Mice lacking melanin-concentrating hormone receptor 1 demonstrate increased heart rate associated with altered autonomic activity

Annika Åstrand, Mohammad Bohlooly-Y, Sara Larsdotter, Margit Mahlapuu, Harriet Andersén, Jan Tornell, Claes Ohlsson, Mike Snaith, and David G. A. Morgan. Mice lacking melanin-concentrating hormone receptor 1 demonstrate increased heart rate associated with altered autonomic activity. Am J Physiol Regul Integr Comp Physiol 287: R749–R758, 2004. First published May 6, 2004; 10.1152/ajpregu.00134.2004.—Melanin-concentrating hormone (MCH) plays an important role in energy balance. The current studies were carried out on a new line of mice lacking the rodent MCH receptor (MCHR1−/− mice). These mice confirmed the previously reported lean phenotype characterized by increased energy expenditure and modestly increased caloric intake. Because MCH is expressed in the lateral hypothalamic area, which also has an important role in the regulation of the autonomic nervous system, heart rate and blood pressure were measured by a telemetric method to investigate whether the increased energy expenditure in these mice might be due to altered autonomic nervous system activity. Male MCHR1−/− mice demonstrated a significantly increased heart rate [24-h period: wild type 495 ± 4 vs. MCHR1−/− 561 ± 8 beats/min (P < 0.001); dark phase: wild type 506 ± 8 vs. MCHR1−/− 582 ± 9 beats/min (P < 0.001); light phase: wild type 484 ± 13 vs. MCHR1−/− 539 ± 9 beats/min (P < 0.005)] with no significant difference in mean arterial pressure [wild type 110 ± 0.3 vs. MCHR1−/− 113 ± 0.4 mmHg (P > 0.05)]. Locomotor activity and core body temperature were higher in the MCHR1−/− mice during the dark phase only and thus temporally dissociated from heart rate differences. On fasting, wild-type animals rapidly downregulated body temperature and heart rate. MCHR1−/− mice displayed a distinct delay in the onset of this downregulation. To investigate the mechanism underlying these differences, autonomic blockade experiments were carried out. Administration of the adrenergic antagonist metoprolol completely reversed the tachycardia seen in MCHR1−/− mice, suggesting an increased sympathetic tone.

MELANIN-CONCENTRATING HORMONE (MCH) is a peptide synthesized exclusively in the lateral hypothalamic area and zona incerta (6, 40) that has been implicated in the hypothalamic regulation of energy balance. Levels of MCH mRNA are regulated by altered energy balance in animal models such as fasting and in models with impaired leptin signaling (30). Intracerebroventricular (30) and intrahypothalamic (1) injection of MCH increases food intake, and mice overexpressing MCH are hyperphagic and obese (22). Conversely, mice lacking a functioning MCH gene are hypophagic and lean (37).

Two receptors for MCH have been identified. The first, MCHR1, was originally characterized as an orphan receptor, SLC-1 (15). MCH was subsequently identified as the cognate ligand by a number of groups (5, 10, 21, 35, 38). It is distributed extensively in the central nervous system, with a distribution consistent with a role in energy balance (34). It demonstrates high sequence homology among species investigated so far. The second receptor, MCHR2, was identified by a number of groups using low-stringency homology searching or 5′/3′-rapid amplification of cDNA ends (3, 15, 24, 31, 33, 44). This receptor is expressed as a functional receptor in dogs, nonhuman primates, and humans but is expressed as a pseudogene in rabbits and guinea pigs and is absent in rodent species (42).

Recently two groups have reported the phenotype of mice lacking the only known rodent receptor for MCH (11, 23). These have a similar phenotype to those lacking MCH itself. However, one striking difference is the lack of hypophagia in MCHR1 null mice; instead these mice eat slightly more than their wild-type littermates, suggesting that the lean phenotype of these animals is, to a large degree, brought about by an increase in energy expenditure. Indeed, a significantly raised 24-h energy expenditure has been recorded in these animals using indirect calorimetry.

Increasing circumstantial evidence suggests that MCH may alter energy balance not only by increasing food intake but also by decreasing energy expenditure consistent with an altered level of autonomic nervous system activity. Thus a recent report suggests that intracerebroventricular MCH might decrease body temperature and brown fat uncoupling protein (UCP-1) (16), whereas ob/ob mice lacking MCH showed an increased level of brown fat UCP-1 and an increased body temperature compared with control ob/ob animals (36). In addition, sympathetic projections to a number of organs have been retrogradely traced to the lateral hypothalamic area, and projections to the brown fat have even been traced to MCH-containing cells (26).

In this study we investigate diurnal variations in energy expenditure of MCHR1−/− mice and compare it to variations in heart rate and blood pressure activity. We have also investigated the autonomic mechanisms underlying differences between MCHR1−/− mice and wild-type controls, using pharmacological blockade of sympathetic and parasympathetic nervous systems.

