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The effect of TNF α on food intake and central insulin sensitivity in rats

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Abstract

Circulating and tissue levels of the proinflammatory cytokine tumor necrosis factor α (TNF α) are elevated in obesity. TNF α interferes with insulin signaling in many tissues and also plays a causal role in the anorexia that accompanies severe challenges to the immune system. The interactions between TNF α and insulin in the control of eating are less well known. The present study evaluated the role of TNF α in the central nervous system control of food intake by insulin in adult male Long Evans rats. We first determined the ability of several doses of TNF α injected into the 3rd cerebral ventricle (i3vt) to reduce food intake in male rats. Subsequently, we assessed the ability of a subthreshold dose of TNF α to modulate the effect of i3vt insulin on food intake in male rats fed a low-fat chow or a high-fat (HF) diet. TNF α administered i3vt dose-dependently reduced food intake in rats fed a standard low-fat chow diet. Moreover, a low, sub-threshold dose of TNF α diminished the reduction in food intake by insulin in rats maintained on a chow diet, but enhanced insulin action in rats maintained on a HF diet. These data suggest that the interaction of TNF α with central insulin varies with nutritional and/or dietary conditions.

Keywords

food intake; inflammation; tumor necrosis factor; insulin; brain

The incidence of obesity and related co-morbidities affects individuals of all ages and both sexes and has become a world-wide epidemic. Consequently, it is essential that therapeutic approaches to overcome this crisis be developed, and numerous pharmacological targets are currently under investigation. In this regard, it has become increasingly clear that obesity is accompanied by low-grade inflammation, suggesting a link between metabolism and the immune system [1-5]. Both macrophages and adipocytes within adipose tissue secrete numerous cytokines, and as adipose tissue expands, the levels of proinflammatory cytokines also increase, both within the adipose tissue itself as well as systemically [6]. This inflammation has been hypothesized to play a causal role both in adipose tissue expansion

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via direct actions of cytokines on adipose tissue [4,7] and in the development of obesity-related co-morbidities, such as insulin resistance, through insulin-desensitizing actions at the insulin receptor in several tissues [8].

Tumor necrosis factor α (TNF α) is one such proinflammatory cytokine and a major mediator of innate immune reactions. As obesity increases, TNF α levels in plasma and in adipose tissue also increase, and this has been implicated in many symptoms of the metabolic syndrome [3,6]. In peripheral tissues, TNF α reduces insulin sensitivity by impairing insulin signal transduction [8,9]. On the other hand, TNF α also mediates the cachexia that accompanies cancer and severe infections, and there is evidence that TNF α acts within the central nervous system, particularly within the hypothalamus, to contribute to this catabolic condition [10-15].

The findings that TNF α levels directly correlate with the level of adiposity, and that TNF α acts within the CNS to promote negative energy balance, are reminiscent of what is known about the pancreatic hormone insulin. It is impossible to write about the central actions of insulin without mention of the work of Steve Woods, the person celebrated in this Festschrift. His studies were seminal in elucidating how insulin communicates the level of energy stored in the body to the brain and how it helps control food intake and defend a particular level of adiposity [16-22]. A more recent revelation is that many different factors influence the sensitivity of the brain to insulin. For example, when animals are maintained on a high-fat (HF) diet, brain insulin sensitivity decreases while the expression of inflammatory markers, including TNF α , within the CNS increases [23-25]. Given that TNF α is well-known to impair systemic insulin signaling [8,9] and that both insulin and TNF α act within the hypothalamus to control eating, it is reasonable to hypothesize that TNF α and insulin interact at the level of the hypothalamus. Some evidence in fact suggests that this may be the case [15,26,27].

The studies presented here were inspired by our discussions with Steve Woods. We investigated the ability of i3vt administered TNF α to modify the effects of insulin on food intake in male rats fed a low-fat or a high-fat diet. As TNF α impairs insulin signaling in peripheral tissues, one logical hypothesis is that a low dose of TNF α would similarly reduce brain insulin sensitivity, resulting in less suppression of food intake in response to centrally-administered insulin. On the other hand, the fact that both TNF α and insulin, when administered individually into the brain, reduce food intake, might suggest the converse hypothesis that the two peptides would have an additive or even synergistic effect when administered in combination.

Methods

Animals

Adult Long Evans rats (Harlan, Indianapolis, IN) were individually housed in tub cages with bedding and maintained on a 12-hour light, 12-hour dark cycle (lights on at 0100 h) and given *ad libitum* access to water and food unless otherwise noted. Rats weighed 415-515 g at the start of the experiments and were fed either standard rodent chow (Harlan Teklad [LM485], Indianapolis, IN; 3.1 kcal/g; ~5% fat) or a high-fat (HF) diet (Research Diets, New Brunswick, NJ; 4.54 kcal/g; ~41% fat). All procedures were approved by the University of Cincinnati Institutional Animal Care and Use Committee.

