Arcuate NPY neurons and energy homeostasis in diet-induced obese and resistant rats

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Levin, Barry E. Arcuate NPY neurons and energy homeostasis in diet-induced obese and resistant rats. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R382–R387, 1999.—The neuropeptide Y (NPY) neurons in the hypothalamic arcuate nucleus regulate and are regulated by short-term changes in energy homeostasis. Both outbred and inbred strains of rats that develop diet-induced obesity (DIO) or are diet resistant (DR) when fed a diet relatively high in energy, fat, and sucrose content (HE diet) were used to study arcuate NPY mRNA expression during long-term changes in energy balance. Outbred, chow-fed obesity-prone rats had 59% higher NPY levels than obesity-resistant rats. After 14 wk on HE diet, DIO rats had 17% lower NPY levels than DR rats made comparably obese on a highly palatable diet. When switched to chow, obese DR rats spontaneously reduced their intake and their body weights fell to control levels in association with a 10% decrease in NPY levels. DIO rats lost weight only with energy restriction associated with a 21% increase in their NPY levels. When again fed ad libitum, the weight and NPY levels in the rats returned to those of unrestricted DIO rats. Chow-fed, inbred DIO rats weigh more and are fatter than age-matched inbred DR rats. As with outbred DIO rats fed the HE diet, inbred DIO rats had 20% lower NPY levels than DR rats. Thus preobese, outbred DIO rats have high levels of NPY message that are not susceptible to metabolic regulation. When obesity develops in both inbred and outbred rats, the levels of NPY mRNA fall but become responsive to alterations in energy availability.

leptin; insulin; energy intake; energy balance; body weight

FEW NEURONS IN THE BRAIN are as responsive to short-term alterations in energy homeostasis as the neuropeptide Y (NPY)-containing neurons in the arcuate nucleus of the hypothalamus (13, 23). These neurons project to the paraventricular nucleus (1), where they play a critical role in the regulation of energy homeostasis (2, 21, 25). They appear to be important central integrators of various metabolic signals from the periphery because they respond to changes in energy intake (13, 23) and to insulin (22), leptin (23), and glucose (unpublished observation). They are in turn involved in energy intake, expenditure, and storage (2), including autonomic nervous system activity (25) and insulin release (25). Thus, whereas they are not the only regulators of energy homeostasis, they are certainly important in the normal animal. However, most studies have examined the role of these neurons in energy homeostasis only over relatively short periods of time or in genetically obese animals in which obesity is already a preexisting condition (22, 24).

The current studies were instituted to assess the regulation of arcuate NPY message in an animal model of diet-induced obesity (DIO), where animals can be studied both before and after the expression of obesity produced by feeding a diet relatively high in energy and fat content (HE diet). About one-half of an outbred population of male Sprague-Dawley rats develops DIO on an HE diet. The remainder are diet resistant (DR), gaining no more weight than chow-fed controls (20). Furthermore, obesity-prone rats can be separated from DR rats before the exposure to HE diet and the development of obesity because they excrete more norepinephrine in the urine (10). We previously showed that obesity-prone rats overexpress arcuate NPY message and that this message is affected by neither energy restriction nor short-term overfeeding (13). In addition, we selectively have inbred rats from this outbred population for the DIO and DR traits (14). The resultant substrains breed true to their weight-gain phenotypes and provide another method of assessing the long-term regulation of body weight and associated factors such as arcuate NPY expression.

Our recent study (17) showed that once obesity is fully established, outbred DIO rats avidly defend their elevated body weights (Fig. 1). When forced to lose weight by restricting their energy intake, they quickly regained lost weight when subsequently given free access to food. On the other hand, when DR rats were made obese by exposure to a highly palatable diet, they spontaneously reacquired their lower body weights when subsequently switched to a diet of low palatability and fat content. This differential response to dietary content occurred despite identical levels of carcass fat, plasma leptin, and insulin in the DIO and obese DR rats. That study suggested that there might be some central “set-point” for the regulation of body weight that was genetically predetermined but expressed only in the presence of appropriate dietary exposure. During the course of those studies we also examined the brains of those animals for the expression of arcuate nucleus NPY mRNA levels as a measure of the long-term regulation of this peptide. Those results and those from the brains of animals from our inbred colonies of DR and DIO rats are reported here.

