Sexual Dimorphism in Cafeteria Diet-Induced Hypertension Is Associated with Gender-Related Difference in Renal Leptin Receptor Down-Regulation

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ABSTRACT
Plasma leptin levels are elevated in obesity suggesting a pathophysiologic role of this hormone in obesity and related disorders, such as hypertension. Furthermore, despite excess leptin levels, leptin satiety action is blunted in obesity suggesting the occurrence of central leptin resistance. As leptin acts on the kidney to induce natriuresis, renal leptin receptor alterations could lead to a defect in sodium excretion and hence to hypertension. Therefore, the present study investigated renal leptin receptor (Ob-Ra and Ob-Rb) mRNA and leptin binding capacities in diet-induced hypertension. Feeding male, female, and testosterone-treated female rats a cafeteria diet for 10 weeks increased body fat mass, plasma insulin, and leptin levels. Furthermore, although male and testosterone-treated female cafeteria-fed rats were hypertensive, the female rats fed the same diet failed to develop elevated blood pressure. In renal medulla, Ob-Ra and Ob-Rb mRNA levels were unchanged after cafeteria diet feeding in all groups; however, binding analysis revealed Ob-R protein down-regulation exclusively in hypertensive rats. Moreover, renal Ob-R densities were inversely correlated to plasma leptin concentrations in male rats and testosterone-treated female rats but not in female rats. These findings demonstrate the existence of differences in renal Ob-R binding capacities, which are correlated to hypertension.

Obesity is associated with profound alterations of cardiovascular functions including an increase in blood pressure. Elevated plasma leptin levels, a hormone mainly produced by the adipose tissue, is occurs frequently in obesity and related disorders in humans (Caro et al., 1996). Initially considered as a satiety factor, leptin also exerts sympathetic, renal, and metabolic effects that could contribute to the altered cardiovascular functions occurring in obesity (Haynes et al., 1997, Mark et al., 1999). Leptin can affect blood pressure through two opposite mechanisms: 1) enhancement of the sympathetic tone (Haynes et al., 1997), leading to an increase in blood pressure; and 2) promotion of natriuresis (Jackson et al., 1997) and nitric oxide vasorelaxant effect (Lembo et al., 2000; Vecchione et al., 2002), which both contribute to lower blood pressure. However, chronic effect of leptin appears to be predominately pressor. Indeed infusion of leptin at rates that raise plasma leptin to levels similar to those found in obesity, increases mean arterial pressure (Shek et al., 1998). Furthermore transgenic mice overexpressing leptin develop elevation of blood pressure (Aizawa Abe et al., 2000). Elevated plasma leptin concentrations have also been described in hypertensive patients (Agata et al., 1997). Leptin could therefore be one of the pathophysiological candidates linking obesity to hypertension (Mark et al., 1999; Aizawa Abe et al., 2000; Hall et al., 2000; Ogawa et al., 2002).

Leptin acts through binding to leptin receptor, Ob-R, which is expressed as various splice variants and is a member of the extended class I cytokine receptor family. Both the short Ob-Ra isoform and the full-length Ob-Rb variant are expressed in the kidney (Hoggard et al., 1997; Serradeil-Le Gal et al., 1997). In the obese Zucker rat, an attenuated diuretic and natriuretic response to systemic leptin infusion has been described (Hoggard et al., 1997). Acute administration of leptin acts on the kidney to promote natriuresis and diuresis (Jackson et al., 1997; Serradeil-Le Gal et al., 1997). In the obese Zucker rat, an attenuated diuretic and natriuretic response to systemic leptin infusion has been described (Villareal et al., 1998). Because obese Zucker rats have a mutation in the Ob-R (Takaya et al., 1996) leading to reduced signal transduction, renal leptin resistance may explain the attenuated natriuretic and diuretic response observed in this strain. Localization studies showing existence of the Ob-R in the renal inner medulla (Hoggard et al., 1997; Serradeil-Le Gal et al., 1997) further support the requirement of the receptor in the mechanisms whereby leptin stimulates natriuresis. It must be noted that the elevated blood
pressure in obese Zucker rats compared with lean control rats was associated with a marked increase in sodium reabsorption (Alonso-Galicia et al., 1996). As obesity is associated with resistance to leptin effects, the occurrence of leptin resistance in the kidney can also be reasonably postulated in the obese state and thus can contribute at least partly to the obesity hypertension. We have previously shown that overexpression induced by cafeteria diet feeding is associated with sexual dimorphism in the development of hypertension (Coatmellec-Taglioni et al., 2000, 2002). Indeed, male cafeteria-fed rats developed hypertension, whereas female rats fed the same diet are overweight but normotensive (Coatmellec-Taglioni et al., 2002). Moreover this sexual dimorphism of the cafeteria diet-induced hypertension was reversed by testosterone imprinting of female rats at birth (Plut et al., 2002).

