High and Low Activity Rats: Elevated Intrinsic Physical Activity Drives Resistance to Diet-Induced Obesity in Non-Bred Rats

Claudio E. Perez-Leighton1,2,5, Kelsey Boland3, Charles J. Billington4,5 and Catherine M. Kotz1,3,5,6

Objective: Humans and rodents show large variability in their individual sensitivity to diet-induced obesity (DIO), which has been associated with differences in intrinsic spontaneous physical activity (SPA). Evidence from genetic and out-bred rat obesity models shows that higher activity of the orexin peptides results in higher intrinsic SPA and protection against DIO. Based on this, we hypothesized that naturally occurring variation in SPA and orexin signaling is sufficient to drive differences in sensitivity to DIO.

Design and Methods: Orexin expression, behavioral responses to orexin-A, basal energy expenditure and sensitivity to DIO were measured in in non-manipulated male Sprague-Dawley rats selected for high and low intrinsic SPA.

Results: Male Sprague-Dawley rats were classified as high-activity or low-activity based on differences in intrinsic SPA. High-activity rats showed higher expression of prepro-orexin mRNA, higher sensitivity to behavioral effects of orexin injection, higher basal energy expenditure and were more resistant to obesity caused by high-fat diet consumption than low-activity rats.

Conclusion: Our results define a new model of differential DIO sensitivity, the high-activity and low-activity rats, and suggest that naturally occurring variations in intrinsic SPA cause differences in energy expenditure that are mediated by orexin signaling and alter DIO sensitivity.

Obesity (2013) 21, 353-360. doi:10.1002/oby.20045

Introduction

Humans and rodents show large variability in their individual sensitivity to diet-induced obesity (DIO) (1-6) and lower DIO sensitivity has been correlated with higher levels of intrinsic spontaneous physical activity (SPAINT) (7-9). SPAINT is defined as “physical activity that does not qualify as voluntary exercise” (10,11). In rodents, SPAINT is measured as spontaneous ambulatory movement plus rearing in an open field over a long period of time (i.e., 24 h) after adaptation to the new environment to avoid a confound effect of initial exploratory activity (11-14). Currently, the neurobiological control of SPAINT is understood as a distributed brain network involving multiple neuropeptide systems, which includes the orexins/hypocretins (10,15). There are two orexin peptides (orexin-A/hypocretin-1, OXA, and orexin-B/hypocretin-2, OXB) and two receptors (orexin/hypocretin receptor 1, OX1R, and orexin/hypocretin receptor 2, OX2R) (16,17). The orexin neurons are located in the lateral hypothalamic and perifornical areas and have efferents to multiple brain sites (18,19) with varying orexin receptors (OXR) expression levels (20-23).

Activation of the OXR promotes negative energy balance, probably through an increase and/or maintenance of SPAINT. This view of orexin function is supported by multiple lines of evidence: mice deficient in orexin neurons show lower levels of physical activity, hypophagia, and obesity (24); overexpression of the orexin peptides leads to resistance against DIO (25); injection of orexin peptides in several brain sites, such as the rostral lateral hypothalamus (rLH) and substantia nigra (SN) increases SPA (12,13,26) and systemic injection of OXR antagonists decreases SPA (27). These data show that higher orexin signaling leads to higher SPAINT resulting in resistance to obesity. Our previous work in the obesity prone (OP) and obesity resistant (OR) out-bred rats (28) supports this hypothesis, as the OR phenotype shows higher SPAINT levels, higher orexin

Disclosure: The authors declared no conflict of interest.

Funding agencies: The Department of Veterans Affairs, the Minnesota Obesity Center and grant no. DK078985 from the National Institute of Diabetes and Digestive and Kidney Diseases.

Additional Supporting Information may be found in the online version of this article.

Received: 18 January 2012 Accepted: 1 August 2012 Published online 3 October 2012. doi:10.1002/oby.20045
behavioral responsivity (14) and long-term DIO resistance (9). However, the OP and OR rats are not bred for differences in SPA but for differences in weight gain after an obesogenic diet (28). Thus, it is possible that higher SPA_{INT} in OR rats is a trait that cosegregates with DIO resistance. Therefore, the OP/OR model does not conclusively prove that differences in SPA_{INT} are sufficient to drive differential DIO sensitivity. Together, these data suggest the variation in SPA_{INT} in non-bred rodent populations and the potential to impact DIO sensitivity remains unknown.

