Dbh(−/−) mice are hypertensive, have altered circadian rhythms, and have abnormal responses to dieting and stress

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We used mice deficient in dopamine β-hydroxylase [Dbh(−/−)] and their littermate controls [Dbh(+/−)] to examine the role of epinephrine (Epi) and norepinephrine (NE) in the maintenance of cardiovascular parameters during 7 days of caloric restriction and acute exposure to environmental stress. Cardiovascular parameters of the mice were monitored using blood pressure radiotelemeters at an ambient temperature of 29°C. Under normal conditions, Dbh(−/−) mice had a low heart rate, were severely hypertensive, and displayed an attenuated circadian blood pressure rhythm. Upon 50% caloric restriction, Dbh(+/−) mice exhibited decreases in heart rate and mean blood pressure. However, the blood pressures of Dbh(−/−) mice did not fall significantly in response to caloric restriction, and the bradycardia associated with caloric restriction was attenuated in these mice. In response to an open-field test, the blood pressure and heart rate of Dbh(+/−) mice increased substantially and rapidly, whereas Dbh(−/−) mice had blunted changes in blood pressures and no change in heart rate. These data suggest a primary role of Epi and NE in mediating the hypotension induced by dieting. Furthermore, Epi and NE play a smaller, but still significant, role in the bradycardia induced by caloric restriction. In contrast, Epi and NE are required for the tachycardia in an open field but are not required for the increase in blood pressure.

hypertension: blood pressure; food restriction; sympathetic nervous system; radiotelemetry; dopamine β-hydroxylase-deficient mice

MATERIALS AND METHODS

Animals. Homozygous control mice [Dbh(+/−)] and Dbh(−/−) mice (6 mo old, ~22 g) were bred at the University of Washington and shipped to Williams College for cardiovascular assessment. Animals were maintained on a 12:12-h light-dark cycle, dark from 5:00 P.M. to 5:00 A.M. All animal studies were approved by the Williams College Institutional Animal Care and Use Committee. Dbh(+/−) mice have normal catecholamine levels (29, 31) and were used as controls for all experiments.

Implantation of blood pressure telemeters. Mice (n = 7 for each group) were anesthetized initially with 5% isofluorane in an oxygen stream and maintained on 1–2% isofluorane. Mice were kept on a heating pad (38°C) throughout implantation of blood pressure transducers (PAC20; Data Sciences International) in the left common carotid artery (26). Mice were maintained on a heating pad for 48 h after the surgery and then housed at 29°C for 1 wk to allow time for recovery.

Baseline and caloric restriction cardiovascular data collection. Data from the blood pressure telemeters were recorded at 500 Hz. Between 5:00 P.M. and 4:00 P.M. on the next day, 2-min data streams
RESULTS

Baseline cardiovascular parameters. Dbh(−/−) mice exhibited striking differences in cardiovascular parameters compared with heterozygote littermates. A typical blood pressure tracing is shown in Fig. 1A, taken during the dark cycle for both animals. Systolic, mean, diastolic, and pulse pressures were significantly lower in Dbh(−/−) mice compared with Dbh(+/−) littermates (Table 1) within both the dark cycle (active period) and light cycle (inactive period). The Dbh(+/−) mice exhibited normal circadian rhythms, with elevated heart rate, blood pressures, and activity during the dark cycle (Fig. 1B and Table 1). In contrast, although the Dbh(−/−) mice showed circadian rhythms in heart rate and activity, the differences in blood pressure between the light and dark cycle were minimal but statistically significant. The SDIBI was calculated from the blood pressure tracings. The SDIBI of Dbh(−/−) mice was significantly higher than in Dbh(+/−) mice during both the dark and light cycles (Table 1).