METHODS

Animals and Experimental Protocol

Outbred DR and DIO rats. The metabolic data in the animals that served as the source for brains used in these experiments have been previously reported (17) and are
reproduced in part in Fig. 1 and Table 1. The experiment began with 98 outbred male, Sprague-Dawley rats (Charles River Laboratories), which were kept at 23–24°C on a 12:12-h light-dark cycle with lights on at 0600. They were brought into the facility at 225–250 g and kept on Purina rat chow (no. 5001) and ad libitum for 1 wk. A subset of 18 rats was placed in metabolic cages, and their urine specimens were collected for determination of urinary norepinephrine levels to discriminate DR- from DIO-prone rats (10). The six rats with the highest (DIO prone) and six with the lowest (DR prone) norepinephrine levels were then killed by decapitation between 0800–1000 for collection of trunk blood and brains. Rats were killed when their body weights matched the DIO-Ad lib group and the DR-HE diet rats after 2 wk (week 16). At this point, five to six rats from each of the four experimental groups (chow, DR-HE diet, DR-Ensure, DIO) were killed at 0800–1000. Their trunk blood was taken for plasma glucose, insulin, and leptin levels, the retroperitoneal fat depots were removed and weighed, and the brains were taken for arcuate NPY message determinations. Rats were allowed free access to food and water before being killed.

Weight loss phase. At this point (week 14) all rats in all groups were switched to ad libitum intake of chow for 1 wk. During this week (week 15) the now obese DR-Ensure rats spontaneously reduced their energy intake on chow to less than one-half of the ingested energy they had consumed on the HE-Ensure diet combination during the preceding week. These rats were permitted ad libitum access to chow for the remainder of the study. Because DIO rats did not reduce their intake of chow comparably to obese DR rats during the first week after the switch from HE diet to chow, a subset of 13 DIO rats had their intake on chow restricted to the spontaneous intake of the DR-Ensure rats over the prior week (DIO-Restrict). The remaining DIO rats (n = 12) were allowed ad libitum access to chow and were designated DIO-Ad lib. DR-Ensure body weights dropped continuously on ad libitum chow intake and reached those of chow-fed controls and DR-HE diet rats after 2 wk (week 16). At this point, 13 DIO rats were fed chow or high energy (HE) diet alone [diet resistant (DR)-HE diet, DR-Ensure rats were fed the HE diet ad libitum for weeks 14–20; DR-Ensure: DR rats were fed HE diet plus Ensure ad libitum for weeks 2–14 and then chow ad libitum for weeks 14–20; diet-induced obesity (DIO) Ad Lib: DIO rats were fed the HE diet ad libitum for weeks 2–14 and then chow ad libitum for weeks 14–20; DIO Restrict: DIO rats were fed HE diet ad libitum for weeks 2–14, chow ad libitum for weeks 14–15, chow restricted to the intake of DR-Ensure rats for weeks 15–17, and then chow ad libitum for weeks 17–20. Data are from Levin and Keesey (17).

Weight gain phase. At the onset of these diet studies the remaining 80 rats were 10 wk of age. Food intake and body weights were measured for 1 wk on chow, and then all rats were switched to a high-energy (HE) diet ad libitum. This diet was composed of 8% corn oil, 44% sweetened condensed milk, and 48% Purina rat chow (Research Diets) and contains 4.47 kcal/g with 21% of the metabolizable energy content as protein, 22% as fat, and 64% as carbohydrate. The remaining 17 DR rats were kept on HE diet (DR-HE diet rats). All rats were kept on their respective diets until the body weights of the DR-Ensure rats were statistically the same as DIO rats (week 4–14). At this point, five to six rats from each of the four experimental groups (chow, DR-HE diet, DR-Ensure, DIO) were killed at 0800–1000. Their trunk blood was taken for plasma glucose, insulin, and leptin levels, the retroperitoneal fat depots were removed and weighed, and the brains were taken for arcuate NPY message determinations. Rats were allowed free access to food and water before being killed.