The aim of the present study was to investigate the renal leptin receptor in hypertension induced by cafeteria feeding. Therefore, renal leptin receptor mRNA and binding capacities were analyzed in male and intact or neonatally androgenized female cafeteria-fed rats. We show here that renal Ob-R down-regulation only appears in hypertensive rats.

Materials and Methods

Animal Procedures. Testosterone treatment: female Sprague-Dawley rat pups were injected subcutaneously with 1 mg of testosterone propionate in olive oil at birth (Plut et al., 2002). Cafeíneria diet: male, female and testosterone-treated female Sprague-Dawley rats with an average weight of 100 g were divided into two groups and maintained at room temperature with a 12-h light/dark cycle. Rat pups with an average weight of 100 g were divided into two groups and injected subcutaneously with 1 mg of testosterone propionate in olive oil at birth (Plut et al., 2002). Cafeteria diet-induced hypertension was reversed by testosterone imprinting of female rats at birth (Plut et al., 2002). The aim of the present study was to investigate the renal leptin receptor in hypertension induced by cafeteria feeding. Therefore, renal leptin receptor mRNA and binding capacities were analyzed in male and intact or neonatally androgenized female cafeteria-fed rats. We show here that renal Ob-R down-regulation only appears in hypertensive rats.

Results

Animal Characteristics. As shown in Table 1, after 10 weeks of cafeteria diet feeding, fat pad weight was increased in male, untreated female, and testosterone-treated female rats. As previously observed, male cafeteria-fed rats were hypertensive, whereas female cafeteria-fed rats remained normotensive (Coatmellec-Taglioni et al., 2000, 2001). However, when female rats treated with testosterone at birth are...
fed with a cafeteria diet, they share an increase in blood pressure similar to male rats. Fasting plasma insulin levels were higher in the cafeteria-fed animals than in the group on regular chow. Moreover, an increase in plasma leptin level was detected in cafeteria groups.

**Binding Studies.** Whereas binding leptin can be detected in cultured glomerular endothelial cells (Wolf et al., 1999), specific $^{125}$I-leptin binding was almost undetectable in crude cortical preparation from rat kidney as observed by Serradeil Le Gal et al. (1997). Therefore, we investigate Ob-R binding sites in crude membranes from the renal medulla. The expression of Ob-R protein in these crude membrane preparations was demonstrated by Western blot analyses using an anti-Ob-R goat polyclonal antibody (Fig. 1). The major protein detected had a molecular mass of ~120 kDa, and this immunoreactive band completely disappeared in the presence of the specific peptide (Fig. 1). Using the same antibody, Shioda et al. (1998) detected a major 120-kDa band in the rat hypothalamus where Ob-Ra and Ob-Rb are expressed. Thus, our result does not exclude the presence of Ob-Rb in membranes of renal medulla.

Saturation experiments showed that $^{125}$I-leptin specific binding to crude renal medullary membranes was saturable in both control and cafeteria-fed rats (Fig. 2). Scatchard analysis of the binding data gave linear plots with high-affinity dissociation constant ($K_d$) that was similar in all groups, indicating that leptin binds to one single class of binding sites. Competition binding analysis (Fig. 3) further showed that unlabeled leptin inhibited $^{125}$I-leptin binding in a dose-dependent manner yielding to $K_i$ values consistent with the $K_d$ values derived from saturation binding experiments.