Caloric restriction. Previous reports by us (26) and others (38, 39) have demonstrated decreases in mouse heart rate and blood pressure in the dark cycle in response to 7 days of 50–60% caloric restriction. We used the Dbh(−/−) mice and littermate controls to determine whether Epi and NE were required for altered cardiovascular parameters during caloric restriction. Dbh(+/−) mice ate 11.8 ± 0.5 kcal/day, whereas Dbh(−/−) mice ate a nonsignificantly different amount of 12.3 ± 0.3 kcal/day during the ad libitum feeding. Mice were calorically restricted at 50% of normal intake (6 kcal/day) for 7 days at 29°C. Dbh(+/−) mice lost 2.3 ± 0.6 g body wt and Dbh(−/−) mice lost 2.4 ± 0.6 g body wt over the 7 days. For analysis of cardiovascular parameters, data from the last two 24-h periods during caloric restriction were averaged and compared with baseline data listed in Table 1. Dbh(+/−) mice exhibited significant drops in all blood pressure values (mean blood pressure shown in Fig. 2) and heart rate (Fig. 2) in the dark cycle in the absence of any significant changes in locomotor activity. Mean blood pressure fell an average of 13.7 ± 3.6 mmHg and heart rate fell an average of 126 ± 23 beats/min.
in the dark cycle. In agreement with earlier studies of wild-type mice (38), blood pressures of \(Dbh^{+/+}\) mice were not changed during the light cycle with caloric restriction. \(Dbh^{-/-}\) mice, however, exhibited no statistical change in blood pressures in response to caloric restriction (\(P > 0.05\) vs. precalaric restriction pressures), although there was a trend for lowered mean blood pressure during both the light and dark cycles. In contrast to the observation that caloric restriction lowered mean blood pressure during both the light and dark cycles. The difference between the last 2 days of the caloric restriction period and the last 2 days before the caloric restriction period was calculated for each animal and averaged. \(\ast P < 0.05\) testing ad libitum vs. caloric restriction within the same genotype and within the same light-dark cycle. AU, arbitrary units. \(\ast P < 0.05\) testing \(Dbh^{+/+}\) vs. \(Dbh^{-/-}\) within the same light-dark cycle. \(\ast P < 0.05\) testing light cycle vs. dark cycle within the same genotype. \(\dagger P = 0.065\) light cycle vs. dark cycle for systolic pressure of \(Dbh^{-/-}\).

### DISCUSSION

Using mice deficient in DBH, an enzyme that is required for synthesis of Epi and NE, we determined the impact of the lack of these catecholamines on the cardiovascular response to a bout of caloric restriction. A secondary goal was to characterize the cardiovascular response of mice to an open-field stressor. One limitation in these studies is that, in addition to release from the adrenal medulla and sympathetic nerve endings, Epi and NE are neurotransmitters in the central nervous system (reviewed in Ref. 7). Thus the present experiments cannot

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### Table 1. Cardiovascular parameters of \(Dbh^{+/+}\) and \(Dbh^{-/-}\) mice during the dark and light cycles

<table>
<thead>
<tr>
<th></th>
<th>Heart Rate, beats/min</th>
<th>Systolic Blood Pressure, mmHg</th>
<th>Mean Blood Pressure, mmHg</th>
<th>Diastolic Blood Pressure, mmHg</th>
<th>Pulse Pressure, mmHg</th>
<th>Activity, AU</th>
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</thead>
<tbody>
<tr>
<td><strong>Dark cycle</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>(Dbh^{+/+})</td>
<td>512 ± 16 *†</td>
<td>112.3 ± 2.6 *†</td>
<td>99.4 ± 2.1 *†</td>
<td>85.4 ± 1.8 *†</td>
<td>27.0 ± 1.6 *</td>
<td>8.7 ± 1.2†</td>
</tr>
<tr>
<td>(Dbh^{-/-})</td>
<td>449 ± 27 *†</td>
<td>89.6 ± 2.0</td>
<td>78.5 ± 2.1 *†</td>
<td>67.8 ± 2.0</td>
<td>21.8 ± 1.0</td>
<td>6.3 ± 0.7†</td>
</tr>
<tr>
<td><strong>Light cycle</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>(Dbh^{+/+})</td>
<td>408 ± 16 *</td>
<td>98.1 ± 1.9 *</td>
<td>85.6 ± 1.6 *</td>
<td>72.9 ± 1.8 *</td>
<td>25.2 ± 1.0 *</td>
<td>2.5 ± 0.3*</td>
</tr>
<tr>
<td>(Dbh^{-/-})</td>
<td>366 ± 23</td>
<td>85.6 ± 1.3</td>
<td>73.7 ± 1.5</td>
<td>62.8 ± 1.4</td>
<td>22.7 ± 0.5</td>
<td>1.9 ± 0.3</td>
</tr>
</tbody>
</table>