The current studies were designed to test the following hypothesis: natural (i.e., unmanipulated) differences in SPA_{INT} are sufficient to drive differences in DIO sensitivity. Furthermore, we hypothesized that orexin function might underlie the variation in SPA_{INT}. In these studies, we selected male Sprague-Dawley (SD) rats for differences in ambulatory SPA_{INT} levels and tested their energy expenditure (EE), prepro-orexin mRNA expression, behavioral responses to OXA injections in rLH and SN, and DIO sensitivity. Our work defined a new model of differential DIO sensitivity: high-activity (HA) and low-activity (LA) rats. These results suggest that naturally occurring variations in SPA_{INT} are related to differences in activity of the orexin peptides and are sufficient to drive differences in EE and protect against DIO.

Methods

Animals

Male SD rats (Charles River, Kingston, NY; 200-250 g at arrival) were individually housed in hanging-wire cages with a 12-h light/dark cycle (lights on at 06:30 AM) at 21-22°C. Animals were given 1 week of acclimation to housing conditions with food and water ad libitum. The experiments were approved by the Local Institutional Animal Care and Use Committee at the Minneapolis VA Medical Center.

Diet

High fat (HF, D12451; 45% kcal from fat) and low fat (LF, D12450B; 10% kcal from fat) from Research Diets (New Brunswick, NJ) and standard diet (Harlan Teklad 8604) were used. Food and water were available ad libitum.

Peptides

Orexin-A (American Peptides, Sunnyvale, CA) was dissolved in artificial cerebrospinal fluid (aCSF, Harvard Apparatus, Holliston, MA), aliquoted and kept at −20°C until needed.

Body weight, fat and lean mass, and food intake measurements

Body weight (g) and food intake corrected by spillage (g) were determined every other day. Total fat mass (FM) (g) and lean mass (LM) (g) were measured by quantitative magnetic resonance (29,30) using the EchoMRI-900 scan (Echo Medical Systems, Houston, TX).

Measurement of SPA_{INT}

SPA was measured in a 17.0 by 17.0 inches squared acrylic cage surrounded by three 16-beams sets of infrared activity sensors (Med Associates, St. Albans, VT). Two sets of arrays were in the x-y plane and the third set was elevated 3 inches above the x-y plane. Movement was recorded by beam breaks with 100 ms resolution.

SPA_{INT} levels were measured over a 24 h period after 24 h habitation to the recording cages. This length of habitation time is sufficient to produce stable SPA_{INT} measurements (Supporting Information Figure 1). Ambulatory SPA_{INT} levels are reported in min as time spent moving lateral plus time spent rearing. Stereotypic SPA_{INT} is defined as time spent performing any partial-body movements (i.e., grooming) within a defined space around the animal (3.25 × 3.25 inches). Measurement of ambulatory and stereotypic SPA_{INT} for classification of rats as HA or LA was the first test conducted in all animals after acclimation to the housing facilities.

Indirect calorimetry

Indirect calorimetry (IDC) and SPA were recorded simultaneously in an air-tight SPA cage of the same dimensions and configuration as described previously. Oxygen consumption and carbon dioxide production were measured by using a customized, high-precision, single-chamber indirect calorimeter (Columbus Instruments, Columbus, OH). SPA was recorded as described in the previous section. Thermogenesis was calculated from oxygen consumption and carbon dioxide production and is reported as the average kcal per h over 24 h. During IDC recordings, animals had food and water available ad libitum. Rats (N = 32) were acclimated for 7 days to the IDC SPA cages. Next, SPA and IDC were recorded for three consecutive 24 h periods.
Response to OXA in rostral lateral hypothalamus (rLH) and substantia nigra pars compacta (SN) injection of orexin-A (OXA). SPA after injection of orexin was measured for 2 h postinjection after 1 h acclimation to the testing chambers. A: Ambulatory (AMB) and stereotypic (STER) SPA in high activity (HA) and low activity (LA) rats used for analysis (for rLH, HA, N = 13; LA, N = 14; for SN, HA, N = 11; LA, N = 7). No significant differences in ambulatory or stereotypic SPA were found in HA (ambulatory, W = 99, P = 0.813; stereotypic, W = 105, P = 1.000) or LA rats (ambulatory, W = 52, P = 0.276; stereotypic, W = 68, P = 0.84) selected for orexin injections in rLH or SN. Line: P < 0.05 for the Mann-Whitney-Wilcoxon test.