Weight loss phase. At this point (week 14) all rats in all groups were switched to ad-libimum intake of chow for 1 wk. During this week (week 15) the now obese DR-Ensure rats spontaneously reduced their energy intake on chow to less than one-half of the ingested energy they had consumed on the HE-Ensure diet combination during the preceding week. These rats were permitted ad-libimum access to chow for the remainder of the study. Because DIO rats did not reduce their intake of chow comparably to obese DR rats during the first week after the switch from HE diet to chow, a subset of 13 DIO rats had their intake on chow restricted to the spontaneous intake of the DR-Ensure rats over the prior week (DIO-Restrict). The remaining DIO rats (n = 12) were allowed ad-libimum access to chow and were designated DIO-Ad lib. DR-Ensure body weights dropped continuously on ad-libimum chow intake and reached those of chow-fed controls and DR-HE diet rats after 2 wk (week 16).

Table 1. Body weights, retroperitoneal fat depot weights and plasma leptin levels, and hypothalamic arcuate nucleus NPY areas from in situ hybridization studies during weight gain, loss, and regain

<table>
<thead>
<tr>
<th>Body wt, g</th>
<th>RP Pad wt, g</th>
<th>Plasma Leptin, ng/ml</th>
<th>NPY Area, mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chow</td>
<td>556 ± 12</td>
<td>7.8 ± 1.7</td>
<td>5.4 ± 1.25</td>
</tr>
<tr>
<td>DR-HE diet</td>
<td>548 ± 20</td>
<td>12.9 ± 1.9</td>
<td>14.6 ± 1.4</td>
</tr>
<tr>
<td>DR-Ensure</td>
<td>626 ± 21</td>
<td>21.8 ± 1.1</td>
<td>20.0 ± 1.6</td>
</tr>
<tr>
<td>DIO Restrict</td>
<td>636 ± 25</td>
<td>21.7 ± 1.6</td>
<td>19.3 ± 5.0</td>
</tr>
<tr>
<td>Chow</td>
<td>575 ± 12</td>
<td>11.5 ± 1.6</td>
<td>10.2 ± 2.4</td>
</tr>
<tr>
<td>DR-HE diet</td>
<td>559 ± 13</td>
<td>13.2 ± 1.8</td>
<td>11.1 ± 1.9</td>
</tr>
<tr>
<td>DR-Ensure</td>
<td>582 ± 17</td>
<td>19.6 ± 1.3</td>
<td>15.7 ± 2.1</td>
</tr>
<tr>
<td>DIO-Ad lib</td>
<td>637 ± 19</td>
<td>18.7 ± 1.4</td>
<td>16.0 ± 1.4</td>
</tr>
<tr>
<td>DIO-Restrict</td>
<td>580 ± 16</td>
<td>13.9 ± 1.4</td>
<td>5.4 ± 0.72</td>
</tr>
<tr>
<td>Chow</td>
<td>601 ± 12</td>
<td>13.8 ± 1.8</td>
<td>14.2 ± 1.9</td>
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<tr>
<td>DR-HE diet</td>
<td>582 ± 14</td>
<td>9.6 ± 1.2</td>
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<td>DR-Ensure</td>
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<tr>
<td>DIO-Ad lib</td>
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<td>17.8 ± 1.5</td>
</tr>
<tr>
<td>DIO-Restrict</td>
<td>655 ± 19</td>
<td>19.0 ± 1.7</td>
<td>14.2 ± 2.9</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5–6 rats/group in all 3 phases. Rats were fed chow or high energy (HE) diet alone (diet resistant (DR)-HE diet, diet-induced obesity (DIO)) or HE diet ≥ Ensure (DR-Ensure) for 12 wk (phase 1). All rats were then switched to chow. DR-Ensure rats voluntarily restricted energy intake by 50% and intake of DIO-Restrict rats was matched to DR-Ensure intake. These rats were killed when their body weights matched those of chow-fed controls (phase 2). Then DIO-Restrict rats were given ad-libimum access to chow until their body weights matched the DIO-Ad lib group and the remaining rats were killed (phase 3). RP, retroperitoneal; NPY, neuropeptide Y. In a given data set, values with different superscripts in each data subset are significantly different at P < 0.05 by post hoc test with correction for multiple comparisons after significant inter-group differences were found by ANOVA.
time six rats from each of the DR-HE diet, DR-Ensure, and chow-fed control groups were killed for trunk blood, retroperitoneal adipose depot weights, and brains. Body weights in the DIO-Restrict rats reached those of chow-fed controls and both DR groups after 1 wk of ad libitum and 2 wk of restricted food intake (week 17). At that time six rats in the DIO-Ad lib and DIO-Restrict groups were killed for assay at 0800–1000, after being allowed free access to food and water overnight.

