Glucagon-like peptide 1 receptor induced suppression of food intake, and body weight is mediated by central IL-1 and IL-6

Rozita Shirazi[a], Vilborg Palsdottir[a], Jim Collander[a], Fredrik Anesten[a], Heike Vogel[a], Fanny Langlet[b], Alexander Jaschke[c], Annette Schürmann[c], Vincent Prévot[b], Ruijin Shao[a], John-Olov Jansson[a,1], and Karolina Patrycja Skibicka[a,1]

[a]Department of Physiology, Institute of Neuroscience and Physiology, Sahlgrenska Academy at the University of Gothenburg, SE-405 30 Gothenburg, Sweden; [b]Inserm U837, Jean-Pierre Aubert Research Centre, University of Lille 2, 59000 Lille, France; and [c]Department of Experimental Diabetology, German Institute of Human Nutrition Potsdam-Rehbruecke, D-14558 Nuthetal, Germany

Edited by Marc Y. Donath, University Hospital Basel, Basel, Switzerland, and accepted by the Editorial Board August 9, 2013 (received for review April 11, 2013)

Glucagon-like peptide 1 (GLP-1), produced in the intestine and the brain, can stimulate insulin secretion from the pancreas and alleviate type 2 diabetes. The cytokine interleukin-6 (IL-6) may enhance insulin secretion from β-cells by stimulating peripheral GLP-1 production. GLP-1 and its analogs also reduce food intake and body weight, clinically beneficial actions that are likely exerted at the level of the CNS, but otherwise are poorly understood. The cytokines IL-6 and interleukin 1β (IL-1β) may exert an anti-obesity effect in the CNS during health. Here we found that central injection of a clinically used GLP-1 receptor agonist, exendin-4, potently increased the expression of IL-6 in the hypothalamus (11-fold) and the hindbrain (4-fold) and of IL-1β in the hypothalamus, without changing the expression of other inflammation-associated genes. Furthermore, hypothalamic and hindbrain interleukin-associated intracellular signals [phosphorylated signal transducer and activator of transcription-3 (pSTAT3) and suppressor of cytokine signaling-1 (SOCS1)] were also elevated by exendin-4. Pharmacologic disruption of CNS IL-1 receptor or IL-6 biological activity attenuated anorexia and body weight loss induced by central exendin-4 administration in a rat. Simultaneous blockade of IL-1 and IL-6 activity led to a more potent attenuation of exendin-4 effects on food intake. Mice with global IL-1 receptor gene knockout or central IL-6 receptor knockdown showed attenuated decrease in food intake and body weight in response to peripheral exendin-4 treatment. GLP-1 receptor activation in the mouse neuronal Neuro2A cell line also resulted in increased IL-6 expression. These data outline a previously unidentified role of the central IL-1 and IL-6 in mediating the anorexic and body weight loss effects of GLP-1 receptor activation.

POMC | DVC | hypothermia

Glucagon-like peptide 1 (GLP-1) is an incretin hormone secreted from intestinal endocrine L-cells and also from pancreatic α-cells. Its ability to stimulate insulin secretion and regulate blood glucose has been used as a treatment for type 2 diabetes. Importantly, GLP-1 and its long-lasting analogs reduce food intake and body weight (see ref. 1 for review). These effects have been regarded as of potential clinical relevance for successful treatment of obesity. There is limited knowledge regarding the mechanisms behind the anorexic effect of GLP-1, but it is likely exerted at the level of the CNS (2–4). Central GLP-1 receptors (GLP-1R) are distributed throughout the CNS energy-balance-regulating areas, including the hypothalamus and hindbrain (5). GLP-1–producing neurons in the nucleus of the solitary tract are likely the main source of the endogenous ligand to the central GLP-1Rs (6, 7). Peripherally applied long-lasting analogs, due to their ability to cross the blood brain barrier (8, 9), can also engage the central GLP-1R populations, making these CNS receptors a relevant clinical target. Even though the contribution of the central GLP-1Rs to energy balance regulation is clear, the understanding of the neural pathways and mechanisms mediating the intake inhibitory effects of GLP-1R agonists is limited.

A number of findings suggest a potential link between GLP-1 and cytokine signaling outside of the CNS. Indeed, elevated interleukin-6 (IL-6) levels in the blood, particularly the IL-6 secreted by the skeletal muscle, increase both secretion and production of GLP-1 from intestinal L-cells and pancreatic α-cells (10). Moreover, peripheral GLP-1 may mediate beneficial effects of IL-6 on blood glucose and the capacity of β-cells to secrete insulin (10). GLP-1 may also interact with interleukin-1β (IL-1β) (11, 12). However, there are no studies on possible interactions between GLP-1 and IL-6 and IL-1β at the level of the brain. IL-1 and IL-6 are key regulators of the inflammatory response (13, 14), influencing metabolism and behavior during illness, including the induction of fever and loss of appetite and mobility (15–17). However, during the last decade, evidence has accumulated that both the IL-1 and IL-6 systems may play an integral role in healthy animals to regulate the metabolic function. In support of this idea, mice lacking IL-1R or IL-6 develop late-onset obesity as well as disturbed glucose metabolism (18–23). When both IL-1β and IL-6 are lacking, mice develop obesity and hyperphagia much earlier than that seen in mice lacking only either IL-1 or IL-6 activity (24), potentially suggesting a cooperative