Stereotaxic surgery

Seven days after arrival, rats were anesthetized with 0.1 ml/100g body weight intraperitoneal (ip) injections of a ketamine (70 mg/kg)/xylazine (2 mg/kg) mixture. Subsequently, 22-gauge guide cannulas (Plastics One Inc., Roanoke, VA) were

stereotactically implanted into the 3rd cerebral ventricle (i3vt). Briefly, bregma and lambda were situated at the same vertical coordinate and cannula tips were positioned on the midline, 2.2 mm posterior to bregma and 7.5 mm ventral to the dura mater. The cannulas were then fixed to the skull using dental acrylic and anchor screws. Obturators extending 0.5 mm beyond the cannula tract were inserted. When all rats had returned to their pre-surgical body weights, cannula placement was confirmed by i3vt infusion of 10 µg of NPY in 1 µl normal saline 3 h prior to the onset of dark and monitoring the rats' food intake over a 60-min period. Animals that did not eat at least 2 g of chow within 60 min were excluded from the study.

TNFα

Prior to the experiments, stock solutions of murine TNFα (R&D Systems, Minneapolis MN) were made at a concentration of 10 ng/µl in 0.2% BSA/PBS. On experimental days, the murine TNFα stock solutions were diluted to the appropriate concentrations (0, 0.3, 1, 3 and 10 pg/µl, equivalent to: 0, 17.6, 58.2, 176.5 and 588.2 pM) in 0.2% BSA/PBS and delivered i3vt in a volume of 1 µl. Similarly, insulin (100 U/ml; Novolin R Regular human insulin, Novo Nordisk, Princeton, NJ) was diluted to the appropriate concentration of 10 U/ml (equivalent to 60 µM) in saline on the day of the experiment.

Experiment 1

A cohort of male rats maintained on chow received each dose of TNFα (0, 0.3, 1, 3 and 10 pg i3vt) in an individually randomized order with 3 non-injection days occurring between successive injections. On each experimental day, food hoppers were removed from cages and weighed 3 h prior to the onset of dark. At 45 min prior to the onset of the dark, body weights were recorded and rats received a 1 µl i3vt injection of vehicle or vehicle plus TNFα which was slowly infused with a Hamilton Syringe. Food hoppers were returned at the onset of dark and weighed at 4 and 24 h after infusion.

Experiment 2

A separate group of rats from the same cohort as in Experiment 1 was fed chow or the HF diet for 2 weeks before receiving i3vt cannulas. After recovery from the surgery and verification of cannula placement, there were 4 experimental treatments with at least 3 non-injection days occurring between treatment days. Each rat received each treatment in an individually randomized order. On experimental days, rats were fasted 4 h prior to the dark and received two 1 µl i3vt injections, spaced 5 min apart, just before dark: vehicle (saline)/vehicle (BSA/PBS), insulin (10 mU)/vehicle, vehicle/TNFα (1 pg, based on Experiment 1), or insulin/TNFα. Food intake was assessed after 4 and 24 h and body weight was assessed after 24 h.

Data Analysis

Data were analyzed using Statistica (StatSoft, Tulsa, OK, USA). Food intakes were assessed using a repeated measures analysis of variance (ANOVA). Main effects and interactions were assessed with Student-Newman-Keuls test.

Results

i3vt TNFα dose-dependently reduces food intake in rats

In Experiment 1, young adult male rats were used to determine an optimal sub-threshold dose of TNFα for use in the subsequent experiment. As depicted in Figure 1, i3vt TNFα dose-dependently reduced food intake at both 4 and 24 h in rats maintained on low-fat chow. Both the 3 and the 10 pg doses effectively reduced food intake at the 24-h time-point and the

10 pg dose was also effective at the 4-h time-point ($p < 0.05$; Figure 1). Based on this, a dose of 1 pg of TNF α was selected as a sub-threshold dose for use in Experiment 2.

TNF α modulates central sensitivity to insulin's anorectic actions

The TNF α alone (1 pg) had no effect on food intake or body weight on rats maintained on either the chow or the HF diet, indicating that the dose was indeed sub-threshold (Figures 2A-C). Consistent with previous studies by Woods and colleagues, insulin alone at a dose of 10 mU reduced 24-h food intake ($p < 0.05$; Figure 2B) and body weight ($p < 0.05$; Figure 2C) in rats maintained on the chow diet, but failed to do so in rats maintained on the HFD [17, 24, 28]. The combination of insulin plus TNF α blunted insulin's ability to reduce 24-h food intake and body weight in rats maintained on chow and enhanced its ability to reduce food intake and body weight in rats maintained on the HFD (Figures 2A-C).

Discussion

The present experiments represent an initial foray to consider how TNF α interacts with factors that act in the brain to regulate energy balance, in particular insulin. TNF α binds to two distinct receptors, the type 1 (TNFR1 or p55) and type 2 (TNFR2 or p75) TNF α receptors [29], both of which are found in the brain [30]. Activation of these receptors then mediates TNF α 's physiological actions such as the initiation of proinflammatory responses and stimulation of nitric oxide (NO), as well as numerous other functions [29]. With regard to energy balance, stimulation of TNF α receptors plays a well-established role in the cachexia that accompanies certain cancers and severe infections, and it is clear that TNF α 's actions within the central nervous system, particularly within the hypothalamus, mediate this catabolism [10-15]. Consistent with this, the first finding of this set of experiments was that i3vt TNF α dose-dependently reduces food intake in adult male Long Evans rats.