Analysis of these experiments (Figs. 2 and 3), showed no differences in leptin receptor affinity between control and cafeteria male rats (Table 2). However, in male rats, maximal binding capacities were markedly and significantly reduced (66%, $p < 0.05$) after cafeteria feeding (Table 2). Moreover, the total number of leptin binding sites was inversely correlated with plasma leptin levels in these male rats ($r = -0.53, n = 10, p < 0.05$). In contrast, in untreated female rats, both leptin affinity and maximal binding capacities were unchanged after cafeteria diet feeding (Table 2). Moreover, no correlation between plasma leptin levels and renal leptin binding sites was observed in the female rats, regardless of diet ($r = -0.09, n = 10, p = 0.71$). On the other hand, in neonatal testosterone-treated female rats, cafeteria feeding induced a significant decrease in maximal binding capacities (30%, $p < 0.05$) (Table 2), and the total number of leptin binding sites was also inversely correlated with plasma leptin levels in these animals ($r = -0.70, n = 8, p < 0.05$).

**Expression of Long and Short Leptin Receptor mRNA.** To determine whether the effect of cafeteria feeding on $^{125}$I-leptin binding capacities was associated with parallel changes in Ob-R expression levels, reverse transcriptase-PCR analysis of Ob-Ra and Ob-Rb was performed in all experimental groups. As shown in Fig. 4, A and B, no differences occurred in the amount of either Ob-Ra and Ob-Rb mRNA between the control and the cafeteria-fed groups, regardless of sex. Cafeteria diet did not alter Ob-Ra and Ob-Rb mRNA expression in the kidney of testosterone-treated female rats either (90 ± 12 and 85 ± 17 of respective control mRNA level). Moreover, no differences in the size of PCR products were observed between control and cafeteria-fed rats, ruling out the presence of alternative splicing in cafeteria-fed animals, as observed in some genetic forms of obesity (Fig. 4A). Finally, the fragment generated from β-actin primers, which was chosen to span 2 introns, was the only amplified fragment suggesting no genomic DNA contamination (Fig. 4A). Thus, the Ob-R down-regulation found in the kidney of male and testosterone-treated female rats is not related to a decrease in Ob-R mRNA expressions.

**Discussion**

The present study demonstrates that the sexual dimorphism in cafeteria diet-induced hypertension is associated with the same dimorphism in Ob-R down-regulation. Fur-
neonatal testosterone treatment allowed the development of hypertension in female rats fed with the cafeteria diet. These hypertensive female rats also elicit renal Ob-R down-regulation as do hypertensive male rats. Although the mechanisms responsible for the gender differences in hypertension are not clear, there is significant evidence that androgens such as testosterone play an important role in the gender-specific blood pressure regulation (Reckelhoff, 2001). Indeed, hypertension was reduced by castration in SHR and in Dahl and Sabra salt-sensitive rats (Crofton et al., 1993; Gong et al., 1994; Khalid et al., 2001). Furthermore, administration of androgen receptor antagonist, for 10 days after birth, attenuates the hypertension of male adult SHR, whereas neonatal androgenization of female SHR leads to similar hypertension as that in male (Cambotti et al., 1984). All together, these results strengthen the influence of testosterone on the promotion of hypertension. Neonatal testosterone induces specific brain maturational changes, which may promote sensitivity to cafeteria diet, leading to the onset of hypertension.