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Fasting at an ambient temperature below thermoneutrality results in large transient depressions in heart rate, mean arterial pressure, and oxygen consumption in mice (46). This response is thought to be mediated in part via a reduction in sympathetic nervous system activity, and so changes in this response can indicate changes in sympathetic nervous system output. To investigate this we have compared the cardiovascular responses to fasting in MCHR1−/− mice and wild-type controls.

**MATERIALS AND METHODS**

**Animal care.** Knockout animals were generated using R1 ES cells (derived from 129/SvJ). All animals used in these studies had been backcrossed for a minimum of three generations to C57Bl/6. Control animals used in the experiments were always the wild-type littermates of the MCHR1−/− mice. Mice were maintained under a standard 12-h light cycle regime, the relative humidity was between 45 and 55%, the temperature was kept at 20°C, and the animals had ad libitum access to chow and water, with environmental enrichment (in the form of egg cartons, wooden tongue depressors, and cotton nesting pads) present in home cages unless otherwise stated. The study was performed after approval from the local ethical committee for animal experimentation. Mice were crossed to C57Bl/6 females, and genotyping of the offspring was performed from tail biopsies by triplex PCR using a common (for wild-type allele) and two different MCHR1 primers (R-34; Lactamin AB, Stockholm, Sweden) or to cafeteria diet, an ad libitum choice of full-fat cheese (38% fat), almond paste, milk (based on vegetarian butter, sugar, rolled oats, cocoa, and coconut shreds). From 4 wk of age, mice were weighed weekly to the nearest 0.1 g on an electronic scale.

Food intake was measured in 6-wk-old single-housed mice for 6 consecutive days. The mice were housed individually 3 wk before food consumption was measured. Mice were fed standard mouse chow (see above), which had a total energy content of 3.0 kcal/g or a high-fat diet. Cafeteria diet consisted of a number of human food-stuffs, which rapidly became contaminated and dispersed in the cages of animals fed this diet. Therefore, we turned to an alternative high-fat diet for measurement of food intake. This contained 45% of total energy as fat (D12451; Research Diets, New Brunswick, NJ; a total energy content of 4.7 kcal/g). Cages were carefully monitored for spillage of food, which was undetectable.

**Measurements of the body composition.** The total fat content of 12-wk-old mice fed standard chow diet was measured by dual-energy X-ray absorptiometry (DEXA) as previously described (39) using the Norland pDEXA Sabre (Fort Atkinson, WI).

**Indirect calorimetry.** Oxygen consumption (VO\textsubscript{2}), carbon dioxide production (VCO\textsubscript{2}), and activity were measured using an open-circuit calorimetry system (Oxymax, Columbus Instruments International, Columbus, OH). The animals were placed in calorimeter chambers with ad libitum access to normal lab chow and water for 72 h. Environmental enrichment was removed. An air sample was withdrawn for 75 s every 20 min, and the O\textsubscript{2} and CO\textsubscript{2} content were measured by a paramagnetic oxygen sensor and a spectrophotometric CO\textsubscript{2} sensor. These values were used to calculate VO\textsubscript{2} and VCO\textsubscript{2}. Data from the first 24 h were not used in analysis of results to allow acclimatization to the novel environment. Data from corresponding hours during the second and third 24-h periods were combined in 1-h bins. After the 3-day period in the Oxymax system, the body composition of each animal was measured by DEXA, and the lean body mass was calculated. Metabolic rate (kcal/h) was calculated using a rearrangement of the Weir equation (45) as supplied by Columbus Instruments: (3.815 + 1.232RER)× VO\textsubscript{2}, where RER is the respiratory exchange ratio [volume of CO\textsubscript{2} produced per volume of O\textsubscript{2} consumed (both ml·kg\textsuperscript{-1}·min\textsuperscript{-1})] and VO\textsubscript{2} is the volume of O\textsubscript{2} consumed per hour. Data for the last two 24-h periods of the experiment were combined and analyzed by two-way ANOVA. An eight-channel activity monitor, forming part of the Oxymax system, provided ambulatory counts for each channel.

**Remote measurement of temperature, heart rate, and blood pressure.** Core body temperature, heart rate, mean arterial pressure, and locomotor activity were continuously monitored using remote telemetry transmitters (DSI, St. Paul, MN) on individually housed 8-wk-old male mice at room temperature. Transmitters were implanted intraperitoneally under isoflurane (Forene) anesthesia. For measurement of blood pressure, a catheter was inserted in the carotid artery. Mice were allowed to recover for at least 14 days postsurgery. All measurements were carried out in the home-cage environment, and environmental enrichment was available in all studies except those involving fasting.