Weight regain phase. Once the body weights of the DIO-Restrict rats reached those of the other groups (week 17), they were given ad libitum access to chow and followed for an additional 3 wk. By this time their body weights had reached those of the DIO-Ad lib group, and all the remaining rats in each of the five experimental groups (n = 5 or 6/group) were killed for assay (week 20) at 0800–1000, after being allowed free access to food and water overnight.

Inbred DR and DIO rats. These rats came from our resident colony of animals inbred from the parent Charles River Sprague-Dawley rat colony of outbred rats (14). They were selectively inbred for the characteristics of maximal or minimal weight gain on the HE diet. Here, 20 male DR and 20 male DIO inbred rats were used at 10 wk of age. After initial weighing, one-half of each phenotype was placed on chow and the other half on HE diet for a total of 12 wk. After the 12-wk diet exposure, they were killed between 0800–1000, after being allowed free access to food and water overnight. Their trunk blood was taken for plasma glucose, insulin, and leptin levels, and their retroperitoneal fat depots were removed and weighed. Brains were taken for arcuate NPY message determinations.

In situ hybridization for arcuate prepro-NPY mRNA. Brains were processed for in situ hybridization by minor modifications of previously described methods (13). Briefly, the 511-bp probe (derived from the original probe of Higuchi et al. (6) and kindly supplied by Dr. Jack Bruno), was subcloned into a pBluescript SK (+) vector at an EcoRI site. Radiolabeled cRNA was synthesized in vitro from BamHI linearized plasmids. Sense and antisense probes were transcribed with T3 and T7 promoters, respectively, using 35S-UTP (1,000 Ci/mmol, New England Nuclear). The probes were hydrolyzed in 0.5 M NaHCO3 for 30 min. Frozen sections of brain were freed from the cryostat and coated with gelatin and fixed in 4% paraformaldehyde. They were treated with acetic anhydride for 10 min and dehydrated through six steps of graded ethanol solutions. Prehybridization was carried out at 50°C for 30 min and then hybridized with labeled sense and antisense probes at 50°C overnight. After treatment with RNAase A, sections were washed, dehydrated, dried, and opposed to SB-5 X-ray film (Kodak) for 3 days. The resulting autoradiograms were read by an observer unaware of the experimental groupings of the brains using computer-assisted densitometry (Drexel). Areal measures were made in the mid-portion of the arcuate nucleus, which shows maximal alterations of NPY mRNA expression during metabolic perturbations (21). Readings from the sections with the three largest areas were averaged for comparison between DIO and DR rats. Optical density readings were also made within these areas but the product of optical density times area did not alter the results. Thus results are given as area alone.

Assays of glucose, insulin, and leptin. Samples of trunk blood were collected into heparinized tubes, and the plasma was removed for assay. Glucose was assayed by automated glucose oxidase method (Beckman), and both insulin and leptin were analyzed by radioimmunoassays (Linco) using antibodies to authentic rat insulin and leptin, respectively.

Statistics. In each assay for NPY mRNA in situ hybridization, brains from at least one animal from each experimental group were always run in the same assay. One-way ANOVA was used for single point measures of terminal body and retroperitoneal fat depot weights, plasma glucose, insulin, and leptin and NPY levels at the end of each phase (gain, loss, regain) in the outbred animals. In the inbred animals, two-way ANOVA was used to compare data among different phenotypes and diet groups because all animals in this study were killed at the same time. When significant intergroup differences were found by ANOVA (P ≤ 0.05), post hoc comparisons were carried out using Tukey’s multiple-comparison tests.

RESULTS
Outbred DR and DIO Rats

Chow-fed rats. Of the group of 12 chow-fed outbred rats selected by their 24-h urinary norepinephrine levels as being DR prone (1.12 ± 0.13 μg) vs. DIO prone (1.85 ± 0.14 μg, P = 0.05), there were no significant differences in plasma leptin levels (DIO prone 3.69 ± 0.30 μg vs. DIO prone 3.85 ± 0.46 ng/ml). However, similar to the previous study (13), DR-prone rats here had significantly elevated (59%) areas of NPY mRNA in the arcuate nucleus (0.569 ± 0.055 mm2) compared with DR-prone rats (0.357 ± 0.016 mm2, P = 0.01).

