular administration of WIN significantly blunted this effect (Fig. 1C). Although vehicle-infused rats suppressed hGP by 85% during the clamp, rats infused with WIN only suppressed hGP by 52% (Fig. 1D), indicating impaired hepatic insulin action. Of note, the rate of glucose utilization (R_d) in peripheral tissues, such as muscle and fat, tended to be higher in the WIN-infused animals (Fig. 1E), which could account for the fact that we did not find differences in the GIR (Supplementary Fig. 1B). These data demonstrate that the activation of the EC system in the brain is sufficient to induce hepatic insulin resistance.

CNS action of the specific CB₁ receptor agonist ACEA induces hepatic insulin resistance. Because WIN can activate both CB₁ and CB₂ (39), we wanted to test whether it is the activation of CB₁ rather than CB₂ or a drug-specific effect of WIN that impairs hepatic insulin action. Therefore, we infused the highly specific CB₁ agonist ACEA intracerebroventricularly while performing hyperinsulinemic, euglycemic clamp studies (Fig. 1A). Blood glucose was controlled throughout the clamp, and hyperinsulinemia was achieved in all rats (Fig. 1B and Supplementary Fig. 1C). As with intracerebroventricular WIN, intracerebroventricular ACEA infusion tended to elevate hGP at baseline and resulted in a significantly higher hGP during the hyperinsulinemic clamp (Fig. 1F). Therefore, intracerebroventricular ACEA infusion significantly impaired the insulin-induced suppression of hGP (66% with vehicle vs. 52% with ACEA) (Fig. 1G). It is noteworthy that the R_d in the intracerebroventricular ACEA-infused group tended to be higher, as seen with intracerebroventricular WIN infusion (Fig. 1H), which could explain why the GIR was not different despite the markedly blunted suppression of hGP (Supplementary Fig. 1D). These data confirm our above finding that central EC system activation induces hepatic insulin resistance, further highlighting the importance of brain CB₁ in regulating hepatic insulin action. **Central CB₁ activation impairs hepatic glucose fluxes without affecting hepatic insulin signaling.** To determine whether the impaired hepatic insulin action seen with intracerebroventricular WIN and ACEA were because of deficits in hepatic insulin signaling, we performed Western blot analyses of livers harvested at the end of the hyperinsulinemic clamps (Fig. 2A and B). Phosphorylation and expression of AKT and glycogen synthase kinase 3 (GSK3), major downstream targets of insulin signaling, were unchanged with either WIN or ACEA infusions. These findings suggest that central CB₁ activation impairs hGP independent of hepatic insulin signaling, consistent with our observations that both central insulin and leptin (40) acutely suppress hGP without altering hepatic insulin signaling.

Because AMP-activated protein kinase (AMPK) is believed to play an important role in regulating hGP (41), we also assessed AMPK phosphorylation. We found that ACEA infusion moderately decreased AMPK phosphorylation (Fig. 2B), and although we were not able to detect this difference with intracerebroventricular WIN infusion, it may in part contribute to the hepatic insulin resistance induced by central EC system activation.

Central EC system activation impairs the ability of systemic insulin to suppress WAT lipolysis. Increased lipolytic flux from WAT to the liver could provide a mechanism for the observed hepatic insulin resistance since glycerol released from WAT can act as a substrate for gluconeogenesis in the liver (42). Because we have demonstrated recently that brain insulin regulates systemic lipolysis (10) and studies have shown that lipolytic flux can regulate hGP (5,6), we investigated whether central EC system activation might induce peripheral lipolysis, consistent with impaired adipose tissue insulin action. To this end, we assessed R_a of glycerol during hyperinsulinemic, euglycemic clamp studies by infusing [²H-5]glycerol. The euglycemic clamp protocol used here enables us to study the effects of central EC system activation on lipolytic flux while controlling circulating insulin and glucose, two major regulators of lipolysis in adipocytes.

During baseline, intracerebroventricular WIN-infused rats tended to have elevated R_a glycerol (Fig. 3A), although