Significance

There is a growing interest in the gut- and hindbrain-produced hormone glucagon-like peptide 1 (GLP-1), and GLP-1–targeting drugs are in clinical trials for treatment of obesity, and already in the clinic for treatment of type 2 diabetes. Therefore, the implications of information arising from our study are clinically relevant and considerable. GLP-1 receptor stimulation decreases feeding and body weight likely via the CNS, effects of unquestioned scientific and clinical importance, considering the alarming rates of obesity. Despite this, there is scarce information about the mediators and mechanisms behind the effects of GLP-1. In this study, we found surprising evidence that two cytokines, interleukin-6 and interleukin-1, mediate antiobesity effects of GLP-1 receptor stimulation at the level of the CNS.

Author contributions: K.P.S. designed research; R. Shirazi, V. Palsdottir, J.C., F.A., H.V., F.L., A.J., R. Shao, and K.P.S. performed research; A.S. and V. Prévot contributed new reagents/analytic tools; H.V., A.S., V. Prévot, R. Shao, and K.P.S. analyzed data; and J.-O.J. and K.P.S. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission. M.Y.D. is a guest editor invited by the Editorial Board.

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[1]To whom correspondence may be addressed. E-mail: karolina.skibicka@neuro.gu.se or john-olov.jansson@neuro.gu.se.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1306799110/-/DCSupplemental.

function of these two interleukins. Body weight regulating effects of IL-6 and IL-1 can be exerted at the level of the hypothalamus (25–28).

Cumulatively, the literature shows that GLP-1, IL-6, and IL-1β all decrease body weight acting at the level of the CNS. In the present study, we aimed to investigate whether GLP-1, IL-6, and IL-1 interact at the level of the CNS in regulation of feeding and body weight. We found that GLP-1R stimulation by the clinically relevant GLP-1 analog [exendin-4 (EX4)] applied centrally dramatically increased hypothalamic and hindbrain IL-6 and IL-1 expression as well as IL-6/1–associated neuropeptides, such as melanocortin, and intracellular signals, such as pSTAT3 and SOCS1. Furthermore, we evaluated whether this interaction at the gene expression level has functional/behavioral consequences. Thus, we assessed whether the effects of GLP-1R activation on food intake and body weight are mediated by IL-6 and IL-1 in both pharmacological and genetic models of blockade/deficiency of IL-6 and IL-1 signaling. The results support a crucial role for IL-6 and IL-1β as downstream mediators of GLP-1–induced anorexia and weight loss.

Results

Central GLP-1R Stimulation Selectively Up-Regulated Hypothalamic and Hindbrain IL-6. Central stimulation of the GLP-1Rs with EX4 resulted in a selective elevation in IL-1β and IL-6 mRNA in the hypothalamus and in IL-6 in the hindbrain, without affecting other cytokines measured, namely TNF and TGFβ1, in rats allowed ad libitum access to food overnight (Fig. 1 *A* and *C*). The detected elevation in mRNA was especially potent for IL-6 in the hypothalamus where EX4 injection was associated with 11-fold higher levels of IL-6 mRNA. IL-1β mRNA was elevated threefold compared with vehicle-injected control but only in the hypothalamus. The pattern of results was similar for rats with overnight restricted access to food, although only the increased expression of IL-6 in the hypothalamus reached statistical significance after EX4 injection compared with vehicle-treated control (Fig. 1 *B* and *D*). Consistent with previous reports, the expression of IL-1β was not altered in the hippocampus (Fig. S1). The overall level of variability in the expression of all genes was higher in the rats under restricted food access. Food restriction alone did not significantly change the expression levels of any of the cytokines measured in the current study. The size of EX4 effect, however, was dampened in the food-restricted group. This regulation of cytokine induction by nutritional status is likely in place to allow for a tighter control of energy expenditure in a situation of a nutrient shortage.

GLP-1R Stimulation Induces Interleukin-Associated Intracellular Signals. Hypothalamic and dorsal vagal complex (DVC) phosphorylation of signal transducer and activator of transcription 3 (STAT3) was increased after central EX4 injection in a rat (Fig. 2 *A* and *B*). Additionally, the protein levels of hypothalamic suppressor of cytokine signaling 1 (SOCS1) but not SOCS2 were elevated by GLP-1R stimulation (Fig. 2 *C* and *D*). The detected levels of SOCS1 and SOCS2 were too low in DVC to allow for quantification. Central EX4 also increased the hypothalamic and hindbrain gene expression for proopiomelanocortin (POMC), a precursor for an anorexic neuropeptide previously associated with central interleukin anorexia (29) (Fig. 2 *E* and *F*). In contrast, the level of other anorexic or orexigenic neuropeptides was not altered by EX4.

