IL-6 ameliorates defective leptin sensitivity in DIO ventromedial hypothalamic nucleus neurons

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Larsen L, Le Foll C, Dunn-Meynell AA, Levin BE. IL-6 ameliorates defective leptin sensitivity in DIO ventromedial hypothalamic nucleus neurons. Am J Physiol Regul Integr Comp Physiol 311: R764–R770, 2016. First published August 17, 2016; doi:10.1152/ajpregu.00258.2016.—Rats selectively bred to develop diet-induced obesity (DIO) have an early onset reduction in the sensitivity of their ventromedial hypothalamic nucleus (VMN) neurons to leptin compared with diet-resistant (DR) rats. This reduced sensitivity includes decreased leptin receptor (Lepr-b) mRNA expression, leptin receptor binding, leptin-induced phosphorylation of STAT3 (pSTAT3), and impaired leptin excitation (LepE) of VMN neurons. When administered exogenously, the pancreatic peptide, amylin, acts synergistically to reduce food intake and body weight in obese, leptin-resistant DIO rats by increasing VMN leptin signaling, likely by stimulation of microglia IL-6, which acts on its receptor to increase leptin-induced pSTAT3. Here, we demonstrate that incubation of cultured VMN neurons of outbred rats with IL-6 increases their leptin sensitivity. Control, dissociated DIO VMN neurons express 66% less Lepr-b and 75% less Bardet Biedl Syndrome-6 (BBS6) mRNA and have reduced leptin-induced activation of LepE neurons compared with DR neurons. Incubation for 4 days with IL-6 increased DIO neuron Lepr-b expression by 77% and BBS6 by 290% and corrected their defective leptin activation of LepE neurons to DR levels. Since BBS6 enhances trafficking of Lepr-b to the cell membrane, the increases in Lepr-b and BBS6 expression appear to account for the corrected reduction in leptin-excitation of DIO LepE neurons to that of control DR rats. These data support prior findings suggesting that IL-6 mediates the leptin-sensitizing effects of amylin on VMN neurons and that the inherent leptin resistance of DIO rats can be effectively reversed at a cellular level by IL-6.

R764

Reduced sensitivity to signaling properties, anorectic, and thermogenic effects of leptin, i.e., leptin resistance, is a regular accompaniment of obesity in animals (11, 37) and humans (5). Such resistance likely contributes to the fact that, despite having high levels of leptin, obese individuals continue to maintain an elevated level of adiposity and caloric intake which does not respond to the normal regulatory negative feedback of endogenous leptin and other hormones (5, 11, 37) or to leptin given exogenously (27, 37). Studies by Roth et al. (27) demonstrated that pharmacological administration of amylin, a peptide which is produced by the pancreatic β-cell and coreleased with insulin in response to food intake, caused weight loss in obese rats and humans, an effect that acted synergistically to increase the effects of coadministered leptin (27, 33, 35). While amylin has its primary short-term satiating effect in the area postrema, amylin’s enhancement of leptin’s effect on weight loss appears to be mediated in the ventromedial nucleus of the hypothalamus (VMN) where amylin increases signaling downstream of the long-form of the leptin receptor (Lepr-b) by enhancing leptin-induced phosphorylation of STAT3 (27, 36) and also increases binding of leptin to its cell surface receptor (36). We later demonstrated that this amylin effect was mediated by stimulation of VMN microglia to produce the cytokine IL-6, which acts on its own IL-6/gp130 receptor complex to phosphorylate STAT3 to amplify the activation produced by leptin (20). Thus, amylin can be thought of as a leptin sensitizer, which acts via microglia-produced IL-6 to enhance leptin signaling downstream of its receptor.