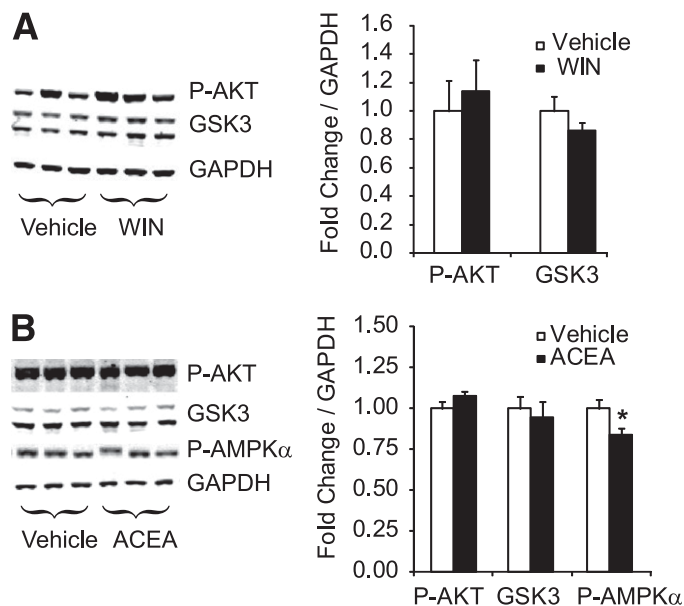


FIG. 2. Central CB₁ activation impairs hepatic insulin action without affecting hepatic insulin signaling. A and B: Western blot analyses of livers from clamped rats infused with vehicle or WIN (A; $n = 8$) or infused with vehicle or ACEA (B; $n = 7$). Membranes were probed with antibodies against phospho-AKT, GSK3, GAPDH, or phospho-AMPK. Representative blots are on the left, and quantifications are on the right. Data were normalized to GAPDH expression and are expressed as fold change compared with vehicle \pm SE. * $P > 0.05$.

this did not reach statistical significance. However, during the clamp, hyperinsulinemia suppressed lipolysis in vehicle-infused rats, but not in intracerebroventricular WIN-infused rats (Fig. 3A). Indeed, intracerebroventricular vehicle-infused rats suppressed R_a glycerol by 47% during moderate hyperinsulinemia, whereas intracerebroventricular WIN-infused rats only suppressed R_a glycerol by 7% (Fig. 3B). These data show that, in addition to the blunted suppression of hGP with systemic hyperinsulinemia, intracerebroventricular WIN infusion impairs the ability of insulin to suppress lipolysis. The inability of the WIN-infused animals to restrain lipolysis could contribute to the impaired suppression of hGP, because it has been shown that lipolytic flux can regulate hGP (5,6). This is highlighted by an inverse correlation between R_a glycerol and hGP suppression (Fig. 3C), and although correlation does not prove causation, it raises the possibility that the impaired suppression of lipolysis is involved in the mechanism regulating hGP.

To investigate the molecular mechanisms responsible for the elevated lipolytic flux in WIN-infused animals, we performed Western blot analyses on WAT from clamped rats. We assessed the activation states and protein expression of several key lipolytic enzymes, including comparative gene identification-58 (CGI-58), HSL, ATGL, and perilipin (43). Perilipin and CGI-58 are lipid droplet-associated proteins. Upon phosphorylation, perilipin facilitates the access of lipolytic enzymes, such as ATGL and HSL, to the lipid droplet and is believed to release CGI-58, which in turn activates ATGL. ATGL hydrolyzes triacylglycerols to diacylglycerols, which are then further hydrolyzed to monoacylglycerols by HSL (44). We found that intracerebroventricular WIN infusion increased perilipin phosphorylation and CGI-58 expression (Fig. 3D), suggesting that ATGL is activated. We also saw consistent