In one experiment, knockout (n = 7) or wild-type (n = 8) male mice were implanted with PA-C20 transmitters (DSI). Data on heart rate and mean arterial pressure were collected every 30 s and averaged in 10-min bins over a 72-h period. The average for each bin from the same point over 3 days was calculated, and these values were used to estimate 24-h diurnal rhythms. A further experiment was carried out using knockout (n = 8) or wild-type (n = 8) male mice implanted with ETA-F20 transmitters (DSI) to monitor baseline body temperature. As mean arterial pressure showed only small variations, ETA-F20 transmitters, which deliver ECG-data as well as blood pressure, were used for further experiments described below.

Another set of experiments was designed to compare autonomic control of heart rate in MCHR1−/− and wild-type mice. Animals were implanted with ETA-F20 telemetry transmitters and allowed to recover as above before undergoing four treatments, each separated by a recovery period of at least 3 days. On each treatment day animals were randomly assigned to one of four treatment groups and received intraperitoneal administration of either 0.9% saline vehicle (10 ml/kg), methylscopolamine (0.5 mg/kg), metoprolol (5 mg/kg), or a combination of methylisopropamide (0.5 mg/kg) and metoprolol (5 mg/kg), so that each animal received all four treatments in random
order. The doses of metoprolol and methylscopolamine were chosen after consultation with the literature (e.g., Refs. 17–19) and in-house dose-finding experiments in wild-type mice from the same supplier. Data were collected from each animal for a 2-h baseline period and then for a further 2 h after intraperitoneal injection of the autonomic blocker. Data were averaged over 15-min bins, and the data for each bin were compared by one-way ANOVA and post hoc Tukey’s test.

In the final experiment, data were collected for 72 h from wild-type 
\( n = 7 \) and MCHR1\(^{-/-} \) 
\( n = 8 \) mice implanted with ETA-F20 telemetry transmitters. Recording was started at 1600 on day 1, and baseline measurements were recorded for 24 h, during which time animals had ad libitum access to food and water. Food was removed at 1600 on the second day, and animals were fasted for 24 h. Access to water was ad libitum during this period. Their recovery from the fast was monitored for 24 h, starting at 1600 on day 3. Average heart rate for each animal was compared under fasted and nonfasted conditions. Episodes of reduced heart rate and body temperature were defined as 5-min bins in which the average value was \( > 3 \) SDs from the mean value seen during the dark period on the previous nonfasted day. The total number of such bins and the time to onset of the first episode were calculated for each animal, and the average values for wild-type and knockout animals were compared by \( t \)-test.

**RESULTS**

**Generation and characterization of MCHR1\(^{-/-} \) mice.** The targeting construct for generation of MCHR1\(^{-/-} \) mice is shown in Fig. 1A. R1 ES cell clones carrying the truncated allele were selected by Southern analysis (Fig. 1B) and used to generate MCHR1-null mice. Real-time PCR with MCHR1 specific primers on the brains from homozygous mice showed that a null mutation had been generated (Fig. 1C), with MCHR1 expression undetectable in the homozygous MCHR1\(^{-/-} \) animals. In the brains of the heterozygous mice, the expression of MCHR1 was reduced by approximately one-half, indicating that loss of one allele is not compensated by an increase in expression from the other (Fig. 1C). Homozygous MCHR1 mutant mice were represented in the litters from heterozygous crosses at the normal Mendelian frequency. MCHR1\(^{-/-} \) mice were viable into adulthood, fertile, and appeared normal by gross inspection.

As previously demonstrated for alternative lines (11, 23), male MCHR1\(^{-/-} \) mice maintained with ad libitum access to food and water were compared with their wild-type counterparts for baseline heart rate and body temperature. Figure 1D shows that both parameters were significantly increased in the MCHR1\(^{-/-} \) mice. In addition, the percentage of time spent in the active phase of the circadian cycle was also increased in the MCHR1\(^{-/-} \) mice, as shown in Figure 1E. These results support the hypothesis that MCHR1 is a key regulator of sympathetic activity.
chow diet gained less body weight compared with their wild-type littermates. By 14 wk of age, male MCHR1−/− mice were ~10% lighter than wild-type controls (P < 0.05; Fig. 2A). Male heterozygous mice of the same age also had a significant reduction (~7%) in body weight compared with wild-type animals (P < 0.01, data not shown). MCHR1-deficient females showed a similar pattern of reduction in weight gain compared with wild-type littermates, but this difference did not reach statistical significance (Fig. 2B).

Wild-type male mice fed a cafeteria diet showed dramatically increased body weight compared with mice fed normal chow. MCHR1 deficiency imparted dramatic resistance to this diet-induced obesity (Fig. 2, A and B). At the age of 14 wk, knockout mice were ~20% lighter than wild-type littermates (P = 0.001 for males, P < 0.05 for females). The weight of the MCHR1−/− mice fed a cafeteria diet did not differ significantly from that of wild-type males fed a normal chow diet. MCHR1-deficient females demonstrated a similar resistance to diet-induced obesity (data not shown).