GLP-1R Stimulation in Mouse Neuroblastoma Cell Line. Neuro2A cells were used to determine whether the GLP-1/IL-6 interaction can be localized to neurons. Neuro2A cells were found to express GLP-1R and IL-6 mRNA, and incubation with EX4 resulted in an increased expression of IL-6 (Fig. 2*G*).

Central IL-6 and IL-1 Are Necessary Mediators of the GLP-1R–Induced Food Intake and Body Weight Suppression. EX4 administration led to a 50% reduction in food intake irrespective of the IL-6 antibody (ab) coadministration at 4 h postinjection. Thus, IL-6

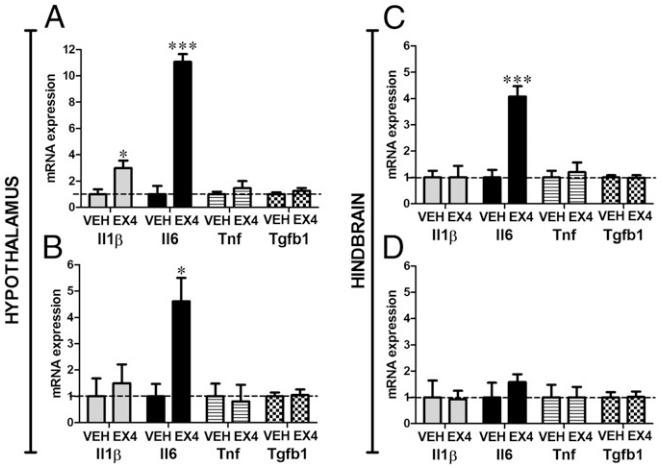

Fig. 1. EX4 markedly increased hypothalamic and hindbrain IL-6 expression. mRNA levels of cytokines in ad-libitum–fed (*A* and *C*) and food-restricted (*B* and *D*) rats following intracerebroventricular (i.c.v.) EX4 treatment. Data are normalized to β-actin and expressed as relative quantity compared with vehicle treatment. n = 9–11/treatment condition. Data are expressed as mean ± SEM. $^{*}P < 0.05$,$^{***}P < 0.005$.

mediation was not required for short latency EX4-induced anorexia (Fig. 3*A*). In contrast to the early intake measurement, IL-6 proved to be a necessary signaling element for the later anorexic effects of EX4 (4–22 h; Fig. 3*B*) where the IL-6ab administration led to a complete reversal of the EX4-induced anorexia. IL-6ab administration also led to a partial attenuation of the EX4-induced weight loss (Fig. 3*C*). Signaling at the IL-1R was not required for the short-latency (up to 4 h) EX4-mediated anorexia (Fig. 3*D*). In contrast, the IL-1 was a necessary mediating element for the longer-term anorexic effects of EX4 (4–22 h) where a blockade of IL-1R led to a complete reversal of the EX4-induced anorexia (Fig. 3*E*). Furthermore, blockade of the IL-1R also led to a reversal of the EX4-induced weight loss (Fig. 3*F*).

Combined Interaction of Central GLP-1R Stimulation on Food Intake and Body Weight with IL-1 and IL-6. To elucidate whether the lack of interaction at early time points results from a potential redundancy between IL-1 and IL-6, a simultaneous IL-6 and IL-1R blockade was tested against EX4. This resulted in a more pronounced reversal of EX4-induced intake and body weight reduction compared with either one of the interleukin blockades alone (Fig. 4 *A–D*). Combination blockade also led to an interaction with a much shorter latency (noted already at 1 h) than that for each of the individual blockades alone (<4 h).

Interaction of the Central GLP-1R Effect on Core Temperature and Activity with IL-6 and IL-1. Because some studies report a *reduction* of motor activity after EX4 treatment and hypoactivity is also associated with interleukins, we wanted to confirm that the behavioral/intake results obtained here are not simply due to an attenuation of EX4-induced behavioral suppression. Surprisingly, in one of the three experiments using EX4, an *increase* in activity was noted (Fig. S2 *A* and *B*). The remaining experiments, however, detected no such change (Fig. S2 *C–F*). Furthermore, when data from all three experiments for the vehicle and EX4 treatment were pooled, no significant differences were detected in motor activity. Because both GLP-1 and interleukins can affect thermoregulation, we extended the interaction studies to measure core temperature. EX4 reliably induced a hypothermic response whether injected alone or in combination with IL-6 and IL-1 (Fig. S3). Single interleukin blockade did not alter this hypothermia (Fig. S3). Body temperature records were additionally analyzed beyond the initial 4-h postinjection period to determine