We have shown that the selectively bred diet-induced obese (DIO) rat has early onset leptin resistance before it becomes obese. This resistance manifests as a reduced expression of the Lepr-b gene (21, 22), binding of leptin to its cell surface receptor (13), leptin-induced phosphorylation of STAT3 (4, 21), hypothalamic development (4), anorexia, and thermogenesis (9). Amylin overcomes this resistance and produces significant weight loss when coadministered with leptin in obese selectively bred DIO rats (34). At the cellular level, VMN neurons from DIO rats exhibit reduced activation by leptin compared with neurons from selectively bred diet-resistant (DR) rats (14). We postulated that, since this synergistic effect of amylin and leptin appears to be mediated by IL-6 in the VMN, prolonged exposure of DIO VMN neurons to IL-6 would correct their resistance to the activating effects of leptin. We further postulated that this correction might do so by increasing the production of Lepr-b and/or increasing its transport to and residence in the neuronal cell membrane. Thus, we first demonstrated that cultured VMN neurons retained their sensitivity to leptin and that exposure to increasing concentrations of IL-6 increased the leptin sensitivity of VMN neurons from outbred rats. We next demonstrated that 4 days of exposure to IL-6 increased the sensitivity of DIO VMN neurons to the activating effects of leptin comparably to that in DR neurons and that such exposure increased the expression of both Lepr-b and Bardet-Biedl Syndrome (BBS) protein 6, one of a family of proteins known to act as chaperones that facilitate the transport of Lepr-b to the cell surface (10, 29).

METHODS

Animals. Animals were housed at 23–24°C on a 12:12-h light-dark cycle (lights off at 1000). Male Sprague-Dawley rats (Charles River Laboratories) and selectively bred DR and DIO rats from our in-house colonies were used between 21 and 28 days of age. All rats were
weaned at 21 days of age onto Purina Rat Chow (rat chow) and fed chow and water ad libitum. All work was in compliance with the Animal Care and Use Committee of the East Orange Veterans Affairs Medical Center.

Culture of dissociated VMN neurons and assessment of leptin responsiveness and sensitivity. At postnatal day (P) 21–P28, ketamine/xylazine-anesthetized rats were perfused with cold oxygenated perfusion solution. Brains were removed and sectioned through the hypothalamus with a vibratome (300 μm) in oxygenated semifrozen perfusion solution. The VMN was bilaterally punched, and neurons were dissociated, as previously described (16, 17, 19). Briefly, neurons were centrifuged, and the pellet was resuspended with 200 μl Neurobasal-A media (Invitrogen, Carlsbad, CA) containing 2.5 mM glucose, 1 mM L-lactate, 0.23 mM l-pyruvate, 2% B27, 0.5 mM L-glutamine, and 100 units/ml penicillin/streptomycin. Neurons were plated on coverslips in a 6-well plates containing 2 ml of the above media and incubated at 37°C, 5% CO2. Three sets of neurons from bilateral punches from four neonates were then equally aliquoted. Three aliquots were assessed for leptin responsiveness after 4–6 h (“freshly dissociated”), and three or four aliquots were placed into separate wells for culture for 1, 2, or 4 days in culture and then assessed for leptin responsiveness.

Individual VMN neurons were assessed for leptin-induced alterations in intracellular calcium ([Ca2+]i), oscillations as a surrogate for leptin-induced activation or inactivation of VMN neurons held at 2.5 mM glucose, as previously described (12, 14, 17, 19). Neurons were classified as leptin excited (LepE) if the area under the curve (AUC) for [Ca2+]i was more than 30% above baseline over the 10 min following sequential addition of 0.1, 1, and 10 fM leptin (National Hormone & Pituitary Program, Torrance, CA) to the media for 10 min at each concentration. Neurons were defined as leptin inhibited (LepI) if the AUC was 30% below baseline for 10 min or as having a biphasic response to leptin (LepB) if AUC varied from both greater and less than 30% from baseline over the 10 min after leptin addition. Leptin sensitivity was defined as the percent change in [Ca2+]i, AUC relative to baseline in response to leptin. All neurons were incubated with 20 nM glutamate terminally to ensure that they were functionally viable.

Effects of IL-6 exposure on dissociated VMN neuron responses to leptin. Neurons from bilateral VMN punches from four P21 male Sprague-Dawley rats were dissociated, and two aliquots were assessed within 4–6 h (freshly dissociated). Another 12 aliquots (2 aliquots at each day and vehicle vs. IL-6 concentration) were cultured for 1, 2, or 4 days in the presence of vehicle (PBS) or 10, 100, or 400 ng/ml IL-6 (Sigma) added once on the day of dissociation and twice a day on the following 3 days. Neurons in each group (vehicle vs. cultured in IL-6) were then assessed for the percentage of neurons responding to leptin (0.1, 1, and 10 fM) as being LepE, LepI, or LepB following the above criteria using calcium imaging. Additional neurons from two rats were cultured for 4 days in the presence of IL-6 (200 ng/ml) and then categorized as LepE neurons were assessed for their leptin sensitivity.