To determine the total body fat content of MCHR1-deficient mice, we assessed adiposity by DEXA. At the age of 16 wk, in animals fed a standard chow diet, total body fat was decreased by ~45% in MCHR1−/− male mice compared with wild-type control male mice (P < 0.05; Fig. 2C).

Daily food intake of 6-wk-old mice was measured over a 6-day period. As demonstrated in previous studies, daily food intake of MCHR1−/− mice on normal lab chow was increased compared with the wild-type controls. Male homozygous mice ingested 8.3 ± 2.5% more calories than wild-type siblings over a 24-h period (P < 0.005).

Measurement of high-fat food intake was carried out using a commercially available pelleted high-fat diet. Although the magnitude of the diet-induced obesity in wild-type mice was not so great as that seen with the cafeteria diet, similar resistance to diet-induced obesity was seen in MCHR1−/− mice (at 14 wk MCHR1−/− 26.4 ± 1.0 g vs. wild type 31.5 ± 1.6 g, P < 0.05, n = 10). In contrast to intake of normal chow, the caloric intake of male MCHR1−/− fed a high-fat diet did not differ from that of controls (MCHR1−/− 12.3 ± 1.9 g vs. wild type 12.0 ± 0.6 kcal/mouse−1·day−1).

Energy expenditure, locomotor activity, heart rate, body temperature, and mean arterial pressure. Data detailing differences between wild-type and MCHR1−/− mice are summarized in Table 1. Energy expenditure was calculated both per kilogram body mass and per kilogram lean mass. In these experiments, MCHR1−/− mice showed a significantly increased caloric expenditure per kilogram body mass compared with wild-type animals as analyzed by two-way ANOVA (Fig. 2D) but not by t-test comparison of mean caloric expenditure (Table 1). When analyzed per kilogram lean body mass, similar results were seen, although this did not reach statistical significance (data not shown). Locomotor activity as measured in the Oxymax system was increased during the dark phase but not during the light phase in MCHR1−/− mice [locomotor activity: 24-h period, wild type 294 ± 33 vs. MCHR1−/− 419 ± 48 beam breaks (P < 0.05); dark phase, wild type 405 ± 36 vs. MCHR1−/− 579 ± 53 beam breaks (P < 0.05); light phase, wild type 173 ± 21 vs. MCHR1−/− 245 ± 35 beam breaks (P = 0.1)] (Fig. 3A).

We used implanted telemetry chips to measure the 24-h profile of locomotor activity, heart rate, temperature, and mean arterial pressure in 8-wk-old MCHR1−/− and wild-type male mice (Fig. 3, B–D). The MCHR1-deficient mice showed increases in all these parameters compared with controls by two-way ANOVA, but these increases were not uniform throughout the light/dark cycle. Of the parameters measured, only heart rate differed significantly between MCHR1−/− mice during both dark and light phases, as well as over the entire 24-h period (24-h period: wild type 495 ± 4 vs. MCHR1−/− 561 ± 8 beats/min (P < 0.001); dark phase: wild type 506 ± 52.
Table 1. Phenotypic analysis of energy balance parameters in MCHR1−/− mice and wild type littermates

<table>
<thead>
<tr>
<th></th>
<th>Wild Type</th>
<th>MCHR1−/−</th>
<th>P</th>
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<td>Locomotor activity, beam breaks/10 min</td>
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<tr>
<td>Light</td>
<td>173±21</td>
<td>245±35</td>
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<tr>
<td>Dark</td>
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<td>579±53</td>
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<td>8</td>
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<tr>
<td>24 h</td>
<td>294±33</td>
<td>419±4</td>
<td>&lt;0.05</td>
<td>8</td>
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<td>Core temperature, °C</td>
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<td></td>
<td></td>
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<tr>
<td>Light</td>
<td>36.4±0.1</td>
<td>36.5±0.1</td>
<td>NS</td>
<td>8</td>
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<tr>
<td>Dark</td>
<td>37.3±0.1</td>
<td>37.6±0.1</td>
<td>&lt;0.005</td>
<td>8</td>
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<tr>
<td>24 h</td>
<td>36.8±0.1</td>
<td>37.0±0.1</td>
<td>&lt;0.05</td>
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<tr>
<td>Heart rate, beats/min</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Light</td>
<td>484±13</td>
<td>539±9</td>
<td>&lt;0.005</td>
<td>8</td>
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<tr>
<td>Dark</td>
<td>506±8</td>
<td>582±9</td>
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<tr>
<td>24 h</td>
<td>495±4</td>
<td>561±8</td>
<td>&lt;0.001</td>
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<tr>
<td>Mean arterial pressure, mmHg</td>
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<tr>
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<td>107±2.4</td>
<td>108±2.3</td>
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<tr>
<td>Dark</td>
<td>113±2.7</td>
<td>117±3.4</td>
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<td>8</td>
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<tr>
<td>24 h</td>
<td>110±0.3</td>
<td>113±0.4</td>
<td>NS</td>
<td>8</td>
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<tr>
<td>Body weight (15 wk), g</td>
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<tr>
<td>Male chow</td>
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<td>27.9±0.4</td>
<td>&lt;0.0005</td>
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<td>Male cafeteria</td>
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<td>35.6±2.0</td>
<td>&lt;0.001</td>
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<td>Female chow</td>
<td>24.1±0.6</td>
<td>23.4±0.3</td>
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<td>Female cafeteria</td>
<td>36.4±3.1</td>
<td>26.1±1.5</td>
<td>&lt;0.05</td>
<td>6</td>
</tr>
<tr>
<td>Energy expenditure, kcal-min−1·kg total body wt−1</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Light</td>
<td>221.1±10.9</td>
<td>230.0±6.0</td>
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<tr>
<td>Dark</td>
<td>242.0±12.7</td>
<td>272.0±13.8</td>
<td>=0.16</td>
<td>4</td>
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<tr>
<td>24 h</td>
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<td>251.7±6</td>
<td>=0.19</td>
<td>4</td>
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<tr>
<td>Energy expenditure, kcal-min−1·kg lean body wt−1</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Light</td>
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<td>245.1±6.4</td>
<td>NS</td>
<td>4</td>
</tr>
<tr>
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<td>292.1±14.4</td>
<td>NS</td>
<td>4</td>
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<td>Body composition, g</td>
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<tr>
<td>Fat</td>
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<td>2.0±0.2</td>
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<td>Lean</td>
<td>32.9±1.2</td>
<td>29.8±1.2</td>
<td>NS</td>
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</table>