Weight gain phase. The remaining 80 outbred 10-wk-old DIO and DR rats were identified retrospectively by their respective weight gains after 2 wk on HE diet. After 14 wk on this diet DR rats fed Ensure and DIO rats fed the HE diet had similar body and retroperitoneal pad weights and plasma leptin levels (Fig. 1, Table 1). Despite these similarities, DIO rats had 23% lower arcuate NPY mRNA levels than the comparably obese DR rats (Table 1). Both DIO and obese DR rats had heavier body and fat pad weights and higher plasma leptin levels than both DR rats on HE diet and chow-fed controls (Fig. 1, Table 1). Although DR rats fed the HE diet alone gained the same amount of weight as chow-fed controls, they had 170% higher leptin levels and 164% heavier retroperitoneal pad weights (17) than chow-fed controls. Their NPY levels were comparable to the obese DR rats fed Ensure but were 13% higher than chow-fed controls.

Weight loss phase. When the obese DR rats that previously had been fed Ensure were switched to ad libitum intake of chow, they spontaneously decreased their energy intake by 60% (17) and their body weights (but not their retroperitoneal fat depot weights or leptin levels) fell to those of both the DR rats previously fed HE diet and the chow-fed controls within 2 wk (Fig. 1, Table 1). By contrast, during the first week after the switch from HE to chow diet, DIO rats decreased their intake by only 8% (17) and their body weight gains showed only a transient decline for 1–2 wk followed by a continued weight gain for the remainder of the study (Fig. 1, Table 1). Given this marked difference in response to diet switching, an additional set of DIO rats was restricted to the intake of the obese DR rats after their switch from Ensure to chow. Once restricted, these DIO rats lost weight comparably to the DR-Ensure rats and after 2 wk had body weights and retroperitoneal pad weights comparable to both groups...
of DR rats and to chow-fed controls. However, the differences between the spontaneous constraint of energy intake and body weight in DR-Ensure rats vs. the involuntary restriction required to produce comparable weight loss in DIO rats was reflected in differences in both plasma leptin and arcuate NPY mRNA levels. In the DR-Ensure rats lep tin levels and fat pad weights remained elevated, whereas NPY were comparable to DR rats switched from HE diet. On the other hand, energy restriction in DIO rats was associated with a markedly lower level of plasma leptin and higher NPY expression than all other groups. Despite differences in retroperitoneal pad weights and plasma leptin levels, NPY levels were comparable between DIO rats fed chow ad libitum and both groups of DR rats.

Weight regain phase. Once the previously obese DR rats (DR-Ensure) spontaneously dropped their body weights to those of nonobese DR rats (DR HE) they also maintained their lowered body weights and their arcuate NPY levels comparably for the entire 6 wk after being switched to chow. However, their fat depot weights and plasma leptin levels remained somewhat elevated even after 6 wk on chow (Fig. 1, Table 1). On the other hand, the restricted DIO rats immediately regained their lost body weights when given ad libitum access to chow. After 3 wk their NPY levels, along with fat pad weights and leptin levels, were comparable to those of ad libitum chow-fed DIO rats, as well as chow-fed and both groups of DR rats.

Inbred DR and DIO Rats

Chow-fed rats. Inbred DR and DIO rats (Table 2) were monitored here from 10–22 wk of age, having all been fed chow from weaning. As in a previous study (14) the inbred DIO rats here were already 15% heavier than the age-matched DR rats at 10 wk of age. In keeping with their increased metabolic efficiency (14), the DIO rats became even more obese than DR rats fed chow over the next 12 wk. They gained 30% more weight than DR rats, had 156% heavier retroperitoneal pads, and plasma glucose, insulin, and leptin levels were 11, 59, and 132% higher than chow-fed DR rats, respectively. These chow-fed DIO rats had a 20% lower level of plasma leptin and higher NPY levels comparably for the entire 6 wk after ad libitum feeding.