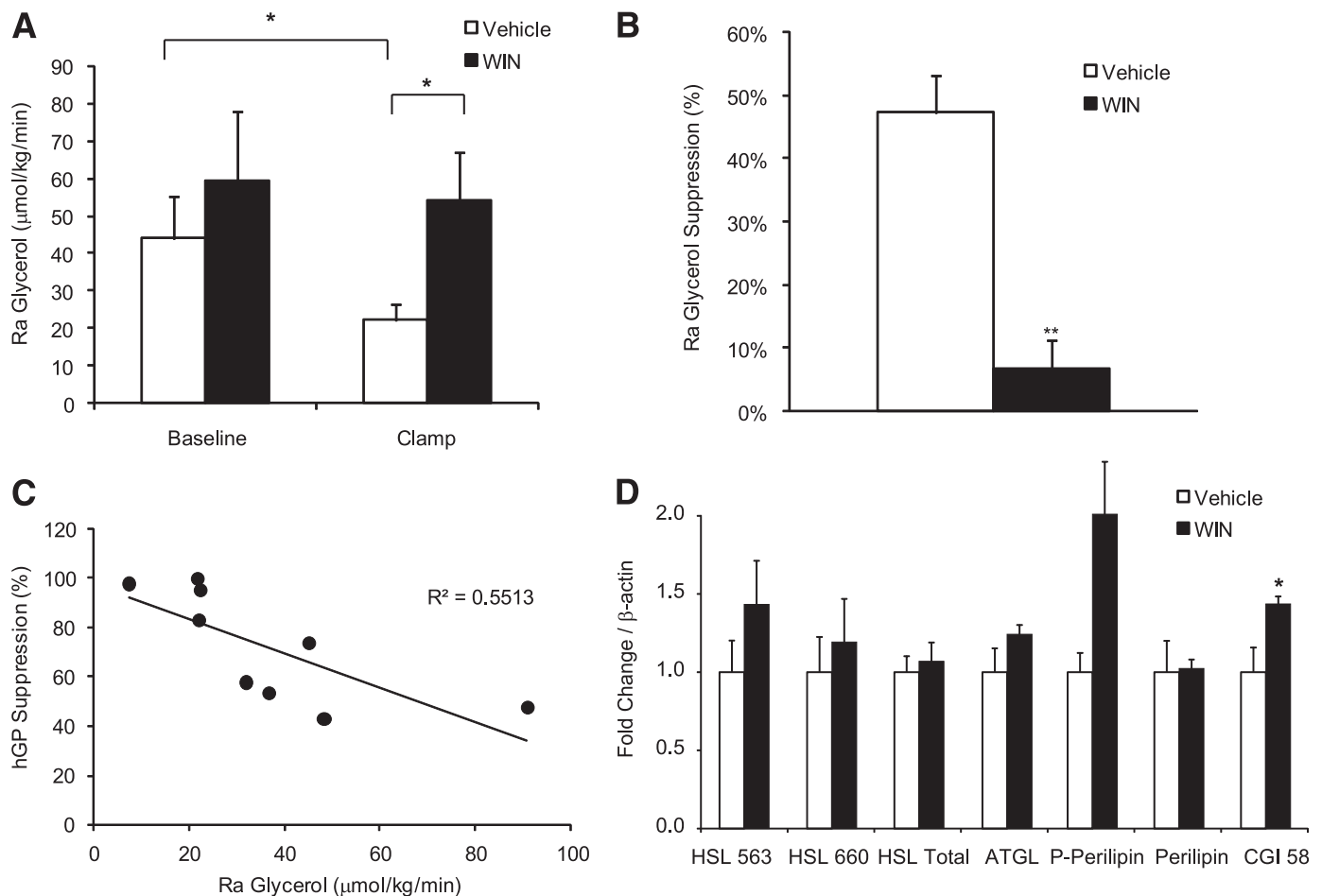


FIG. 3. Central CB₁ activation blunts insulin-mediated suppression of lipolysis. **A:** R_a glycerol in plasma before and during hyperinsulinemic clamps in intracerebroventricular vehicle and WIN-infused rats ($n = 4$). **B:** Percent suppression of R_a glycerol during the clamp ($n = 4$). **C:** Correlation between hGP suppression and R_a glycerol ($n = 4$, $P < 0.05$). **D:** Western blot analyses of lipolytic protein expression and phosphorylation in WAT from clamped rats. Data are normalized to β -actin and expressed as fold change compared with vehicle ($n = 8$). Data are presented as means \pm SE. * $P < 0.05$, ** $P < 0.01$.

trends for intracerebroventricular WIN infusion to increase activating serine phosphorylation sites on HSL. Overall, this increased activation of several key lipolytic enzymes could contribute to the elevated lipolytic flux seen with intracerebroventricular WIN infusion.

Central CB₁ blockade ameliorates overfeeding-induced insulin resistance. We next wanted to understand the role of brain CB₁ in a pathophysiological state of insulin resistance. We induced insulin resistance by exposing SD rats to HFD for 3 days and allowing them to overeat (Supplementary Fig. 2A). This model is characterized by hepatic insulin resistance, whereas peripheral glucose utilization is not yet reduced (45,46). Thus this is a model of early insulin resistance lacking secondary confounding factors, including impaired glucose utilization in muscle and WAT and a chronic proinflammatory state, that are associated with long-standing insulin resistance and diabetes. One of the key defects in this model is impaired hypothalamic insulin action, which is a failure of MBH-infused insulin to suppress hGP (13).

To test whether increased EC tone in the CNS is responsible for the impaired insulin action, we blocked EC signaling in the brain by infusing rimonabant intracerebroventricularly while elevating circulating insulin levels and controlling glucose with a hyperinsulinemic, euglycemic clamp (scheme in Fig. 4A, insulin and glucose

levels in Supplementary Fig. 2B and C). During baseline, hGP was similar between groups, whereas during the clamp the rats infused with rimonabant had significantly lower hGP than HFD vehicle rats (Fig. 4B). Thus although HFD vehicle-treated rats suppressed hGP by only 53%, rats infused with rimonabant suppressed hGP by 79% (Fig. 4C). It is noteworthy that intracerebroventricular rimonabant infusion tended to decrease the R_d (Fig. 4D) and, although not statistically significant, it complements the elevation in R_d seen with CB₁ activation by WIN and ACEA infusions. Intracerebroventricular rimonabant mildly increased the GIR (Supplementary Fig. 2D), although this did not reach statistical significance. These data demonstrate that central CB₁ blockade can restore hepatic insulin action, ascribing a critical role to increased EC tone in the brain in the development of early insulin resistance.