Values are means ± SE. MCHR1−/−, mice lacking melanin-concentrating hormone receptor 1.
8 vs. MCHR1−/− 582 ± 9 beats/min (P < 0.001); light phase: wild type 484 ± 13 vs. MCHR1−/− 539 ± 9 beats/min (P < 0.005)]. The telemetry chip system was a much less sensitive measure of locomotor activity than the Oxymax system, with a number of bins during the light phase recording no locomotor activity and no significant difference (NS) in the average number of movements between genotypes [24-h period: wild type 3.57 ± 0.18 vs. MCHR1−/− 5.06 ± 0.22 (P = NS); dark phase: wild type 5.33 ± 0.28 vs. MCHR1−/− 7.82 ± 0.34 (P = NS); light phase: wild type 1.83 ± 0.20 vs. MCHR1−/− 2.33 ± 0.25 (P = NS)]. Nevertheless, a very similar overall pattern was recorded, with genotype giving a significant effect by two-way ANOVA, and with locomotor activity increased during the dark phase to a greater extent than during the light phase in MCHR1−/− mice. Body temperature was increased during the dark phase but not the light phase in MCHR1−/− mice [24-h period: wild type 36.8 ± 0.1 vs. MCHR1−/− 37.0 ± 0.1°C (P < 0.05); dark phase: wild type 37.3 ± 0.1 vs. MCHR1−/− 37.6 ± 0.1°C (P < 0.005); light phase: wild type 36.4 ± 0.1 vs. MCHR1−/− 36.5 ± 0.1°C (P = NS)]. Average mean arterial pressure showed no significant difference between MCHR1−/− mice and wild-type mice [24-h period: wild type 110 ± 0.3 vs. MCHR1−/− 113 ± 0.4 mmHg (P > 0.05)].

Thus there appeared to be temporal differentiation between the effects on temperature, mean arterial pressure, and locomotor activity, where the difference was more marked during the dark phase, and the effect on heart rate, which was apparent throughout the 24-h period. As the effect on mean arterial pressure was relatively minor, telemetry chips measuring heart rate but not mean arterial pressure were used for experiments on the intrinsic heart rate and fasting responses of MCHR1−/− mice described below.

**Autonomic blockade and regulation of heart rate.** The intraperitoneal injection procedure caused a rapid, transient increase in heart rate, which returned to a steady level after ~1 h. Treatment with sympathetic and parasympathetic blockade altered heart rate in both wild-type and MCHR1−/− mice during the 3-h period after administration (Fig. 4A), but due to the transient injection effect, comparison of strains during the period after administration has been discounted, and statistical