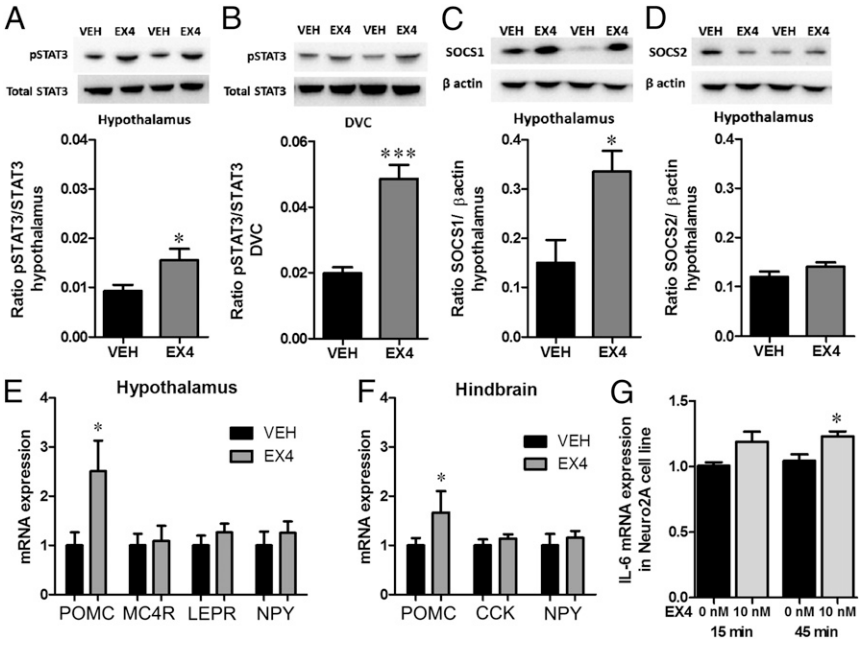

Fig. 2. Interleukin-associated intracellular signals and neuropeptides are elevated by GLP-1R stimulation. Hypothalamic (*A*) and DVC (*B*) phosphorylation of STAT3 was increased after central EX4 injection in an ad-libitum–fed rat. Also, the protein levels of hypothalamic SOCS1 (*C*) but not SOCS2 (*D*) were elevated by GLP-1R stimulation. Hypothalamic and hindbrain mRNA expression of Pomc, a precursor for an anorexic neuropeptide previously associated with central interleukin anorexia (*E* and *F*), is also elevated in response to EX4. In contrast, the level of other anorexic or orexigenic neuropeptides was not altered by EX4. Neuropeptide Y, Npy; choleocystokinin, Cck; melanocortin 4 receptor, Mc4r; leptin receptor, Lepr. n = 6–11/treatment condition. (*G*) Effect of GLP-1R stimulation with EX4 on IL-6 expression in mouse neuroblastoma cell line (Neuro2A). Incubation of Neuro2A cells with EX4 resulted in a significant elevation of IL-6 mRNA. n = 3/treatment. Data are expressed as mean ± SEM. $^{*}P < 0.05$,$^{***}P < 0.005$.

whether the temperature interaction might follow the late-onset interaction for food intake described above. Although the hypothermia was found to persist until the onset of the dark cycle (additional 4 h from the period shown in Fig. S3), the antagonist remained unsuccessful at preventing it. In contrast, a partial attenuation of this response was achieved via a combined IL-6 and IL-1 blockade (Fig S3 *E* and *F*).

Response to GLP-1R Stimulation in IL-1R1– or IL-6Rα–Deficient Mice. IL-1R1$^{-/-}$ mice—results obtained with a pharmacological central blockade of IL-1R in rat indicated that IL-1 signaling is required for the effect of GLP-1R stimulation (Fig. 3). To confirm this finding, GLP-1R responsiveness was tested in mice lacking IL-1R1 (IL-1R1$^{-/-}$ mice; B6.129S7-*Il1r1*^{tm1Imx}/J) and the wild-type (WT) control mice (C57BL/6J). The results mirrored those obtained in a central IL-1R blockade in the rat study. In WT mice, peripheral injection of EX4 reduced food intake at both an early (6 h) and a later time point (22 h) and body weight compared with saline injection (Fig. *5A*). In contrast, IL-1R1$^{-/-}$ mice reduced only their food intake at the early 6-h time point, whereas no significant effect of EX4 was detected at the 22-h time point (Fig. 5*B*). The IL-1R1$^{-/-}$ mice did not reduce their body weight in response to EX4 (Fig. 5*B*). Brain IL-6Rα knockdown (KD) mice were used to examine whether the reduction in CNS IL-6Rα resulted in an attenuation of EX4-induced hypophagia. To reduce the CNS expression of IL-6Rα, a Tat-cre fusion protein (or vehicle/saline for control mice) was infused to the lateral ventricle of mice homozygously floxed for IL-6Rα (strain B6; SJL-*Il6ra*$^{tm1.1Drew}$) (30). Peripheral [intraperitoneal (IP) injection] EX4 induced an early hypophagia in both IL6Rα KD and control mice compared with injection of saline. At the 24-h time point, however, only the control mice showed a significant reduction in food intake (Fig. 5 *C* and *D*). Similarly, IL-6Rα KD mice did not significantly reduce their body weight after IP-injected EX4 whereas the same treatment significantly reduced the body weight of control mice with intact IL-6Rα.