C, D: Ambulatory SPA after OXA injection in rLH (C) and SN (D). E, F: Stereotypic SPA after OXA injection in rLH (D) and SN (E). Line: P < 0.05 for pairwise comparison between HA and LA at each OXA dose; *P < 0.05 for pairwise comparison within HA and LA rats at each OXA dose vs aCSF injection. For (C–F) all P-values were corrected for multiple comparisons (See “Statistical analysis”).

Measurement of SPA after OXA injections was conducted as follows. During each recording session, animals were transported from their home cages to the SPA recording cages and habituated for 1-2 h. Analysis of the time course of response showed this length of habituation was sufficient to produce a stable baseline in both HA and LA rats (Supporting Information Figures 2 and 3). Next, animals were injected unilaterally with either aCSF or OXA (50, 125, 250, and 500 pmol) in a latin-square design to avoid a treatment order confound. At least 48 h elapsed between injections.

Time course analysis showed that SPA during the first 5 min after injection related to the injection procedure itself and was excluded from the data analysis. Thus, SPA is reported as time spent moving for 2 h starting 5 min after injection. After completion of the experiment, accuracy of cannula placement was confirmed by histological methods (13).

Real time PCR analysis

HA and LA rats were euthanized by decapitation between 10:00 AM and 12:00 PM. Food was removed 2 h before euthanasia. Samples from caudal lateral hypothalamus (cLH) were collected as described previously (32), immediately frozen in liquid nitrogen and stored at −80°C.

Total RNA was extracted using TRIzol (15596-026, Invitrogen, Carlsbad, CA), cleaned using the RNeasy micro kit (74034, Qia-gen, Germantown, MD), quantified by UV absorption at 260 nm with the ND-1000 spectrophotometer (ThermoScientific, Wilmington, DE) and stored at −80°C until use. Equal amounts of RNA were used to measure expression levels of prepro-orexin and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA with the LightCycler RNA Master SYBR Green I kit (0306476001, Roche, www.obesityjournal.org

FIGURE 2

FIGURE 2 SPA after rostral lateral hypothalamus (rLH) and substantia nigra pars compacta (SN) injection of orexin-A (OXA). SPA after injection of orexin was measured for 2 h postinjection after 1 h acclimation to the testing chambers. A: Ambulatory (AMB) and stereotypic (STER) SPA in high activity (HA) and low activity (LA) rats used for analysis (for rLH, HA, N = 13; LA, N = 14; for SN, HA, N = 11; LA, N = 7). No significant differences in ambulatory or stereotypic SPA were found in HA (ambulatory, W = 99, P = 0.813; stereotypic, W = 105, P = 1.000) or LA rats (ambulatory, W = 52, P = 0.276; stereotypic, W = 68, P = 0.84) selected for orexin injections in rLH or SN. Line: P < 0.05 for the Mann-Whitney-Wilcoxon test.

C, D: Ambulatory SPA after OXA injection in rLH (C) and SN (D). E, F: Stereotypic SPA after OXA injection in rLH (D) and SN (E). Line: P < 0.05 for pairwise comparison between HA and LA at each OXA dose; *P < 0.05 for pairwise comparison within HA and LA rats at each OXA dose vs aCSF injection. For (C–F) all P-values were corrected for multiple comparisons (See “Statistical analysis”).

Original Article

OBESITY BIOLOGY AND INTEGRATED PHYSIOLOGY

www.obesityjournal.org

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Indianapolis, IN) in a LightCycler 2 thermocycler (Roche). Primer sequences were described previously (14). Efficiency of each PCR reaction was determined using the dilution method (33). Expression levels of prepro-orexin mRNA were normalized against GAPDH and comprising 88% of all animals in the population. Descriptive statistics of the HA and LA rats are shown in Table 1. Importantly, there were no significant differences in total body weight, FM, LM or FM/LM between HA and LA rats at study onset (Table 1), which did not depend on the criteria for selection of HA or LA rats (Supporting Information Table 3), justifying the use of total body weight for correction of EE. For HA/LA classification, reliability of classification was calculated as before, in a sample of 32 male SD rats; rats were classified as HA/LA based on ambulatory SPANt measured consecutively over three 24 h periods.