Leptin receptor mRNA and/or protein down-regulation has been clearly characterized in mouse brain (Lin et al., 2000), rat hypothalamus (Martin et al., 2000) after either high-fat diet or leptin loads. In these previous studies, Ob-R down-regulation was attributed to altered transcription of the re-
receptor gene or mRNA stability. In contrast, the present study demonstrates an obvious leptin receptor protein down-regulation in renal medullary membranes from male cafeteria-fed rats, without changes in Ob-Ra and Ob-Rb mRNA levels. One common well characterized mechanism by which cell surface receptor number can decrease is the ligand-induced receptor internalization and degradation by lysosomal pathway. Two independent groups have recently demonstrated that leptin is able to down-regulate its own receptor through a coat-pitted-dependent mechanism (Barr et al., 1999; Uotani et al., 1999). Because the presently reported decrease in available cell surface Ob-R is inversely correlated to plasma leptin level in the kidney of male rats and testosterone-treated female rats, the receptor down-regulation could be related to excess leptin levels. However, despite a similar rise in plasma leptin levels, renal Ob-R were not down-regulated in female rat kidneys. This suggested the existence of gender-specific leptin sensitivity. The kidney has been shown to be the main site of leptin clearance, where leptin is degraded (Cumin et al., 1997) by a receptor-mediated internalization through a clathrin-mediated mechanism (Barr et al., 1999; Uotani et al., 1999). Because males had greater renal leptin clearance than females (Meyer et al., 1997), more leptin may access to renal Ob-R in male and induce the greatest Ob-R down-regulation. Furthermore androgens, in vivo as in vitro, increase clathrin expression (Prescott et Tindall, 1998) and may therefore participate in the greatest sensitivity of male to Ob-R down-regulation. Gender is also a major determinant of plasma leptin in human (Considine et al., 1996). Although women are likely to have a greater body fat mass than men, leptin levels rose more rapidly as a function of body mass index in women than in men (Kennedy et al., 1997). Therefore, higher levels of leptin are required in women to achieve similar biological endpoints. This suggests that there may be a difference in the biological homeostatic set point for obesity in women. Soluble leptin receptor is the major leptin-binding protein in the plasma (Lammert et al., 2001). Obesity is associated with decreasing levels of circulating soluble leptin receptor, but a gender difference was observed in soluble leptin receptor levels, which were higher in obese men than in obese women (Ogier et al., 2002). Overexpression of soluble leptin receptor leads to an improved weight-reducing effect of leptin in ob/ob mice (Huang et al., 2001) suggesting that these receptors affect bioavailability and functionality of leptin. Therefore, an eventual gender difference in soluble leptin receptors in cafeteria-fed rats can be responsible for renal leptin sensitivity.

On the other hand, because leptin receptor down-regulation only occurred in hypertensive rats, these alterations might have also appeared as a consequence of renal disease induced by hypertension. Indeed, the kidney is one of the main sites of blood pressure control, and hypertension is generally associated with renal damage (Guyton et al., 1981). Moreover, male animals are at greatest risk of developing renal diseases and exhibit a more rapid progression of renal injury (Reckelhoff and Granger, 1999). Whatever the causes of the renal Ob-R down-regulation, this alteration may reduce natriuresis and renal nitric oxide production, which both contribute to leptin hypertensive actions (Jackson and Li, 1997; Vecchione et al., 2002). In summary, this study provides some evidence that renal leptin receptor down-regulation is associated with a diet-induced hypertension in Sprague-Dawley rats. This effect does not appear in the kidney of female cafeteria-fed rats. Thus, sexual dimorphism in renal leptin receptor sensitivity and hypertension occurs in Sprague-Dawley rats.

**Table 2**
Effect of cafeteria diet on leptin binding data

<table>
<thead>
<tr>
<th></th>
<th>$B_{max}$ (fmol/mg protein)</th>
<th>$K_d$ (nM)</th>
<th>$K_i$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Males</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>55.36 ± 12.58</td>
<td>2.41 ± 0.99</td>
<td>1.78 ± 0.77</td>
</tr>
<tr>
<td>Cafeteria</td>
<td>18.44 ± 5.58*</td>
<td>1.07 ± 0.17</td>
<td>1.94 ± 0.45</td>
</tr>
<tr>
<td><strong>Females</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>39.05 ± 6.58</td>
<td>2.45 ± 0.75</td>
<td>1.76 ± 0.46</td>
</tr>
<tr>
<td>Cafeteria</td>
<td>50.21 ± 13.72</td>
<td>2.87 ± 0.84</td>
<td>1.43 ± 0.40</td>
</tr>
<tr>
<td><strong>Testosterone-treated females</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>33.50 ± 3.36</td>
<td>1.77 ± 0.26</td>
<td>1.59 ± 0.08</td>
</tr>
<tr>
<td>Cafeteria</td>
<td>23.47 ± 1.62*</td>
<td>2.67 ± 0.50</td>
<td>1.87 ± 0.16</td>
</tr>
</tbody>
</table>

* $p < 0.05$ versus corresponding control.
References