Effects of 4 days of IL-6 incubation on leptin sensitivity and leptin signaling genes in DR vs. DIO VMN LepE neurons. Dissociated VMN neurons from male P21–P28 DR and DIO rats (n = 4 per group) were cultured (4 wells for vehicle vs. IL-6) for 4 days in the presence of vehicle or IL-6 (200 ng/ml) and those that were characterized as LepE by calcium imaging were assessed for their leptin sensitivity (percent increase in [Ca2+]i, AUC) following sequential additions of 0.1, 1, and 10 fM leptin. Following assessment for leptin responsiveness, 120 μl RNA lysis buffer (Ambion, Austin, TX) was added to the neurons, and the supernatant was saved at −80°C for later real-time quantitative PCR (QPCR) determinations (see below).

Expression of genes involved in leptin signaling in dissociated VMN neurons. After determination of leptin sensitivity and lysis of neurons, as above, mRNA was quantified by quantitative PCR, as previously described (15, 19, 23). The resultant cDNA was analyzed using TQAqMan MGB primer/probes sets targeting genes for the long form of the leptin receptor (Lepr-b), endospanin-1, Bardet-Biedl syndrome proteins (BBS) 1, 2, 4, and 6. The housekeeping gene cyclophilin was used to correct results for variability in sample size and/or extraction and reverse transcription efficiency. For each gene of interest, the average relative abundance of that gene for all samples in a given experiment was set at 100%, and the data represent the abundance of the gene in a given sample relative to the overall group abundance. (15, 19, 23).

Statistics. One-way and two-way ANOVA were used for gene expression data. ANOVA for repeated measures were used for the concentration-response studies. Post hoc Bonferroni corrections were made for multiple comparisons. Outliers were removed if necessary (Systat, Chicago, IL).

RESULTS

Preliminary studies: effects of days in culture and exposure to IL-6 on dissociated VMN neuron responses to leptin from outbred rats. To ensure that time in culture had no substantial effect on the responses of dissociated neurons to leptin, a preliminary study was carried out in freshly dissociated and cultured neurons from VMN micropunches from outbred Sprague-Dawley rats. In neurons assessed within 4–6 h of dissociation, 62% were LepE, 3% were LepI, and 7% showed a biphasic response to LepB over a concentration range of 0.1, 1, and 10 fM (Table 1). Among LepE neurons, there was a modest (16%) increase in the leptin-induced AUC of intracellular calcium [Ca2+]i, which we have used as a surrogate for neuronal activation (12, 14, 17, 19), through the 100-fold increase in leptin concentration (Fig. 1). When dissociated neurons from the same rats were assessed after 1, 2, and 4 days in culture, there was no substantial change in the percentage of neurons that were excited, inhibited, or had a biphasic response to leptin on days 2 or 4, although on day 1, there were 37% fewer LepE neurons and 50% fewer neurons overall responded to leptin than did freshly dissociated neurons (Table 1). When assessed for sensitivity to leptin (0.1, 1, and 10 fM), there were no statistical differences in AUC between freshly dissociated neurons or those cultured for 1, 2, or 4 days (Fig. 1). These preliminary studies, thus, demonstrate that dissociated neurons

Table 1. Effect of days in culture on dissociated ventromedial hypothalamic nucleus neuron responses to leptin