For all statistical analysis, a P-value of less than 0.05 was considered significant.

Results

Definition of HA and LA phenotype

We calculated a distribution of 24 h ambulatory SPANt in male SD rats on a standard diet (Figure 1A, N = 166 male SD rats). This distribution had a positive skewness (0.053) and was described by a gamma distribution (KS test, D = 0.039, P = 0.95). There was a significant correlation of ambulatory SPANt with LM (ρ = 0.172, P = 0.032) and total body weight (ρ = 0.162, P = 0.042) but not with FM (ρ = −0.001, P = 0.98) or fat to lean mass ratio (FM/LM, ρ = −0.075, P = 0.349).

We used the ambulatory SPANt distribution to define HA (SPANt ≥ 120 min, 63% quantile of the ambulatory SPANt distribution) and LA (SPANt ≤ 90 min, 25% quantile of the ambulatory SPANt distribution). While this criterion is arbitrary, it results in significant differences in SPANt between HA and LA rats (Figure 1B) and comprises 88% of all animals in the population. Descriptive statistics of the HA and LA rats are shown in Table 1. Importantly, there were no significant differences in total body weight, FM, LM or FM/LM between HA and LA rats at study onset (Table 1), which did not depend on the criteria for selection of HA or LA rats (Supporting Information Tables 1-3). Although we defined the HA/LA phenotype based on ambulatory SPANt, SD rats also show stereotypic physical activity (See “Methods” section for definition of ambulatory and stereotypic SPA). There was a small, but statistically significant linear correlation between ambulatory SPANt and stereotypic SPANt (ρ = 0.241, P = 0.002). Likewise, there was a small, but statistically significant difference in stereotypic SPA between HA and LA rats (W = 1609.5, P = 0.014, See Table 1).

In a subset of SD rats, we repeated the measure of ambulatory SPANt one week apart and calculated the reliability of classification over the course of 2 weeks. Reliability was calculated as number of HA/LA rats that were classified in the same category based on different SPANt measurements. As the SPANt from HA and LA populations do not follow a normal distribution, bias confidence intervals for the mean and median were calculated by bootstrap analysis.

During the first day of IDC recording, we observed a reduction in the range of 24 h ambulatory SPANt (Figure 3A) compared with SPANt recorded in non-IDC SPA cages (Figure 1A). Therefore, we classified HA and LA rats using the same quantiles from the ambulatory SPANt distribution recorded in the IDC SPA cages as used in all other experiments (25% quantile for LA and 63% for HA rats). SPA and EE were recorded for a total of three 24 h periods. EE was corrected with a linear regression analysis over total body weight independently for HA and LA rats (36,37) using the averages of uncorrected EE (kcal/h) and body weight (g) over three consecutive 24 h periods of recording. At the end of the IDC recordings, we measured FM and LM in a subset of rats and found no significant differences between HA and LA rats in these measurements (Supporting Information Table 3), justifying the use of total body weight for correction of EE. For HA/LA classification, reliability of classification was calculated as before, in a sample of 32 male SD rats; rats were classified as HA/LA based on ambulatory SPANt measured consecutively over three 24 h periods.

Statistical analysis

All statistical analyses were done with R software version 2.11.1 (34). Data are presented as mean ± standard error of the mean. Effects of DIO on HA and LA rats were analyzed with a 2-way ANOVA with phenotype (HA/LA) and diet (HF/LF) as independent variables. Analysis of SPAN after OXA injections was done using a 2-way repeated measures ANOVA with dose of orexin as the independent variable and dietary group as a repeated measure. For all statistical analysis, a P-value of less than 0.05 was considered significant.

Analysis of reliability of HA/LA classification was performed on data from 29 male SD rats for which SPANt levels were measured twice with the method of Hochberg (35).
of SD rats as HA/LA to be 72.85%; See “Statistical analysis” section.