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**Fig. 4.** Increased sympathetic activity underlies increased heart rate in MCHR1−/− mice. A: variations in heart rate in 8-wk-old male MCHR1−/− (○) and wild-type (■) mice after administration of vehicle, metoprolol, methylscopolamine, or metoprolol + methylscopolamine. Due to the large transient increase in heart rate seen after injection, statistical comparisons of effects of sympathetic and vagal blockade were carried out on data collected between 1 and 1.25 h after administration (B). MCHR1−/− mice (filled bars) showed a higher heart rate than wild-type animals (open bars). This appeared to be due to the dominance of sympathetic tone in these animals compared with dominant parasympathetic tone in wild-type animals. Data are the average ± SE from 7–8 animals of 3 time points in each 15-min bin. *P < 0.05 vs. wild-type saline, †P < 0.05 vs. MCHR1−/− saline as measured by 1-way ANOVA and post hoc Tukey’s test at the individual time point.
analysis was carried out on data gathered between 1 and 1.25 h postinjection (Fig. 4B). During this period, intraperitoneal vehicle-treated male MCHR1−/− mice had a significantly higher heart rate than wild-type animals receiving a similar treatment [wild type 355 ± 30 vs. MCHR1−/− 502 ± 24 beats/min (P < 0.001)]. Treatment with the sympathetic blocker metoprolol had no effect on the heart rate of wild-type animals but significantly reduced the tachycardia seen in MCHR1−/− mice [wild type 385 ± 30 beats/min (P > 0.05 vs. wild type saline); MCHR1−/− 417 ± 15 beats/min (P < 0.01 vs. knockout saline; P > 0.05 vs wild type metoprolol)]. Treatment with methylscopolamine significantly increased the heart rate of wild-type animals and also caused a significant increase in the knockout animals [wild type 522 ± 28 beats/min (P < 0.001 vs. wild type saline); MCHR1−/− 550 ± 22 beats/min (P < 0.01 vs. MCHR1−/− saline; P < 0.05 vs. wild type methylscopolamine)]. Treatment with a combination of methylscopolamine and metoprolol showed that there was no significant difference in intrinsic heart rate between wild-type and MCHR1−/− mice and that this was intermediate between the basal heart rates of the two mouse strains [wild type 477 ± 23 beats/min (P < 0.001 vs. wild type saline); MCHR1−/− 451 ± 21 beats/min (P < 0.01 vs. MCHR1−/− saline; P > 0.05 vs. wild type methylscopolamine + metoprolol)].

Effect of fasting on heart rate and body temperature. As above, MCHR1−/− mice showed a significantly higher heart rate compared with wild-type mice, both during the fed and fasted periods (fed period: wild type 518 ± 12 vs. MCHR1−/− 561 ± 10 beats/min; P < 0.05; fasted period: wild type 447 ± 14 vs. MCHR1−/− 536 ± 23 beats/min; P < 0.01). Transient falls in heart rate were seen in both wild-type and MCHR1−/− mice (Fig. 5A); however, the overall average heart rate during the 12-h after removal of food decreased significantly more in the wild-type mice than in the MCHR1−/− mice (wild type 70 ± 13 vs. MCHR1−/− 25 ± 14 beats/min, P < 0.05). This difference in average heart rate represented both an increase in latency to the first profound drop in heart rate, as assessed by a 3-SD difference from the mean value for the fed period (MCHR1−/− 7.7 ± 1.1 h after fasting vs. wild type 3.3 ± 1.4 h after fasting, P < 0.05) and in the overall time spent with depressed heart rate, as assessed by the number of 15-min bins where heart rate was >3 SDs below the average for the fed period (MCHR1−/− 21 ± 6.9 vs. wild type 44 ± 5.4 bins, P < 0.05).

Body temperature also showed transient and profound falls during the fasting period (Fig. 5B), and there was a temporal relationship between drops in heart rate and body temperature. Again, MCHR1−/− mice had a small but statistically significant elevation in body temperature during both fed and fasted phases (fed period: wild type 36.7 ± 0.1 vs. MCHR1−/− 36.9 ± 0.05°C, P < 0.05; fasted period: wild type 35.1 ± 0.2 vs. MCHR1−/− 36.1 ± 0.3°C, P < 0.05). Similar transient falls in average body temperature during the 12-h after removal of food were seen in both wild-type and MCHR1−/− mice (Fig. 5B). Although this did not reach significant difference, there was a trend toward a greater decrease in average body temperature in the wild-type animals (wild type 1.5 ± 0.24 vs. MCHR1−/− 0.78 ± 0.27°C, P = 0.055). This difference represented both an increase in latency to the first profound drop in temperature (wild type 2.7 ± 1.0 vs. MCHR1−/− 6.9 ± 1.5 h after fasting, P < 0.05) and in the overall time spent with depressed body temperature (wild type 63 ± 10 vs. MCHR1−/− 33 ± 7 bins, P < 0.05).

Fig. 5. MCHR1−/− mice show a reduced cardiovascular response to fasting. Heart rate (A and B) and core temperature recordings (C and D) from 8-wk-old male wild-type (A and C) and MCHR1−/− (B and D) mice during 24-h periods with ad libitum access to food (open symbols) or with food removed from cages (filled symbols). Data shown are from a representative animal because the timing of large, transient drops in heart rate and body temperature varied between individuals. Data are the average ± SE from a representative animal of 12 time points in each 1-h bin.
DISCUSSION