<table>
<thead>
<tr>
<th>Day</th>
<th>LepE</th>
<th>LepI</th>
<th>LepB</th>
<th>NLep</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>67 ± 1(41)a</td>
<td>42 ± 6 (35)b</td>
<td>19 ± 4 (16)c</td>
<td>12 ± 3 (11)d</td>
</tr>
<tr>
<td>Day 1</td>
<td>76 ± 4 (37)a</td>
<td>9 ± 1 (4)c</td>
<td>4 ± 1 (2)d</td>
<td>11 ± 3 (6)b</td>
</tr>
<tr>
<td>Day 2</td>
<td>74 ± 2 (32)a</td>
<td>9 ± 1 (4)c</td>
<td>5 ± 1 (2)d</td>
<td>14 ± 2 (6)b</td>
</tr>
<tr>
<td>Day 4</td>
<td>74 ± 2 (32)a</td>
<td>9 ± 1 (4)c</td>
<td>5 ± 1 (2)d</td>
<td>14 ± 2 (6)b</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE percent of total responses at a given day with number of neurons assessed in parentheses. Freshly dissociated neurons (Day 0) from ventromedial hypothalamic nucleus (VMN) micropunches from four outbred P21 Sprague-Dawley rats were assessed for their responsiveness to leptin (0.1, 1, 10 fM) in 2.5 mM glucose and compared to responses to comparable neurons cultured of 1, 2, and 4 days. For this study, neurons from three separate aliquots from freshly dissociated neurons (Day 1) and from 3 or 4 wells on Days 1, 2, and 4 in culture were assessed for leptin responsiveness using calcium imaging to characterize them as LepE (leptin excited), LepI (leptin inhibited), LepB (biphasic leptin response), and NLep (no leptin response). In rows with superscript letters, percent responding neurons in each category with differing letters differed by P = 0.05 or less when compared by one-way ANOVA and post hoc t-test using Bonferroni correction for multiple comparisons.

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Dissociated ventromedial hypothalamic nucleus (VMN) neurons from four outbred P21 Sprague-Dawley rats were assessed for their responsiveness to leptin (0.1, 1, and 10 fM) in 2.5 mM glucose and compared with the responses to comparable neurons cultured for 1, 2, and 4 days. Those characterized as LepE were assessed for their sensitivity to 0.1, 1, and 10 fM leptin. Data are expressed as means ± SE percent increases in [Ca^{2+}]_i. AUC from baseline in response to leptin. There were no statistical differences among the concentration responses in freshly dissociated vs. cultured neurons compared by one-way ANOVA for repeated measures.

Retain their responsiveness to leptin over at least 4 days in culture.

Next, preliminary studies to assess the effects of culturing outbred rat VMN neurons for 4 days with IL-6 were carried out with vehicle vs. 10, 100, and 400 ng/ml IL-6 and subsequent measurement of responses of the various classes of leptin-responsive neurons to 10 fM leptin on day 5 (Table 2). In these studies, there was a progressive increase in the percentage of LepE neurons (i.e., the percentage of VMN that were excited by leptin), with increasing concentrations of leptin, with little obvious effect on LepI or LepB neurons to leptin. This suggests that 4 days of culture in IL-6 had its primary effect on increasing the number of LepE neurons, which responded to leptin. Of note, the percentage of LepE relative to LepI neurons in vehicle-treated neurons in this study was, for unclear reasons, less than that in neurons cultured for 4 days in Table 1. Nevertheless, an additional study was carried out in dissociated VMN neurons from outbred rats, which were incubated for 4 days with vehicle vs. 200 ng/ml IL-6 and assessed for sensitivity (percent increase in [Ca^{2+}]_i; AUC) of LepE neurons to increasing doses of leptin (Fig. 2). Incubation with IL-6 for 4 days significantly increased the [Ca^{2+}]_i AUC responses to leptin by 20–43% over a range of 0.1–10 fM leptin compared with neurons incubated with vehicle.

Effects of 4 days of IL-6 incubation on leptin sensitivity and leptin signaling genes in DR vs. DIO VMN LepE neurons. Dissociated VMN neurons from P21 DR and DIO rats were cultured in the presence of vehicle or 200 ng/ml IL-6 for 4 days and then assessed for their sensitivity to increasing concentrations of leptin (0.1, 1, and 10 fM) in 2.5 mM glucose using calcium imaging (Fig. 3). In confirmation of our previous results in freshly dissociated neurons (12), DIO VMN neurons were less sensitive to increasing concentrations of leptin than were those from DR rats after 4 days of incubation in vehicle. However, incubation in IL-6 for 4 days increased the sensitivity of DIO LepE neurons to the level of DR neurons incubated with vehicle, while IL-6 incubation actually decreased the sensitivity of DR neurons to the level of that seen in vehicle-treated DIO neurons.