Discussion

GLP-1R stimulation has been successfully used to modulate glucose levels in patients with type 2 diabetes. Notably, GLP-1 analogs are being investigated as a potential treatment for obesity due to their ability to decrease feeding and body weight. This effect is likely exerted via GLP-1Rs in the CNS, but the GLP-1R mechanism of action remains to be elucidated. In this study, we provide evidence that both IL-6 and IL-1β are important downstream mediators of the anorexic and the weight loss effects of the central GLP-1R stimulation. Two different experimental paradigms, both pharmacological blockade of the central IL-6 or IL-1 signaling and genetically induced whole-body or central IL-1R1 or IL-6Rα deficiency, respectively, resulted in attenuated GLP-1R–induced intake inhibition and weight loss. An enhanced attenuation of the GLP-1R–mediated inhibition of feeding was observed with a simultaneous IL-1 and IL-6 blockade. Furthermore, GLP-1R stimulation with EX4 led to a striking elevation of IL-6 gene expression in two key brain energy balance controlling areas—the hypothalamus and the hindbrain—and to increased IL-1β expression in the hypothalamus. In a mouse neuronal cell line, Neuro2A, incubation with EX4 also resulted in an induction of IL-6, suggesting that the cellular machinery required for this interaction is present in neurons. In line with the detected IL-1 and IL-6 activation, intracellular signals, namely pSTAT3 and SOCS1, associated with these interleukins (31) were elevated in the hypothalamus and the DVC after GLP-1R stimulation.

The mediation of the GLP-1 effect on food intake and body weight by IL-1 and IL-6 demonstrated here fits well with the past literature indicating that these two interleukins are critical for energy balance effects of the fat-produced hormone leptin (18, 32–34). Additionally, numerous studies indicate that food intake and body weight-reducing effects of leptin are also mediated by the central GLP-1 (35, 36). This places GLP-1 as a downstream mediator of the leptin signal. What follows—GLP-1 and IL-1β and IL-6—may all fit downstream of leptin as the results of the present study suggest that IL-1β and IL-6 are downstream of GLP-1. Finally, there is evidence that IL-6 is downstream of IL-1 (37). Based on these data, the simplest interaction model would be a sequential pathway composed of leptin → GLP-1 → IL-1 → IL-6. However, it should be kept in mind that this is a simplistic model, and many of the mentioned factors may work in parallel as well as in series and that additional substances need to be taken into account.

Neuropeptides regulating feeding and body weight that are influenced by GLP-1 as well as IL-1 and/or IL-6 are of interest in relation to the present results. One example is the central melanocortin neuropeptide, a target for both leptin and GLP-1 (38–41). In the hypothalamus, direct activation of IL-1R on the POMC neurons leads to an increase in hypothalamic melanocortins (29). Here, in line with IL-1β induction by GLP-1R activation, we show that POMC, but not other major anorexic/orexigenic

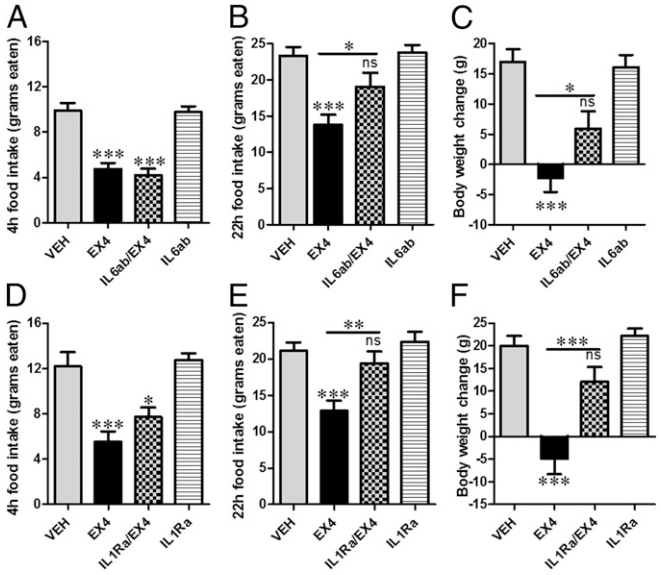

Fig. 3. IL-6ab attenuates food intake and weight loss responses to i.c.v injection of EX4. Anorexic response to central EX4 after 4 h of food access was not altered by IL-6 blockade (*A*). In contrast, the 22 h (overnight) food intake was abolished by the IL-6ab treatment (*B*). The IL-6ab administration led to a complete reversal of the EX4-induced anorexia. Similarly, the EX4-induced weight loss was significantly attenuated by IL-6ab (*C*). Also, IL-1Ra attenuates food intake and weight loss responses to i.c.v. injection of EX4. Anorexic response to central EX4 after 4 h of food access was not altered by IL-1R blockade (*D*). In contrast, the 22-h (overnight) food intake was abolished by the IL-1Ra treatment (*E*), and blockade of IL-1R led to a complete reversal of the EX4-induced anorexia. Similarly, the EX4-induced weight loss was significantly attenuated by IL-1Ra (*F*). For details of statistical analysis, see *SI Results*. Data are expressed as mean ± SEM. n = 12–16/treatment condition. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$.