**Prepro-orexin mRNA expression and rLH and SN orexin responsivity in HA and LA rats**

As genetic manipulation of orexin expression levels directly alters SPINT (24,25), we hypothesized that differences in orexin contribute to differences in ambulatory SPINT. Analysis of the *prepro*-orexin mRNA (17) in rLH samples from HA (N = 18) and LA (N = 9) rats (Figure 1B) showed that HA rats had higher expression of prepro-orexin mRNA compared with LA rats (Figure 1C, Welch t-test, t = 2.73, df = 23.11, P = 0.012).

In the OP/OR model, higher levels of ambulatory SPINT correlate with higher rLH orexin responsivity (14), and orexin regulation of SP is distributed throughout multiple brain sites including the rLH and SN (13). Therefore, we hypothesized that HA rats will have higher rLH and SN orexin responsivity compared with LA rats. Figure 2A and 2B shows the ambulatory and stereotypic SP in HA and LA rats for rLH (HA, N = 14; LA, N = 13; Figure 2A) and SN (HA, N = 11; LA, N = 7; Figure 2B) injections. A 2-way ANOVA with dose of OXA as a repeat measure showed that injection of OXA in rLH and SN significantly increased ambulatory SP (rLH: Figure 2C, F₄,₁₀₀ = 53.42, P < 0.01; SN: Figure 2D, F₄,₆₄ = 6.01, P < 0.01). In rLH, comparison of individual doses of OXA against aCSF injection showed significant differences in HA and LA rats across all doses of orexin-A (Figure 2C). On the contrary, for SN, there were only significant differences in HA rats at higher OXA doses (Figure 2D). As with ambulatory SP, there was a significant effect of the HA/LA phenotype for rLH OXA injections (F₄,₁₀₀ = 7.023, P = 0.0013), but failed to reach statistical significance for SN OXA injections (F₄,₆₄ = 4.02, P = 0.062). Pairwise analysis of stereotypic SP response to OXA in rLH of HA and LA rats showed significant differences only at one dose of OXA (Figure 2E), whereas for SN injections there were no statistical differences between HA and LA rats at individual OXA doses (Figure 2F). Together, these data suggest that differences in rLH and SN orexin responsivity contribute to the differences in ambulatory and stereotypic SP between HA and LA rats, with the rLH effect being more robust.

**Energy expenditure in HA/LA rats**

We used IDC to test the hypothesis that HA rats have higher EE than LA rats. The distribution of ambulatory SPINT over 24 h collected in the IDC cages (Figure 3A) had a smaller range compared with non-IDC SP cages (Figure 1A), despite using cages of the same size (See “Methods” section). Therefore, we classified HA and LA rats from the distribution of ambulatory SPINT collected in the IDC cages using the same quantiles used to define HA/LA rats from the distribution shown in Figure 1A. Similar to the original ambulatory SPINT distribution (Figure 1A), the distribution of ambulatory SP recorded in the IDC cages had a positive skewness (0.054) and was described by a gamma distribution (KS test, D = 0.017, P = 0.83). Despite the reduced range of ambulatory SPINT, there were significant differences in ambulatory SPINT between HA and LA rats (Figure 3B; τ₁₂ = 10.57, P < 0.001). Next, we recorded EE and ambulatory SPINT for two additional 24 h periods. We found no significant differences in body weight or food intake between HA and LA rats across recording sessions (Supporting Information Table 2). Therefore, we used the average values of uncorrected EE and body weight over the three 24 h periods of recording to compare EE between HA and LA rats. Importantly, we maintained the classification of HA and LA rats based on ambulatory SPINT from the first 24 h of recording. Figure 3C shows the best-fit lines for HA and LA rats for uncorrected EE over total body weight averaged over the recording sessions. After adjustment for

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**TABLE 1** Descriptive statistics for SPINT distribution for high and low activity rats

|                         | High activity | Low activity |
|-------------------------|---------------|-------------|
| Mean ambulatory SPINT   | 149.18 [144.0, 155.9] (N = 61) | 71.90 [67.50, 75.75] (N = 41) |
| Median ambulatory SPINT | 143.18 [136.9, 146.7] (N = 61) | 76.22 [69.72, 81.36] (N = 41) |
| Mean stereotypic SPINT  | 97.17 [93.80, 101.92] (N = 61) | 90.85 [85.59, 97.87] (N = 41) |
| Median stereotypic SPINT | 94.63 [90.86, 97.81] (N = 61) | 88.15 [78.22, 91.72] (N = 41) |
| Body weight             | 291.80 ± 5.20 (N = 56) | 282.25 ± 5.35 (N = 39) |
| Fat mass                | 29.00 ± 0.58 (N = 56) | 28.49 ± 0.79 (N = 39) |
| Lean mass               | 235.22 ± 4.04 (N = 56) | 228.2 ± 3.86 (N = 39) |
| Fat mass/lean mass      | 0.124 ± 0.0021 (N = 56) | 0.124 ± 0.0024 (N = 56) |

*a*-Confidence intervals are from bootstrap calculation using bias correction.