After these neurons were assessed for their leptin sensitivity, they were lysed and assessed for their expression of Lepr-b and genes involved in the transport of leptin receptors to the cell membrane; endospanin-1 (6, 30) and Bardet Biedl 1, 2, 4, and 6, (BBS 1, 2, 4, and 6) (7, 26, 29). Relative to the constitutively expressed gene, cyclophilin, DIO VMN neurons cultured for 4 days in vehicle had 66% less expression of Lepr-b and 75% less expression of BBS6 genes compared with DR neurons (Table 3). Given the lack of differences between DIO and DR neuron expression of endospanin-1, BBS 1, 2, and 4, determinations for only Lepr-b and BBS 6, as well as BBS4 as a control, were made after incubation with IL-6. Although IL-6

![Graph](http://ajpregu.physiology.org/)

**Fig. 1.** Effect of days in culture on dissociated ventromedial hypothalamic nucleus (VMN) leptin excitation (LepE) neuron sensitivity to leptin in outbred rats. Freshly dissociated neurons (day 0) from VMN micropunches from four outbred P21 Sprague-Dawley rats were assessed for their responsiveness to leptin (0.1, 1, and 10 fM) in 2.5 mM glucose and compared with the responses to comparable neurons cultured for 1, 2, and 4 days. Those characterized as LepE were assessed for their sensitivity to 0.1, 1, and 10 fM leptin. Data are expressed as means ± SE percent increases in [Ca^{2+}]_i. AUC from baseline in response to leptin. There were no statistical differences among the concentration responses in freshly dissociated vs. cultured neurons compared by one-way ANOVA for repeated measures.

**Table 2.** Effect on leptin responsiveness of culturing dissociated outbred rat VMN neurons with increasing doses of IL-6 for 4 days

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>10 ng/ml</th>
<th>100 ng/ml</th>
<th>400 ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>LepE</td>
<td>22 (9)</td>
<td>31 (10)</td>
<td>52 (20)</td>
<td>75 (21)</td>
</tr>
<tr>
<td>LepI</td>
<td>5 (2)</td>
<td>0 (0)</td>
<td>5 (2)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Biphasic</td>
<td>0 (0)</td>
<td>6 (2)</td>
<td>5 (2)</td>
<td>4 (1)</td>
</tr>
<tr>
<td>NLep</td>
<td>73 (30)</td>
<td>63 (20)</td>
<td>38 (15)</td>
<td>21 (6)</td>
</tr>
<tr>
<td>Total</td>
<td>100 (41)</td>
<td>100 (32)</td>
<td>100 (39)</td>
<td>100 (28)</td>
</tr>
</tbody>
</table>

Dissociated ventromedial hypothalamic nucleus (VMN) neurons from four outbred P21 Sprague-Dawley rats were assessed for their responsiveness to 10 fM leptin in 2.5 mM glucose on day 5 after 4 days of culture in vehicle or 10, 100, or 200 ng IL-6. Neurons from two wells for vehicle and for each concentration of IL-6 were characterized by calcium imaging as LepE, LepI, LepB, and NLep. Data are mean percent of total responses at a given day with number of neurons assessed in parentheses. There are no SE provided since only two sets of neurons per group were assessed.

![Graph](http://ajpregu.physiology.org/)