DIO-Chow DR-HE DIO-HE

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<tbody>
<tr>
<td>Body wt, g</td>
<td>452 ± 22*</td>
<td>546 ± 27*</td>
<td>645 ± 30*</td>
</tr>
<tr>
<td>Body wt gain, g</td>
<td>148 ± 22*</td>
<td>194 ± 28*</td>
<td>256 ± 33*</td>
</tr>
<tr>
<td>RP pad wt, g</td>
<td>5.23 ± 0.50a</td>
<td>13.4 ± 2.8b</td>
<td>24.6 ± 3.0f</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>136 ± 3a</td>
<td>151 ± 5b</td>
<td>152 ± 4p</td>
</tr>
<tr>
<td>Insulin, ng/ml</td>
<td>1.79 ± 0.15a</td>
<td>2.85 ± 0.32b</td>
<td>4.78 ± 0.65e</td>
</tr>
<tr>
<td>Leptin, ng/ml</td>
<td>5.83 ± 0.61b</td>
<td>13.5 ± 1.6b</td>
<td>19.7 ± 1.3p</td>
</tr>
<tr>
<td>NPY area, mm²</td>
<td>0.799 ± 0.033a</td>
<td>0.636 ± 0.021b</td>
<td>0.623 ± 0.020b</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8–10 rats/group. Values with different superscripts differ from each other by P = 0.05 or less by t-test after significant intergroup differences were found by ANOVA.

DISCUSSION

The current study and a prior one (13) show that NPY expression in arcuate neurons is regulated differentially in DIO compared with DR rats as a function of the interaction of genotype and dietary composition. As we previously showed (13), and have confirmed here, outbred preobese DIO rats [identified by high urinary norepinephrine levels (10)] have higher arcuate NPY mRNA levels than DR rats before exposure to HE diet. Furthermore, these NPY levels are not upregulated by restriction of energy intake as they are in DR rats (13). This is similar to the abnormalities of NPY regulation seen in obese rodents with a defective long form of hypothalamic leptin receptors (5, 22, 24). As in these single-gene mutations (5) fully obese DIO rats had markedly increased plasma leptin levels but maintained their body weights at a much higher level than DR rats on comparable diets. But DIO rats differ from these genetically obese rodents in one important way. They are capable of changing their regulation of both arcuate NPY expression and their level of defended body weight when exposed to an HE diet for a prolonged period. Thus the elevated NPY levels of DIO-prone rats were reduced here by 12 wk but not by 2 wk of HE-diet intake (13). Once the DIO phenotype was fully expressed, outbred DIO rats avidly defended their higher body weights and carcass adiposity against diet restriction evi-
restoration (Fig. 1, Ref. 17). This restriction was accompanied by the expected fall in plasma leptin and insulin levels and elevations in arcuate NPY expression that occur in energy-restricted DR rats (5, 13). This full expression of obesity in DIO rats was associated with an actual decrease in NPY expression compared with both obese and nonobese DR rats. In fact, both outbred and inbred DIO rats exhibited this decrease. The inbred DIO rats had lower levels of NPY than DR rats, regardless of whether they were fed chow or HE diet. Whereas this might seem contradictory, inbred DIO rats are so metabolically efficient that they become obese by 10–12 wk of age, even when fed only chow from weaning (Table 2, Ref. 14).

Thus arcuate NPY mRNA expression is abnormally high and not responsive to energy restriction before phenotypic expression of obesity in DIO rats. However, once obesity is fully expressed, NPY levels are depressed and become normally responsive to energy restriction. This decreased arcuate NPY mRNA expression may be associated with decreased NPY release because DIO rats have increased binding to Y2 and/or Y5 receptors in the paraventricular nucleus (27), which receives afferents from arcuate NPY neurons (1). The sustained elevations of plasma levels of both leptin and insulin associated with DIO in both inbred (Table 2, Ref. 14) and outbred rats (17) may be responsible for this decreased arcuate NPY expression. Such sustained high levels of leptin and/or insulin may also be required for normal regulation of energy balance and arcuate NPY mRNA expression in DIO rats. However, comparably obese DR rats did not show this apparent downregulation of NPY expression and did not defend their higher body weight.