peptides, is elevated after GLP-1R stimulation in both hypothalamus and hindbrain. Thus, it is possible that the GLP-1–dependent hypothalamic interleukin induction described here contributes to this elevated melanocortin synthesis and release. Important for the current study is also the fact that IL-1β activates only a subset of hypothalamic POMC neurons selectively responsible for reducing food intake but not fever production. This dissociation of intake suppressive and thermogenic effects is important considering that in the current study the EX4 reduces thermogenesis. Not only the hypothalamus but also the hindbrain might represent the neural substrate underlying the intake and weight-suppressing effect of EX4-induced IL-6. Surprisingly, little is known about the role of IL-6 in the hindbrain. That hindbrain IL-6 is up-regulated by GLP-1R simulation may suggest that hindbrain IL-6, just as the hypothalamic IL-6, contributes to energy balance regulation. Circuitry endemic to the hindbrain is sufficient for the anorexic effects of GLP-1 and EX4 (42), and central (lateral ventricle) injections used in the current study allow easy access of the drugs not only to the hypothalamus but also to the hindbrain via the caudal cerebrospinal fluid (CSF) flow. Thus, hindbrain-produced GLP-1 may avail of hindbrain IL-6 and melanocortin to suppress food intake and body weight.

Although a wealth of data suggests that central IL-6 and possibly IL-1 may be used for the physiological regulation of food intake during health (18–21), IL-6 and IL-1 are also key to infection-induced anorexia (43). Moreover, in the hindbrain GLP-1 signaling appears to mediate the anorexic response to lipopolysaccharide (LPS) (44), which likely involves an intermediary step of interleukin elevation. Thus, it is possible that the connections discovered here between GLP-1 and IL-6 and IL-1 are relevant for anorexia of infection and inflammation, although it seems unlikely to mediate inflammation in general as discussed above.

In both genetically induced and pharmacological blockade of IL-1β or IL-6 alone, the attenuation of EX4-induced anorexia was most prominent at the 22-h food intake measurement, whereas simultaneous blockade of both interleukins also inhibited anorexia at 1, 4, and 22 h after EX4 injection. Integrating these results might suggest that at the early time points there may be a redundancy between IL-1β and IL-6 in mediating the anorexic effects of GLP-1R stimulation, whereas no such redundancy is detected at 22 h. In line with this, combined knockout of IL-6 and IL-1 activity in mice causes an earlier increase in body weight than knockout of either interleukin alone (24).

In the present study, in line with earlier studies (42, 45, 46), we found that EX4 reduced body temperature in the rat. Surprisingly, combined (but not single) blockade of IL-6 and IL-1 partly reversed the hypothermic effect of GLP-1R activation. Body temperature is often elevated by IL-1, with IL-6 having a permissive role. However, IL-1β administration in some circumstances may also be hypothermic (47), making it a possible potential mediator of GLP-1R–induced hypothermia, in line with the present data. Notably, the previously demonstrated IL-1β–induced hypothermia has a similar short onset latency and a similar time course to that observed here after EX4 treatment. Most importantly, however, this finding provides another example where IL-6 and IL-1β could mediate effects of GLP-1R stimulation in the CNS.

The fact that cytokines and inflammation-associated molecules other than IL-6 and IL-1 were not impacted by the GLP-1 analog stimulation in this study may support the claim that GLP-1R activation is likely not linked to a general inflammatory response but rather selectively targets IL-6 and IL-1β. The 11-fold elevation of IL-6 mRNA in the hypothalamus after the central GLP-1R stimulation was nearly twofold that previously reported after an LPS challenge (48). In fact, this places EX4 as one of the most potent known inducers of hypothalamic IL-6. In contrast, the hypothalamic IL-1β mRNA was elevated here to a much lesser extent than what is observed after LPS administration (48)

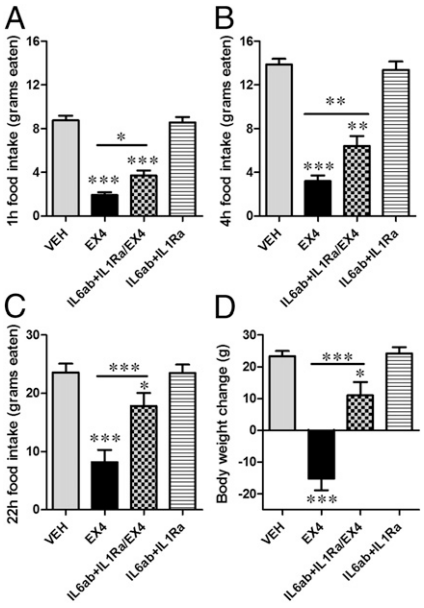

Fig. 4. Simultaneous IL-1R and IL-6 blockade synergistically attenuates food intake and weight loss responses to i.c.v. injection of EX4. Anorexic response to central EX4 after 1 h (*A*), 4 h (*B*), and 22 h (*C*) was significantly attenuated by the combination of IL-1Ra and IL-6ab treatment. Similarly, the EX4-induced weight loss was significantly attenuated by this combination treatment (*D*). For details of statistical analysis, see *SI Results*. Data are expressed as mean ± SEM. n = 11/treatment condition. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$.