**Fig. 2.** Effect of culturing dissociated VMN neurons from four P21 outbred rats for 4 days with vehicle or 200 ng/ml IL-6 and assessing the sensitivity (increase in [Ca^{2+}]_i; AUC) of LepE neurons responding to 0.1, 1, and 10 fM leptin on day 5. Data are expressed as means ± SE for increased in AUC [Ca^{2+}]_i from baseline in response to leptin. *P = 0.013 where the increase in AUC of [Ca^{2+}]_i in LepE neurons in response to increasing doses of leptin were compared for vehicle vs. IL-6-treated cultured neurons by one-way ANOVA for repeated measures.
incubation had no effect on the expression of any genes evaluated in DR rats, such incubation increased Lepr-b expression by 77% and BBS6 expression by 290% in DIO rats compared with their vehicle-treated controls. While IL-6 incubation did not bring DIO Lepr-b expression to the level of DR rats, it did fully increase levels of BBS6 to those of DR rats. These data in dissociated VMN neurons support our previous findings in VMN micropunches, demonstrating reduced Lepr-b expression in DIO vs. DR rats (21, 22) and demonstrate, for the first time, that DIO rats also have reduced expression of BBS6, a gene that promotes occupancy of Lepr-b at the neuronal cell membrane (29). These increases in Lepr-b and BBS6 are likely to explain the salutary effect of IL-6 on increasing defective DIO VMN neuron leptin sensitivity to that of DR rats.

**DISCUSSION**

Amylin increases leptin signaling in the rodent VMN (27, 36). We previously showed (20) that this increase in VMN leptin signaling is due to an amylin-induced increase in VMN microglial IL-6 production, which acts through its IL-6/gp130 receptor to activate STAT3 phosphorylation (1, 18, 28), which is also downstream of Lepr-b, the long, signaling form of the leptin receptor (2, 3). Several of our prior studies have shown that lean selectively bred DIO rats have an early onset of reduced leptin signaling as a major cause of their susceptibility to become obese when the caloric density, fat, and sucrose content of their diet is increased (4, 9, 13, 21, 22). Moreover, amylin administration reverses DIO VMN leptin signaling abnormalities (27, 36) and significantly reduces their obesity that develops on such diets (27, 33, 35). Given the dependence of the amylin-induced increase in VMN leptin signaling on IL-6 and the defective signaling of DIO rats, we postulated that direct incubation of neurons with IL-6 would enhance leptin signaling and specifically do so in VMN neurons from DIO rats. Our initial studies demonstrated that there was no appreciable effect of time in culture on the percent or sensitivity to leptin of VMN LepE, LepI, or LepB neurons in outbred Sprague-Dawley rats and that incubating VMN neurons from outbred rats for 4 days in increasing concentrations of IL-6 selectively increased the percentage of LepE neurons that responded to leptin. Furthermore, incubation with 200 ng/ml of IL-6 also significantly increased the sensitivity of outbred rat LepE neurons to leptin.

Having established the relevance of these baseline conditions in outbred rats, we used them to assess the effects on leptin sensitivity by incubating dissociated VMN neurons from DR and DIO rats in vehicle vs. IL-6. First, we confirmed our previous studies in freshly dissociated neurons showing that DIO VMN neurons have both reduced mRNA expression of Lepr-b (21, 22) and are less sensitive to the activating effects of leptin than are DR neurons (14). In keeping with their reduced sensitivity to the activating effects of leptin, which we reconfirmed here after 4 days in culture, vehicle-treated DIO VMN neurons also had reduced expression of BBS6, one of the family of BBS genes that facilitates occupancy of Lepr-b at the neuronal cell surface (10, 29). Theoretically, this reduced BBS6 expression should lead to reduced occupancy of Lepr-b at the neuronal cell membrane and account for the impaired leptin binding to its receptor in the VMN of DIO rats (13). In keeping with our demonstration that the amylin-induced increase in VMN leptin signaling is IL-6-dependent in outbred rats (20), incubation of DIO VMN neurons for 4 days with IL-6 enhanced Lepr-b mRNA expression and brought their reduced BBS6 expression to DR levels. This was paralleled by increased sensitivity to the activating effect of leptin on DIO LepE neurons to the level of DR LepE neurons. For uncertain reasons, although IL-6 incubation had no effect on Lepr-b or BBS6 gene expression in DR VMN neurons, it did significantly reduce the sensitivity of their Lepr-b neurons to leptin to the level of vehicle-treated DIO rats. Taken together, the current studies support our previous findings demonstrating an important role for IL-6 in amylin-induced increase in VMN leptin signaling (20) by demonstrating that IL-6 has a direct

**Table 3. Effects on expression of genes involved in leptin signaling from incubating dissociated VMN neurons from DIO vs. DR rats with 200 ng/ml IL-6 vs. vehicle for 4 days**