In fact, DR rats regulate their body weight much differently than DIO rats. When fed the HE diet with its moderate levels of fat content (16), they gain the same amount of weight and carcass fat as chow-fed controls over a 12-wk period (16). However, if fed this diet for 14–20 wk, their proportion of carcass fat increases even though their body weights do not differ from chow-fed controls (Table 1, Ref. 15). Thus they can be made obese by prolonged exposure to a high-fat diet (Table 1, Ref. 17). Also, DR rats can be made hyperphagic and obese by exposure to a highly palatable diet (17). However, DR rats will not maintain their higher body weights unless fed a highly palatable diet, whereas DIO rats maintain their higher body weights for months after being switched from HE diet to a low-fat diet (19). Obese DR rats voluntarily inhibited their intake of a low-fat diet until their weights equaled those of nonobese DR rats, and this reduction was not associated with the expected drop in leptin levels or increased arcuate NPY expression associated with energy restriction (5, 13) that was seen in the restricted DIO rats.

Furthermore, previously obese DR rats remained at the lower body weight and gradually brought their carcass adiposity back toward control levels, all the while showing virtually no change in NPY expression. On the other hand, restricted DIO rats quickly increased their intake, body weight, leptin, insulin, carcass adipose, and elevated arcuate NPY levels back to those of never-restricted DIO rats. This suggests that the elevated body weight was the one at which arcuate NPY expression was preferentially regulated. The fact that NPY levels were not decreased in either group of DIO rats 6 wk after their return to chow may be due to the metabolic transition that occurs in these first weeks following the switch from HE to chow diet (16, 19). Energy intake, body weight, glucose metabolism, and sympathetic activity all undergo changes during this period, which then stabilize after several more months (16, 19).

In conclusion, these studies suggest that energy homeostasis is coordinated by at least two separate but interrelated systems. The arcuate NPY system is regulated by leptin and/or insulin and responds primarily to the metabolic needs of the animal. A second system is responsive to palatability and does not require metabolic “need” to drive intake. Energy homeostasis in DIO and DR rats appears to be regulated by differences in these endogenous systems in the face of changes in dietary content. Furthermore, preobese DIO rats appear to be primed to become obese by their elevated levels of arcuate NPY expression. Once the fat content of the diet is increased, the obese phenotype is expressed and the arcuate NPY system appears to function in the expected manner to defend this higher body weight. Thus these studies demonstrate the important interface between genetic background and environmental factors with critical systems in the brain that regulate energy homeostasis.

Perspectives

There is no question that there are multiple, redundant systems involved in the control of energy homeostasis in the body. This, of course, is to be expected since energy balance is critical to the survival of both the species and the individual. The present studies focus on the arcuate nucleus NPY neurons that play a critical role in this process. Whereas it is clear that energy balance can be maintained when NPY is removed during the development of the nervous system (4), this only emphasizes both the redundancy of the systems controlling energy balance and the ability of the brain to undergo developmental plasticity. The present studies show that the adult brain also can undergo plasticity and that this plasticity is critically dependent on both genetic background and environmental exposure. The metamorphosis from “abnormal” to “normal” arcuate NPY mRNA levels and regulation seen in the transition from the obesity-prone to the fully obese DIO rat is only one of a number of brain systems that appear to normalize only with the appearance of obesity. These include α2-adrenoceptors (8, 28), noradrenergic (11) and gluco-sensing (12) systems, and hypothalamic neuronal function (9, 18). This suggests that the DIO rat brain is genetically predisposed to support the obese state but requires exposure to calorically dense diets with elevated fat content for expression of this phenotype. Those systems in the obesity-prone brain appear to prime it to respond to such diets by raising intake.
and metabolic efficiency until the preprogrammed body weight set-point is realized.

Once the obesity-prone rat becomes fully obese, these neural systems appear to function normally as long as no attempt is made to alter body composition. When reduction of the elevated body energy stores is attempted, the DIO animal responds as if it were in a state of starvation and attempts to preserve adipose mass by increasing its metabolic efficiency (3). Obese humans show similar responses to energy restriction despite an apparent excess of energy stores (7). Thus the obese state, once realized, may lead to a succession of plastic changes in brain function that become near permanent. This could account for the poor success rate in the treatment of obesity (26). The current and previous results (17) show that the obese DIO brain will not voluntarily cede any of its excess carcass energy stores, whereas the obese DR brain spontaneously sheds its excess fat once the powerful cue of increased diet palatability is removed. If this applies to humans, it is likely that any pharmacotherapy of obesity will necessitate long-term treatment with drugs that intervene in critical regulatory pathways to prevent the brain from taking the counter-regulatory steps normally involved in the response to perceived starvation.

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