*b*-P-values for comparisons between HA and LA < 0.05.

*c*-P-values for comparisons between HA and LA were not significant.
total body mass, HA rats showed significantly higher EE than LA rats (Figure 3D, $b_{HA} = -2.26$, $P = 0.035$), whereas there were no differences in uncorrected EE between HA and LA rats averaged over IDC recording sessions (Supporting Information Table 2).

**Diet-induced obesity sensitivity in HA/LA rats**

To test the contribution of ambulatory SPAINT levels to DIO resistance HA and LA rats (Figure 4A) were fed a HF (HA, $N = 17$; LA, $N = 10$) or LF (HA, $N = 16$; LA, $N = 9$) diet *ad libitum* for 10 weeks. Analysis of cumulative food intake (Figure 4B) with a 2-way ANOVA showed a significant effect of diet ($F_{1,48} = 18.70$, $P < 0.001$) and no effect of HA/LA phenotype ($F_{1,48} = 0.006$, $P = 0.940$) or their interaction ($F_{1,48} = 1.58$, $P = 0.214$). A similar pattern was observed for FM gain ($\Delta$FM; diet, $F_{1,48} = 18.86$, $P < 0.001$; HA/LA phenotype, $F_{1,48} = 2.71$, $P = 0.106$, [diet × HA/LA phenotype] interaction, $F_{1,48} = 0.00$, $P = 0.998$). Pairwise analysis indicated increased caloric consumption due to HF diet only in HA rats (Figure 3B), whereas HF consumption increased $\Delta$FM in both HA and LA rats (Figure 4C). On the contrary, for lean mass gain ($\Delta$LM, Figure 4D), there was a significant effect of the HA/LA phenotype ($F_{1,48} = 4.30$, $P = 0.043$) but not of diet ($F_{1,48} = 1.51$, $P = 0.22$), or their interaction ($F_{1,48} = 1.63$, $P = 0.21$). As obesity is defined by chronic accumulation of FM and not by changes in overall body weight (38), severity of obesity was measured as accumulation of FM relative to lean mass (Figure 4E, $\Delta$FM/$\Delta$LM). There was a significant effect of HA/LA phenotype ($F_{1,48} = 10.06$, $P = 0.002$), diet ($F_{1,48} = 12.27$, $P = 0.001$) in $\Delta$FM/$\Delta$LM, but no significant interaction ($F_{1,48} = 0.09$, $P = 0.759$). Pairwise analysis indicated higher $\Delta$FM/$\Delta$LM in HA and LA rats fed a HF diet, whereas $\Delta$FM/$\Delta$LM was lower in HA compared with LA rats under a HF or a LF diet (Figure 4E). These data suggest that, independent of diet, HA rats have a higher resistance toward an obese phenotype.