DISCUSSION

Here we demonstrated that elevated EC tone in the brain is sufficient to induce hepatic insulin resistance. By use of pancreatic clamp studies, we showed that central infusions of the CB₁ and CB₂ agonist WIN and the specific CB₁ agonist ACEA impair the ability of systemic hyperinsulinemia to suppress hGP. Conversely, in a model of early insulin resistance characterized by hepatic insulin resistance, central CB₁ blockade was sufficient to restore

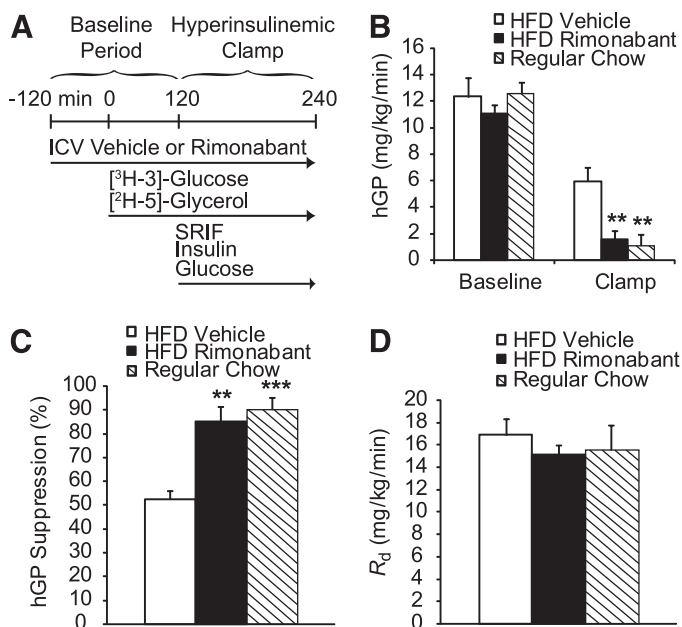


FIG. 4. Intracerebroventricular rimonabant restores hepatic insulin sensitivity after short-term overfeeding. **A:** Experimental protocol. **B:** hGP before and during the clamp. **C:** Percent suppression of hGP during the clamp. **D:** R_d during the clamp. See also Supplementary Fig. 2 and Supplementary Table 2. Data are presented as means \pm SE; $n = 5$. ****** $P < 0.01$, ******* $P < 0.001$.

hepatic insulin sensitivity. After 3 days of HFD, rats develop hepatic and hypothalamic insulin resistance (13). Because animal models of obesity are accompanied by elevated brain and hypothalamic EC levels (20,21), we hypothesized that elevated central EC tone might impair hypothalamic insulin action. By infusing rimonabant intracerebroventricularly, we were able to restore hepatic insulin action caused by overfeeding, emphasizing the relevance of brain CB_1 in the control of hepatic metabolism.

Our second major finding is that central CB_1 activation increases systemic lipolysis. We have recently shown that both systemic lipolysis and hGP can be regulated through brain insulin signaling (10), and because lipolytic flux can affect hGP (5,6), we investigated lipolysis after central EC system activation. The elevation in lipolysis after intracerebroventricular WIN administration increases the flux of glycerol from WAT to the liver, where glycerol would serve as a gluconeogenic substrate, which then contributes to the increased hGP observed during the clamp studies. Therefore, our data support the notion that brain EC tone impairs hypothalamic insulin action not only at the liver but also in WAT.

We measured lipolysis with R_d glycerol through the tracer dilution technique (36), which provides a good estimate of WAT lipolysis (42) for two reasons. First, WAT has very low glycerokinase activity (approximately 1,000-fold lower than liver [47]), and therefore WAT lipolysis results in glycerol release. Second, the liver releases less than 10% of all systemic glycerol (48). Thus, although WAT glycerol release does not entirely account for the R_d glycerol, WAT is the major contributor. We further complement this estimate of whole body glycerol flux by measuring the activation states of HSL and perilipin as estimates of local lipolytic activity. Both measurements support our conclusion that the central EC system regulates lipolysis in WAT.