In this series of studies we show that a third line of MCHR1<sup>−/−</sup> mice has a similar phenotype to that of the MCHR1<sup>−/−</sup> mice lines previously described by Marsh et al. (23) and Chen et al. (11). All three lines have a lean phenotype and are resistant to diet-induced obesity. In each case, increased energy expenditure appears to be an important factor (11, 23) because MCHR1<sup>−/−</sup> mice show a slight increase in food consumption. We also demonstrate that the absence of a functional MCHR1 system leads to an increased basal heart rate independent of increased locomotor activity and that this appears to be caused by increased sympathetic and decreased parasympathetic activity compared with wild-type animals. We also demonstrate that under fasting conditions MCHR1<sup>−/−</sup> mice maintain heart rate and body temperature for longer than wild-type mice before the initiation of transient but profound drops in these two parameters, a response normally involving reduced sympathetic activity. It thus appears that the MCHR1 system is involved in both the tonic regulation of autonomic activity and the fast-induced autonomic suppression of heart rate and body temperature.

MCH has been considered an orexigenic peptide, with intracerebroventricular injection causing increased food intake (10, 32), and MCH antagonists causing acute (41) and chronic reductions (7) of food intake. Thus it was an unexpected result that mice lacking the MCHR1 receptor should be hyperphagic compared with wild-type animals. It is plausible that the increase in food intake is due to compensatory mechanisms acting to rectify the negative energy balance in these animals, and one might even speculate that the compensatory hyperphagia is somewhat impaired because MCHR1<sup>−/−</sup> mice show only a modest increase in food intake, inadequate to compensate fully and maintain a “normal” wild-type body weight. Thus it is possible that the slight compensatory hyperphagia might be masking an effect on food intake responsible in part for the lean phenotype in these animals. This explanation would be in keeping with the reports of reduced food intake after administration of MCHR1 antagonists (7, 41). It is interesting to note that in the semichronic study reported by Borowsky et al. (7), there appears to be a steady reduction in the effect of the MCH antagonist SNAP-7941 on food intake, while no such normalization of body weight loss is seen.

MCHR1<sup>−/−</sup> mice show an increase in dark-phase locomotor activity, accompanied by increased body temperature, in keeping with previous reports (11, 23). However, energy expenditure, as measured by open-circuit calorimetry, is increased during the light phase as well as the dark phase, whereas no increased locomotor activity is seen during the light phase. This suggests that, while increased locomotor activity might be responsible for part of the increased energy expenditure, an increase in basal metabolic rate, or involuntary energy expenditure is also likely to be present. Such an increase in involuntary energy expenditure is likely to be regulated by the autonomic nervous system, and in keeping with altered autonomic regulation we also observed an increase in heart rate in MCHR1<sup>−/−</sup> mice. Like the increase in energy expenditure, this mild tachycardia was present throughout the light/dark cycle, and thus did not appear to be wholly caused by increased locomotor activity.

**Autonomic control of heart rate.** In many cases, factors altering food intake also have a reciprocal effect on energy expenditure (for review, see Ref. 8). In many cases the sympathetic nervous system plays a major role in such changes in energy expenditure. The sympathetic nervous system is made up of multiple differentially regulated segments, and heart rate in particular can be regulated by a number of these, including several that do not appear important in regulation of energy balance (see Ref. 25 for review). However, under a number of circumstances, heart rate and thermogenesis are under coordinate autonomic control, and specific areas of the brain, such as the raphe pallidus, have been shown to control both (9). Thus it appears that under some circumstances heart rate and brown fat thermogenesis might be controlled by the same single unit of the sympathetic nervous system (8). Because both parameters appear altered in MCHR1<sup>−/−</sup> mice, it seems at least possible that the same mechanisms underlie the increased heart rate and a proportion of the increased energy expenditure in MCHR1<sup>−/−</sup> mice. We therefore investigated the autonomic mechanisms underlying the increased heart rate. To do this we measured the effects of sympathetic and parasympathetic blockade on heart rate in MCHR1<sup>−/−</sup> mice using metoprolol and methylscopolamine. This also allowed us to differentiate between autonomic regulation and intrinsic heart rate of MCHR1<sup>−/−</sup> and wild-type mice.

Directly after injection, we observed a transient stress-induced increase in heart rate. This transient increase in heart rate disappeared within 1 h of administration, and heart rate then returned to a steady level. We compared the effects of sympathetic and parasympathetic blockade during this later steady period. Intrinsic heart rate, as measured in the presence of both sympathetic and parasympathetic blockade, was the same for both MCHR1<sup>−/−</sup> and wild-type mice and lay intermediate to the baseline levels for the two strains. In both MCHR1<sup>−/−</sup> and wild-type mice, heart rate was controlled by a balance between sympathetic and parasympathetic inputs. In MCHR1<sup>−/−</sup> mice, it appeared that the sympathetic nervous system was the predominant input. Thus administration of the sympathetic blocker metoprolol reduced heart rate to the level seen in wild-type mice. However, when MCHR1<sup>−/−</sup> mice were treated with both metoprolol and methylscopolamine, they showed a heart rate significantly higher than when treated with metoprolol alone, demonstrating that parasympathetic tone was present in these animals that had been unmasked by metoprolol treatment.