The differences in DIO severity between HA and LA rats suggest differential effects of DIO on ambulatory SPAINT and feeding efficiency. Figure 5 shows the effects of HF and LF feeding on 24 h ambulatory SPAINT. These data were analyzed with a repeated measures ANOVA with diet (HF or LF) and time (pre- or post-feeding) as independent variables. For HA rats, there were no significant differences in SPAINT for diet and time, and no interaction between diet and time ($t_{1,30} = 0.205$, $P = 0.65$; time $t_{1,30} = 0.954$, $P = 0.33$; [diet × time] interaction, $F_{1,30} = 0.0007$, $P = 0.99$). For LA rats, there was no significant effect of diet ($F_{1,11} = 2.07$, $P = 0.17$), but there was a significant effect of time ($F_{1,11} = 22.83$, $P = 0.0005$) and a significant interaction between diet and time ($F_{1,11} = 5.01$, $P = 0.046$). Consistently, pairwise analyses indicated an increase in SPAINT in LA rats fed a LF diet ($P = 0.002$), and no change in SPAINT in LA rats fed a HF diet ($P = 0.15$). Importantly, after completion of the experiment, HA rats showed significantly higher SPAINT than LA rats, independent of diet ($P < 0.05$ for all pairwise comparisons). Figure 6 shows the effects of DIO on feeding efficiency for FM and LM (weight gain per kcal). These data were analyzed with a 2-way ANCOVA using FM or LM prefeeding as a covariate. For FM feeding efficiency (Figure 6A), there was a significant effect of diet ($F_{1,47} = 29.83$, $P < 0.001$), phenotype...
(\(F_{1,47} = 7.40, P = 0.009\)), and covariate (\(F_{1,47} = 16.86, P < 0.001\)) but there was no diet by phenotype interaction (\(F_{1,47} = 0.089, P = 0.76\)). Pairwise analysis indicated a significant difference in FM feeding efficiency in HA and LA rats when fed a HF diet compared with LF diet (Figure 6A). For LM feeding efficiency, there was a significant effect of phenotype (\(F_{1,47} = 6.68, P = 0.013\)), no significant effects of diet (\(F_{1,47} = 1.22, P = 0.27\)), the [diet \times HA/LA phenotype] interaction (\(F_{1,47} = 0.11, P = 0.74\)) or covariate (\(F_{1,47} = 0.049, P = 0.83\)). Pairwise analysis indicated that HA rats had higher feeding efficiency for lean mass weight compared with LA rats only under HF diet feeding (Figure 5B). Together, these results show that HA rats have lower DIO sensitivity than LA rats (Figure 4) and that different diet differentially alter SPA in HA and LA rats (Figure 5). Our results suggest resistance to obesity in HA rats is associated with a preferential ability for accumulation of lean mass under a HF diet (Figure 6B) and lower caloric efficiency for FM under a LF diet (Figure 6A) compared with LA rats.

Discussion

There is extensive evidence suggesting that modulation of orexin expression levels drives SPA\textsubscript{INT} resulting in resistance against DIO. This article tested a critical hypothesis regarding the role of orexin in energy balance: whether naturally occurring variations in SPA\textsubscript{INT} are related to orexin gene expression and are sufficient to drive differences in DIO sensitivity. Our characterization of the HA/LA phenotype in unmanipulated SD rats shows that variations in SPA\textsubscript{INT} are: (1) related to orexin function (Figures 1 and 2) and (2) are sufficient to drive resistance to obesity (Figure 4).

To our knowledge, this is the first article that describes the distribution of ambulatory SPA\textsubscript{INT} (Figure 1) in male SD rats. The reliability of the classification of SD rats as HA or LA using ambulatory SPA\textsubscript{INT} measured in an open-field (See “Methods” section) was calculated as 72.85%. The proposed method for measurement of ambulatory SPA\textsubscript{INT} is relatively time consuming (24 h of adaptation and 24 h of recording), and thus, it would be interesting to establish the minimum conditions of ambulatory SPA\textsubscript{INT} measurement to classify SD rats as HA or LA while retaining similar reliability as observed in this study.

Previous work from our laboratory in a polygenic model of obesity, the obesity-prone (OP) and obesity-resistant (OR) rats (28), showed the OR phenotype correlates with higher SPA\textsubscript{INT} (14). The reported values of SPA\textsubscript{INT} over 24 h for OP and OR rats at 1-2 months were ~102.2 min and 158 min (14). The estimated average ambulatory 24 h SPA\textsubscript{INT} values for LA and HA rats are 71.75 and 145.5 min. Although the values are not directly comparable, in terms of SPA\textsubscript{INT}, the differences in SPA\textsubscript{INT} between groups appear to be larger in the HA/LA phenotype compared with the OP/OR model.

The HA rats have higher expression of prepro-orexin mRNA in cLH (Figure 1C) and higher orexin responsivity than LA rats to rLH and SN orexin injections (Figure 2). The higher expression of prepro-orexin mRNA in HA rats fits with previous evidence showing that downregulation of orexin signaling decreases physical activity (24,27). We evaluated SPA after orexin-A injection in rLH and SN, as these brain sites mediate orexin effects on SPA\textsubscript{INT}. As shown previously, injection of OXA in rLH and SN increases ambulatory SPA (13), but the higher responsivity in HA compared with LA rats in ambulatory and stereotypic SPA was more robust after rLH injection than after SN injection. This difference is intriguing as significant differences in rLH OXA responsivity were also observed in the OP/OR rats (14), although differential OXA responsivity for SN injections between OP and OR rats has not been reported. This suggests the rLH as an important site for the contribution of orexin signaling in controlling SPA in SD rats.