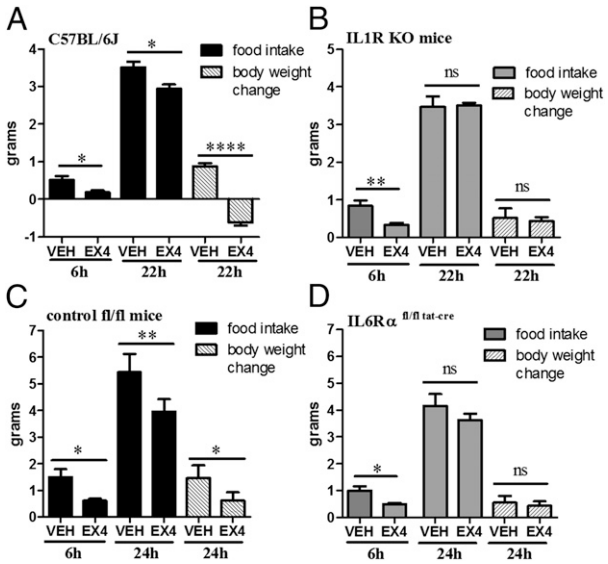

Fig. 5. EX4-induced hypophagia and body weight reduction are attenuated in IL-1R1$^{-/-}$ and IL6Rα$^{fl/fl\ tat\text{-}cre}$ mice. Peripherally (i.p.) injected EX4 reduces intake and body weight in C57BL/6J mice (*A*), although it fails to suppress food intake and body weight over 22 h in IL-1R1$^{-/-}$ (*B*) . Similarly, although IL6Rα$^{fl/fl}$ mice show a clear reduction in food intake and body weight after peripheral EX4 injection, IL6Rα$^{fl/fl\ tat\text{-}cre}$ mice do not display a significant intake or body weight reduction after EX4. Data are expressed as mean ± SEM. $n = 8$ (*A* and *B*), $n = 5$ (*C*), $n = 6$ (*D*)/treatment condition. *$P < 0.05$, **$P < 0.01$, ****$P < 0.0001$ vs. vehicle.

and not altered at all in the hindbrain or the hippocampus. Furthermore, TNFα, normally up-regulated in the hypothalamus after LPS (49), was not altered by EX4. This highlights the specificity of the transcriptional modulation induced by EX4, with a selective up-regulation of IL-6 primarily and IL-1β to a lesser extent. Moreover, locomotor depression is a common manifestation of systemic inflammation. Notably, it is not observed here after administration of EX4 or any of the interleukin-blocking agents. This might further support the conclusion that not only the cytokine profile but also the behavior displayed after EX4 stimulation differs from that observed during inflammatory responses. Thus, overall, the changes observed after EX4 administration differ in many ways from those seen after an inflammatory challenge and may instead reflect a physiologic function during health. In fact, there is growing body of literature to support a role for IL-6 and IL-1 in exerting a tonic-suppressing effect on body weight during health (18–21). In contrast, TNFα, a classic proinflammatory cytokine not enhanced by GLP-1R stimulation in this study, might not inhibit body weight during health (50). Moreover, GLP-1R stimulation has been reported to alleviate CNS inflammation caused by LPS or irradiation (51–53). Taken together, these data are consistent with the hypothesis that central GLP-1R–induced expression of IL-1β and IL-6 mediates suppression of food intake and body weight, more so than general inflammation. This issue is of importance, considering the clinical treatment with EX4 and other GLP-1R–stimulating analogs.

Although an up-regulation of IL-6 and IL-1β in the hypothalamus by EX4 is consistent with the well-documented anorexic and anti-obesity effects of these components acting on the hypothalamus, it might seem somewhat counterintuitive in light of other data showing that EX4 reduces LPS-induced IL-1β production in other brain regions, for example, the cortex (51). However, to bring forth the anti–IL-1 action of EX4 in the cortex, the hippocampus, or the ventral midbrain, a background of an abnormality/perturbation is required. Thus, EX4 alone did not change any of the cytokines or any other inflammatory markers measured in those extrahypothalamic areas at baseline, and the EX4-induced reduction of cytokines was noted only after LPS treatment (51, 52). This lack of effect of EX4 on IL-1 in the hippocampus of rats not given LPS was replicated here to indicate that the EX4-induced IL-1β is restricted to the energy balance-regulating hypothalamus.

Further studies are needed on several important aspects of this report that central IL-6 and IL-1 may be important mediators of the hypophagic and weight loss effect of GLP-1 receptor stimulation. These include determining the hypothalamic/hindbrain subnuclei of the GLP-1R–induced increases of IL-6 and IL-1 expression, as well as the sites of the anorexic and body weight-reducing effects of IL-6 and IL-1. Furthermore, although there was EX4-induced IL-6 expression in a neuronal cell line, GLP-1 in vivo may induce IL-6 and IL-1 expression in glia cells in addition to neurons, or even instead of neurons.