It is interesting that both CB_1 agonists tended to increase peripheral glucose utilization, whereas the antagonist rimonabant tended to decrease this parameter during the intracerebroventricular infusions. Although the effect size was small and our data did not reach statistical significance, the trends were consistent in each of the three studies, raising the possibility that central CB_1 signaling increases peripheral glucose utilization. We speculate that this could be mediated through increased sympathetic outflow to muscle as has been described recently (49), because muscle is the major glucose utilizing organ, although other tissues may be involved. Therefore, with central CB_1 activation, the elevated R_d could compensate for the decreased hGP suppression, resulting in an unchanged GIR. Likewise, in the rimonabant-infused rats, enhanced hGP suppression could be tempered by this decreased R_d , resulting in a moderate overall increase in GIR.

Whether our studies translate to human physiology is unclear. Although the relevance of neuronal insulin signaling in regulating hGP has been established in rodents through pharmacological and genetic studies (4), the physiological relevance of this regulatory pathway remains uncertain in higher species. Cherrington's group recently found that brain insulin does regulate hGP in dogs (50), although the effect magnitude was modest, possibly because of species discrepancies and differences in the experimental design of the clamp studies compared with earlier rodent studies. In humans, insulin regulates neuronal activity (51) and participates in weight regulation (52). However, whether brain insulin participates in the regulation of hGP and/or lipolysis remains unstudied in humans. One of the reasons why brain insulin could be more relevant in rodent physiology than in human is that rodents may exhibit a higher basal neural tone to the liver as has been previously suggested (53).

Our results with brain infusions of the CB_1 antagonist rimonabant differ from those obtained by Nogueiras et al. (32), where prolonged intracerebroventricular rimonabant treatment in a long-standing model of diet-induced obesity did not improve hepatic insulin sensitivity. It is important to point out some differences in the models used in these studies. We studied an early model of high-fat feeding-induced insulin resistance that is chiefly associated with hepatic insulin resistance (45) and characterized by impaired hypothalamic insulin action (13). Thus this model lacks the secondary sequela of long-term high-fat feeding, such as impaired glucose utilization and chronic inflammation. Conversely, Nogueiras et al. studied a model of prolonged high-fat feeding (32) where these complications are likely present and where the peripherally increased EC tone may become more prominent (18,54). Furthermore, our rimonabant dose was different. Whereas Nogueiras et al. (32) infused 5 μ g per day, we infused 6.25 μ g over a 6-h period. Our chosen intracerebroventricular dose was approximately 1% of the widely used dose of 10 mg/kg ip, which was also used in the abovementioned studies (32). It is possible that the intracerebroventricular dose chosen by Nogueiras et al. (32) was not sufficient to reveal effects of isolated brain CB_1 antagonism, or that in a model of longer-standing insulin resistance isolated central CB_1 antagonism is not enough to reverse the metabolic phenotype.

The role of neuronal EC signaling in regulating energy homeostasis has been highlighted in a study where CB_1 receptors were knocked out in CAMKII α expressing neurons, including forebrain and sympathetic ganglions (34).

These mice displayed a lean phenotype and were resistant to diet-induced obesity and insulin resistance. Although these mice were essentially unaffected by rimonabant treatment, the study did not answer the question whether the beneficial effects of pharmacological CB₁ antagonism are mediated through central versus peripheral mechanisms. Our study demonstrates that brain EC signaling does play an important role in regulating lipolysis, hGP, and possibly peripheral glucose utilization.

It has been shown that short-term overfeeding leads to hypothalamic insulin resistance (13). We speculate that this could be due, in large part, to the dysregulation of the EC system. The activation of the EC system in the brain blunts the ability of insulin to suppress both hGP and lipolysis, similar to what we found by infusing an insulin antagonist into the MBH (10). Under normal conditions, insulin could generate a signal in the MBH that is mediated through descending neuronal pathways that innervate WAT (12,55), resulting in the suppression of lipolysis. However, during states of elevated EC tone, ECs may act as retrograde inhibitors of synaptic transmission and thereby impair hypothalamic insulin action, potentially even in the absence of impaired intracellular insulin signaling. By infusing rimonabant intracerebroventricularly, we released this blockade on insulin-induced synaptic transmission and restored hepatic and WAT insulin action. These data strongly suggest that central EC tone plays an important role in the development of hypothalamic insulin resistance. Although our study does not negate prior work suggesting that the EC system has important peripheral effects, we do demonstrate that the central EC system acutely participates in the regulation of systemic glucose and lipid homeostasis. Therefore, a peripherally restricted CB₁ antagonist might not be as therapeutically potent as the brain-penetrating rimonabant, although it is likely that the feared psychiatric side effects would be avoided.

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