Conversely, in wild-type mice, it appeared that parasympathetic inputs predominated. Thus treatment with methylscopolamine alone increased heart rate in wild-type mice to the same level as seen in MCHR1<sup>−/−</sup> mice. Again, it was clear that parasympathetic blockade unmasked a tonic sympathetic activity in wild-type mice because treatment with both metoprolol and methylscopolamine induced a heart rate significantly lower than that seen after treatment with methylscopolamine alone. In summary, in this paradigm, ablation of the MCHR1 gene leads to a state where sympathetic rather than parasympathetic control of heart rate predominates. This is in keeping with the changes expected if altered autonomic regulation were responsible for the changes in energy expenditure in these mice.

It is often stated that sympathetic activity is the predominant autonomic controller of heart rate (for review, see Ref.
18). However, contrary to this finding, we see a predominance of parasympathetic control of heart rate in wild-type mice in our study. A careful review of the literature shows that this is not a unique finding. Indeed there are studies showing a spectrum of autonomic control from predominantly sympathetic (12, 17) to a balance between sympathetic and parasympathetic (43) to predominantly parasympathetic control (2). Other than the differences in autonomic control, perhaps the most pertinent observed difference between these studies is in the resting heart rate. Not surprisingly, the higher the resting heart rate is, the larger is the sympathetic component in the control of heart rate. For example, whereas we observed a resting heart rate of approximately 350–400 beats/min in wild-type animals, Gehrmann et al. (12), using a similar telemetry system, reported resting heart rates over 700 beats/min. A number of variables might contribute to these variations in resting heart rate. These include the different methods of sampling heart rate data (telemetry vs. externalized catheters); the length of time between operation and measurements; administration, novel environment, and handling stress-induced effects; strain, age, and gender effects; and effects of the ambient temperature at which the experiments were carried out. Of these factors, ambient temperature seems a possible unreported variant between studies. Thus in our study although the room temperature was 20°C, well below the thermoneutral temperature for most mice strains, the animals did have access to nesting material in the cages, in keeping with local ethical guidelines and in an attempt to make the surroundings as normal as possible. It seems quite likely that the mice were thus able to maintain thermoneutrality and that this maintains the parasympathetic balance of the autonomic inputs. Nesting material was removed during fasting experiments, and this perhaps explains the smaller differences in heart rate seen between knockout and wild-type animals in these experiments.

**Responses to fasting.** Fasting induces a distinctive pattern of responses in rodents. This includes reductions in heart rate, arterial pressure, VO\(_2\), and body temperature, as well as an initial hyperactivity followed by periods of hypoxia (28, 46, 47). A large body of indirect evidence suggests that decreased sympathetic activity and increased parasympathetic activity underlie this process (4, 13, 14, 27). Given that we had seen altered autonomic activity in MCHR1\(^{-/-}\) mice, we hypothesized that the MCH system might play an important role in mediating the autonomic changes leading to altered cardiovascular parameters during fasting. To test this we measured heart rate and body temperature changes in MCHR1\(^{-/-}\) and wild-type mice during a 24-h fast. In this model we observed a reduced response to fasting in MCHR1\(^{-/-}\) mice.

The cardiovascular elements of the fasting response are not constant but instead occur as transient bouts of profound activities (46). In MCHR1\(^{-/-}\) mice we observed that both the latency to onset of these bouts and the overall time with depressed heart rate and body temperature were reduced in MCHR1\(^{-/-}\) mice. However, we did observe that the typical cardiovascular response to fasting was eventually seen. Thus the data suggest that the MCHR1 system is an important regulator of the physiologic responses to fasting (as its removal slowed the response) but does not represent the one and only mechanism (since the response still eventually occurred).

A number of hypothalamic peptide systems that are known to alter food intake also have reciprocal effects on sympathetic nervous activity (see Ref. 8 for review), a coordinate energy-conserving response to negative energy balance. From this model, it would appear that MCH can be added to that number. It appears that MCH may play an important role in the balance of autonomic nervous system activity, as in its absence there appears to be a switch from predominantly parasympathetic control to sympathetic control. It could be also be speculated that increases in MCH, which occur during fasting and food restriction, play a role in the suppression of sympathetic activity in these states. It remains to be investigated whether direct intracerebroventricular injection of MCH causes a suppression of sympathetic activity and heart rate in rodents, although intracerebroventricular MCH has been shown to have no effect on heart rate in sheep (29). It also remains unproven whether the increased heart rate seen in MCHR1\(^{-/-}\) mice is related to an increase in brown fat thermogenesis in these animals.

In summary, it appears that the MCH system may be an important tonic regulator of sympathetic nervous activity. This may form part of a coordinated response to negative energy balance and may be of importance when considering modulation of this system as a target for body weight control.

**REFERENCES**