Clinically used GLP-1 analogs may provide beneficial effects on food intake and body weight reduction that are likely exerted at the level of the CNS, but the knowledge about how this effect is exerted is limited. Here we report evidence that, surprisingly, two cytokines, IL-6 and IL-1, mediate the suppression of food intake and body weight induced by GLP-1R stimulation. Although this finding is unexpected, it is in line with earlier reports that both IL-6 and IL-1 exert a tonic suppression of body weight in the absence of illness at the level of the CNS/hypothalamus. The current findings increase our knowledge of a widely used group of therapeutics and may result in a more refined treatment of obesity.

Materials and Methods

Animals. Adult male Sprague–Dawley rats (200–250 g, Charles River) were housed in a 12-h light/dark cycle (lights on at 6:00 AM) with regular chow and water available ad libitum in their home cages unless otherwise stated. For details on mouse strain use, see *SI Materials and Methods*. All animal procedures were carried out with ethical permission and in accordance with the University of Gothenburg Institutional Animal Care and Use Committee guidelines. For details of rat lateral ventricle guide cannula implantation, telemetric body temperature, and locomotor transponder surgery, see *SI Materials and Methods*.

Drugs. EX4 and AF 12198 (IL-1R antagonist; IL-1Ra) were purchased from Tocris, and IL-6 antibody (IL-6ab; H-183) was purchased from Santa Cruz (local distributor AH Diagnostics). All centrally injected substances were dissolved in artificial cerebrospinal fluid (aCSF) and stored as aliquots in -20C (EX4 and IL-1Ra) and -4C (IL-6ab). EX4 was used in the current study due to its stability and clinical relevance; EX4 is a long-lasting analog of GLP-1 and a full agonist at the GLP-1R (54).

Central GLP-1R Stimulation Effect on the Hypothalamic and Hindbrain Cytokines, Neuropeptides, and Intracellular Signaling Molecules. Hypothalamic and hindbrain/DVC gene and protein expression after lateral ventricle injection of the GLP-1 analog, EX4, or vehicle (aCSF) [as previously described (55)] was measured via real-time PCR and Western blotting. Antibodies used in Western blotting are described in Table S1. For details, see *SI Materials and Methods* and Table S1.

Interaction of Central GLP-1R Stimulation Effect on Food Intake, Body Weight, and Temperature with IL-6 and IL-1β. To determine whether signaling at the IL-1R or IL-6 (or the combination of the two) mediates anorexic, body weight-suppressing, locomotor, and thermoregulatory effects of central GLP-1R stimulation, rats received four counterbalanced injection conditions over 4 experimental days as described in *SI Materials and Methods*.

IL-1R1$^{-/-}$ Mice. To establish the requirement of IL-1 signaling for the anorexic effect of EX4 in a nonpharmacological model, IL-1R1$^{-/-}$ mice (B6.129S7-*Il1r1*^{tm1Imx}/J) or wild-type controls (C57BL/6J) were peripherally (IP) injected with 0.6 μg EX4 or vehicle in a counterbalanced order. Food intake and body weight were measured at 6 and 22 h.

Brain IL-6Rα Knockdown. A Tat-cre fusion protein, synthesized as described (56), was stereotaxicaly infused to the lateral ventricle according to a previously described protocol (57) to male isoflurane anesthetized mice that were homozygously floxed for IL-6Rα (strain B6; SJL-*Il6ra*$^{tm1.1Drew}$) (30). Tomato expression was decreased in cells surrounding the ventricles following treatment with Tat-cre according to a similar protocol in dtTomato$^{loxP/+}$ mice (Fig. S4). To test the effect of GLP-1R stimulation in these mice, mice were fasted overnight and injected i.p. with 0.3 μg EX4 or 0.1 mL of saline as a control in a counterbalanced order with 48 h between each condition. The

food intake was measured at 6 and 24 h, and the body weight was measured after 24 h. This procedure was repeated 1 wk later, and the values obtained from both sessions were averaged.

Cell Culture. Neuro2A cell cultures were incubated with 10 nM EX4 for up to 45 min, and expression of IL-6 was measured via real-time PCR at 15- and 45-min time points. For details see *SI Materials and Methods*.

Statistical Analysis. All behavioral parameters were analyzed by repeated measures ANOVA followed by post hoc Tukey HSD test as appropriate or Student *t* test where only two conditions were compared. All statistical analyses were conducted using the GraphPad software. Differences were considered significant at $P < 0.05$.

ACKNOWLEDGMENTS. This research was funded by the Swedish Research Council (2011-3054 and K2013-54X-09894-22-3), Avtalet om Läkarutbildning och medicinsk Forskning Göteborg (SU7601 and SU142921), Swedish Foundation for Strategic Research to Sahlgrenska Center for Cardiovascular and Metabolic Research (A305-188), European Union Seventh Framework Programme (FP7/2007-2013) under Grant Agreement 266408 (Full4Health), Deutsche Forschungsgemeinschaft Research Fellowship, Fru Mary von Sydow's Foundation, French Agence Nationale de la Recherche (ANR-09-BLAN-0267), and Foundation pour la Recherche Medicale (Equipe FRM DEQ20130326524).

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