In addition to its catabolic role in the brain, another characteristic of TNF α is that it acts at various peripheral tissues to impair insulin signal transduction [9]. Less clear, however, is whether TNF α interacts with insulin in the brain. In this regard, another finding of these experiments was that a subthreshold dose of i3vt TNF α (1 pg; determined in Experiment 1) reduces sensitivity to the anorectic actions of i3vt insulin in rats fed a normal low-fat chow diet, while it enhances insulin action in rats fed a HF diet. One implication is that when rats are maintained on a relatively low-fat diet, TNF α causes central insulin resistance. Conversely, when rats are fed a HF diet, central TNF α enhances the catabolic action of insulin.

The ability of a low-dose of TNF α to reduce insulin's anorectic actions in rats maintained on chow is consistent with reports by Romanatto *et al.* and Moraes *et al.* in which a low subthreshold dose of TNF α (0.035 pg) administered into the lateral cerebral ventricle attenuated the ability of insulin as well as of leptin to inhibit food intake in male rats maintained on a chow diet [15,26,27]. Conversely, a higher dose of TNF α (350 pg) that did reduce food intake by itself did not interact with insulin's anorectic action [15,26,27].

There is substantial evidence supporting an interaction between hypothalamic inflammation and insulin sensitivity [3,5,23]. Increases in hypothalamic proinflammatory signaling, as occurs with the consumption of a HF diet or with the administration of a sub-threshold dose of TNF α , impairs the sensitivity to the anorexigenic actions of insulin [27]. Moreover, TNF α 's stimulates nitric oxide (NO) production and Moreas *et al.* ascertained that this mediates, at least in part, TNF α 's insulin desensitizing actions in the brain. In this regard, a low dose of TNF α (0.035 pg) stimulates NOS catalytic activity in the hypothalamus in rats and also reduces sensitivity to insulin-induced anorexia in rats, wild-type mice and neuronal nitric oxide synthase (nNOS) knockout mice but fails to do so in inducible nitric oxide

synthase (iNOS) deficient mice, suggesting that nitric oxide (NO) produced by iNOS in response to TNF α reduces central insulin sensitivity [26]. It is possible that a similar mechanism underlies the insulin-desensitizing actions of TNF α in the present studies.

Another important finding of the present studies is that TNF α has divergent effects on the brain's sensitivity to insulin based on the nutritional or dietary conditions. When exposed to a HF diet, the interaction between TNF α and insulin actually becomes positive. In this regard, and also consistent with our results, an increase in hypothalamic inflammation has also been determined to reduce food intake [26]. Administration of TNF α into the lateral cerebral ventricle induces the expression of hypothalamic neuropeptides that inhibit food intake, including proopiomelanocortin (POMC) and corticotrophin-releasing hormone (CRH) to a greater extent than neuropeptides that stimulate appetite [31]. Perhaps when rats are fed a HF diet and subsequently administered the sub-threshold dose of TNF α in combination with insulin, these mechanisms contribute to the enhanced hypophagia. Another possible explanation for these differences based on dietary conditions relates to the role of reactive oxygen species (ROS) in energy balance [31]. Although, ROS are implicated in peripheral insulin resistance [32], insulin's ability to induce ROS in the hypothalamus is a critical step for promoting catabolism [31]. Exposure to a HF diet not only reduces insulin's ability to reduce food intake but also reduces its ability to stimulate ROS [31]. On the other hand, TNF α is a potent stimulator of ROS. Perhaps, this increase in ROS induced by TNF α is then able to reestablish insulin's anorectic action on a HF diet.

In summary, TNF α dose-dependently reduces food intake in male rats fed a standard chow diet. Moreover, when administered directly into the brain, a low, sub-threshold dose of TNF α diminishes insulin-induced anorexia in rats maintained on a chow diet, but enhances insulin action in rats maintained on a HF diet, indicating that the interaction between TNF α and insulin in the brain depends on the dietary conditions.

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Figure 1. Mean 4- and 24-h food intake (kcal) of male Long Evans rats maintained on chow and administered TNF α i3vt at the indicated doses. * = $p < 0.05$. $n = 6/\text{group}$. Bars = 1 SEM.

Figure 2.

Mean 4-h caloric intake (A), 24-h caloric intake (B), and change in body weight (C) of male Long Evans rats 24 h after receiving vehicle or insulin (10 mU) i3vt, followed 5 min later by TNF α (1 pg) or vehicle i3vt. Rats were maintained on chow or HF diet for 2 weeks prior to the start of the experiment. * = $p < 0.05$. $n = 6$ /group. Bars = 1 SEM.