<table>
<thead>
<tr>
<th>Gene</th>
<th>DR Vehicle</th>
<th>DR IL-6</th>
<th>DIO Vehicle</th>
<th>DIO IL-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lepr-b</td>
<td>2.09 ± 0.40</td>
<td>2.59 ± 0.48</td>
<td>0.71 ± 0.35</td>
<td>1.26 ± 0.44</td>
</tr>
<tr>
<td>Endostatin-1</td>
<td>1.08 ± 0.09</td>
<td>ND</td>
<td>0.83 ± 0.06</td>
<td>ND</td>
</tr>
<tr>
<td>BBS1</td>
<td>0.99 ± 0.03</td>
<td>ND</td>
<td>0.86 ± 0.05</td>
<td>ND</td>
</tr>
<tr>
<td>BBS2</td>
<td>1.26 ± 0.06</td>
<td>ND</td>
<td>1.24 ± 0.07</td>
<td>ND</td>
</tr>
<tr>
<td>BBS4</td>
<td>1.78 ± 0.32</td>
<td>1.94 ± 0.20</td>
<td>1.69 ± 0.58</td>
<td>1.59 ± 0.16</td>
</tr>
<tr>
<td>BBS6</td>
<td>1.68 ± 0.33</td>
<td>2.43 ± 0.40</td>
<td>0.43 ± 0.29</td>
<td>1.68 ± 0.26</td>
</tr>
</tbody>
</table>

Dissociated VMN neurons from DR (n = 4) and DIO rats (n = 4) incubated for 4 days with vehicle or IL-6 were first assessed for their leptin sensitivity and then were lysed; their contents were assessed for expression of Lepr-b, BBS4, and BBS6 gene expression relative to cyclophilin. Data are expressed as means ± SE for relative abundance of gene expression. Data with differing superscripts for a given gene differ from each other by P ≤ 0.05 or less when assessed by two-way ANOVA followed by post hoc Bonferroni t-test. ND, not determined.
enhancing effect on leptin sensitivity on cultured VMN LepE neurons from both outbred and selectively bred DIO rats. In DIO rats, this may well be mediated by an IL-6-dependent increase in both Lepr-b and BBS6, both of which would be predicted to increase the occupancy of Lepr-b at the neuronal cell membrane leading to an amelioration of the defective binding of leptin to this cell surface receptor (13) and enhanced downstream signaling and sensitivity to leptin.

Our previous data demonstrating a small (10–15%) decrease in Lepr-b mRNA expression in VMN of DIO, compared with DR rats (21, 22) in the face of a 50% reduction in leptin binding to its receptor in the VMN (13), suggested that this gene expression-binding discrepancy might be due to a defect in trafficking of the receptor to the cell membrane. The BBS proteins comprise a family of chaperone molecules that form a core complex of up to 17 genes. Collectively, these form a core complex known as the “BBSome” that is crucial for ciliary function. This complex associates with the ciliary membrane and functions as a unit that sorts and directs protein and vesicle trafficking to the ciliary membrane, a critical site of receptor-ligand interactions (10). Among these proteins is Lepr-b, which is trafficked between the Golgi apparatus and specific areas of the plasma membrane (29). In humans, mutation of BBS 1, 2, 4, or 6 leads to the Bardet-Biedl syndrome, which is associated with obesity, along with several defects in brain and other organ development (8). In mice, deletion of BBS 1, 2, 4, or 6 genes reduces leptin signaling and promotes obesity (7, 26). While the BBS gene complex leads to functional increases in Lepr-b cell surface expression (29), endospanin-1 is a negative regulator of Lepr-b cell surface expression, which causes Lepr-b to be internalized into early endosomes, which do not recycle back to the trans-Golgi network (6, 30). In DIO VMN neurons incubated with vehicle for 4 days, there were significant differences in gene expression only for Lepr-b and BBS6. Reduced BBS6 expression alone among the BBS genes evaluated, together with reduced Lepr-b expression, should be sufficient to account for our previously demonstrated reductions in leptin binding to its receptor (13), reduced leptin signaling (4, 21), and the current and past (14) demonstration of reduced sensitivity of DIO VMN neurons to the activating effect of leptin on LepE neurons.