We used IDC to measure EE in HA and LA rats for three consecutive 24 h periods (Figure 3). In our laboratory, we use cages of the same size for routine measurements of SPA and IDC. Thus, we currently do not have an explanation for the reduction in range of ambulatory SPA\textsubscript{INT} observed during the IDC recordings (Figure 3A), compared with routine, non-IDC, SPA recordings (Figure 1A). This difference is interesting considering that rats were acclimated for 7 days before the start of the IDC recordings (See “Methods” section) and were eating and gaining weight normally. Considering the reduction in range of ambulatory SPA\textsubscript{INT} during IDC, we classified SD as HA and LA rats using the same quantiles (25% for LA and 63% for HA rats) used for classification of the HA/LA phenotype during routine SPA analysis (Figure 1A), but based on the distribution of ambulatory SPA\textsubscript{INT} as recorded in the IDC cages (Figure 3A). This resulted in a difference of ambulatory SPA\textsubscript{INT} of ~21 min per 24 h between HA and LA rats (Figure 3B). When correcting EE for total body weight (averaged over all three 24 h periods), HA rats have higher EE compared with LA rats (Figure 3C and 3D), which is estimated to be ~2 kcal/h. Thus, despite the reduction in range of ambulatory SPA\textsubscript{INT}, these data show that differences in ambulatory SPA\textsubscript{INT} are reflected in overall EE.

A central aspect of the usefulness of the HA/LA phenotype as a new model for obesity research depends on the robustness of the differential DIO sensitivity between HA and LA rats. Our data suggest that HA rats have lower DIO sensitivity compared with LA rats (Figure 5) and that HA rats have higher total EE than LA rats (Figure 3). Together, this suggests that differences in ambulatory and stereotypic SPA\textsubscript{INT} contribute to their DIO resistance. It is important to note that sensitivity to DIO obesity is most likely to be a multifactorial process, in which SPA\textsubscript{INT} plays a significant role, but is clearly not the only mechanism involved. Thus, the HA/LA
phenotype might prove useful in establishing how variations in SPAINT are correlated with other aspects of the development of obesity.

Under both HF and LF diet feeding, the feeding efficiency in HA rats is reflective of a tendency for a leaner phenotype. When fed a HF or LF diet, HA rats are less efficient in accumulating FM relative to LA rats (Figure 4A). During HF diet feeding, HA rats are more efficient in accumulating lean mass (Figure 4B). Together, these results suggest that during HF diet feeding, HA rats adapt to caloric excess by increasing accumulation of lean mass. Body composition was measured using quantitative magnetic resonance; thus, we suggest the increase in lean mass observed in HA rats represents an increase in lean muscle (29,30), a tissue that is more metabolically demanding than white adipose tissue.

Finally, we measured ambulatory SPAINT before and after HF or LF feeding (Figure 5). Our data show no effect of diet on SPAINT of HA rats. However, LA rats fed a LF diet showed an increase in SPAINT, whereas there was no change in SPAINT in LA rats fed a HF diet. After feeding, and independent of diet, HA rats still showed higher SPAINT than LA rats. These data suggest an interaction between the HA/LA phenotype and effects of age and DIO on ambulatory SPAINT. Longitudinal studies in SD rats showed increased ambulatory SPAINT between 2 and 4 months of age, which matches the time frame used in our studies. This suggests that in LA rats, HF consumption prevents the natural increase in SPAINT at this developmental stage.

In summary, these studies describe a new model of differential DIO sensitivity, the HA and LA rats. This model is based on naturally occurring variations in SPAINT in male SD rats. These data further suggest that differences in orexin signaling contribute to the observed variations in SPAINT and DIO sensitivity. This model should prove useful in future studies that address how differences in orexin and other neuropeptidergic mediators might contribute to individual sensitivity to obesity.© 2012 The Obesity Society

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