Our results raise several issues that remain unanswered. First, it is unclear why IL-6 incubation altered leptin sensitivity in only LepE neurons from outbred rats. Most likely, this was due to the fact that LepI and LepB neurons were present in such small numbers that any changes might have been obscured. Also, we have only four rats for these determinations, so that actual statistical comparisons were not possible. However, in DIO rats, it was quite clear from statistical comparisons that IL-6 incubation did increase leptin sensitivity of LepE neurons. On the other hand, it remains uncertain why IL-6 incubation actually decreased leptin sensitivity in VMN neurons from DR rats, especially since there were no alterations in three of the genes involved in leptin signaling in those neurons. These results in DR neurons suggest that IL-6 incubation might actually have produced “leptin resistance” that was independent of changes in Lepr-b, endospanin 1, or BBS6 expression. We have no easy explanation for this finding since exogenous amylin treatment causes both weight loss and increased VMH leptin signaling in DR rats (36). This suggests that the apparent reduction in cultured LepE neuronal responses to leptin consequent to IL-6 incubation does not have a physiologically relevant effect as far as the weight-reducing responses to amylin. While we have no obvious explanation for these findings, it is important to point out that determination of mRNA expression was carried out in the entire population of VMN neurons, regardless of their leptin response type, while sensitivity in DIO vs. DR neurons was carried out only in LepE neurons. Also, gene expression does not necessarily reflect the expression or functionality of these various proteins, so that the failure to demonstrate differences among them, or even where differences in gene expression were found, cannot be linked unequivocally to protein expression or function. Finally, since these results were obtained in primary cultures of neurons, they are subject to all of the potential pitfalls of such a technique with regard to their in vivo physiological relevance. However, these in vitro data lend strong support to our previous results demonstrating the critical interactions among amylin, IL-6, and leptin signaling, both in vivo and in vitro in the VMN (20).

In summary, the present studies support the hypothesis that DIO rats have reduced leptin sensitivity, both at a single neuron and whole animal levels due to defects in both the production of the Lepr-b and of facilitated transport of those receptors to the neuronal cell membrane. In addition, these studies provide a potential mechanism at the single neuron level by which amylin treatment increases leptin signaling and helps obese, selectively bred DIO rats lose weight (27) by stimulating VMN microglial production of IL-6, which acts downstream of the leptin receptor to enhance its signaling by stimulating activation of phospho-STAT3 (1–3, 18, 28).

Perspectives and Significance

These studies add additional support for a distinctive role for IL-6 as a potential “leptin sensitizer.” While IL-6 is one of several cytokines associated with proinflammatory responses in various tissues, when IL-6 increases selectively, as it does in the plasma after exercise (25), it may have actual anti-inflammatory effects. Thus, while intake of a high-fat diet causes an inflammatory response in association with increased expression of IL-6 in the hypothalamus, this IL-6 rise is accompanied by increases in expression of several proinflammatory cytokines (32). As we have shown previously (20), exogenous amylin treatment produces a selective increase in VMH IL-6 production, to the exclusion of increases in other cytokines. This IL-6 increase is required for the increase in leptin signaling in rat and mouse hypothalamus and the associated reduction in body weight and fat mass. Interestingly, although leptin signaling might not be involved, the anorectic response to GLP-1 appears to be dependent upon both brain IL-6 and the proinflammatory cytokine, IL-1 (31). This suggests that not all combinations of IL-6 with proinflammatory cytokines have a deleterious effect on promoting positive energy balance.

Thus, our current and previous data strongly support a role for IL-6, in isolation, as a direct mediator of enhanced leptin sensitivity in VMN LepE neurons and as a mediator of amylin-induced increases in VMH leptin sensitivity. On the other hand, increases in hypothalamic IL-6, which are accompanied by increases in proinflammatory cytokines, can combine to reduce leptin signaling (32). Overall, our findings suggest that IL-6 might be utilized as a target for development of drugs to enhance leptin signaling in the fight to treat obese individuals.
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REFERENCES