Data are means ± SE. \(Dbh^{-/-}\), dopamine \(\beta\)-hydroxylase-deficient mice; \(Dbh^{+/+}\), littermate control mice; AU, arbitrary units. \(\ast P < 0.05\) testing \(Dbh^{+/+}\) vs. \(Dbh^{-/-}\) within the same light-dark cycle. \(\dagger P < 0.05\) testing light cycle vs. dark cycle within the same genotype. \(\dagger P = 0.065\) light cycle vs. dark cycle for systolic pressure of \(Dbh^{-/-}\).
differentiate whether it is the lack of central nervous system signaling or lack of peripheral SNS signaling that is responsible for the altered cardiovascular parameters exhibited by the Dbh\(^{-/-}\) mice. It is likely because of the latter, however, given the strong link between chemical sympathectomy and chronic low blood pressure (reviewed in Ref. 43).

Cho et al. (2) have previously reported that Dbh\(^{-/-}\) mice have normal left ventricular systolic and diastolic pressures. However, these measurements were obtained from anesthetized mice. In contrast, we found here using radiotelemetry in conscious and freely moving animals that the blood pressures of Dbh\(^{-/-}\) mice were astonishingly low (Table 1 and Fig. 1). The hypotension in Dbh\(^{-/-}\) mice closely mirrors DBH deficiency in humans, who exhibit hypotension and severe orthostatic intolerance (25). These mice also exhibit both a low heart rate and low pulse pressure. The severe hypotension in Dbh\(^{-/-}\) mice is consistent with earlier findings suggesting that blood pressure in mice is heavily influenced by the SNS (13, 14, 17). An interesting aspect of the baseline cardiovascular parameters of the Dbh\(^{-/-}\) mice is the attenuated circadian blood pressure variability in the DBH-deficient mice. Humans (23), rats (15, 22, 37), and mice (20, 26, 36, 39) all exhibit higher blood pressure values and heart rate during the active cycle (light for humans, dark for rodents) relative to the resting cycle. The circadian oscillating system from the suprachiasmatic nucleus appears to be responsible for the circadian cardiovascular parameters, and some studies in the rat have implicated the autonomic nervous system as the mediator of this rhythm (15, 22, 37). In the current study, Dbh\(^{+/+}\) mice exhibited blood pressures 13–14 mmHg higher during the dark cycle than the light cycle (Table 1), much like that previously described for wild-type mice. However, the blood pressure difference between the dark and light cycle of Dbh\(^{-/-}\) mice was blunted to only 4–5 mmHg (Table 1), even though these animals display a normal circadian rhythm in activity levels. Furthermore, the Dbh\(^{-/-}\) mice have a normal circadian rhythm in heart rate (Table 1). These data suggest that sympathoadrenergic catecholamines, and not spontaneous cage activity, play a principal role in the circadian difference in blood pressure. This is consistent with the finding of the circadian rhythm in circulating catecholamines (elevated during the dark cycle) in rodents (5). In contrast, the heart rate difference between the dark and light cycle must be the result of some other cause than Epi and NE and is likely activity related, as has been previously suggested in a rat study using chemical sympathectomy (22).

Fig. 3. Cardiovascular parameters of Dbh\(^{+/+}\) and Dbh\(^{-/-}\) mice before, during, and after exposure to an open field. Mean blood pressure, heart rate, and activity were continuously monitored in these representative mice. “Rest” is the 5-min period in the mouse’s home cage before exposure to the open field. “Open field” is the 5-min period during which the mouse is placed in a 3 ft. × 3 ft. × 3 ft. white box flooded with light. “Recovery” is the 5-min period in the mouse’s home cage immediately after exposure to the open field.

Fig. 4. Cardiovascular parameters of all Dbh\(^{+/+}\) and Dbh\(^{-/-}\) mice tested before, during, and after exposure to an open field. Mice (n = 7 for each genotype) were exposed to an open field as described in Fig. 3. The last 2 min of each condition (rest, open field, recovery) were averaged for each animal. P < 0.05 vs. rest (a) and vs. the open field (b).
The drop in blood pressure that is associated with caloric restriction has long been known to be associated with a drop in SNS activity in rodents and humans (4, 8, 10, 16, 18, 41). With the use of the Dbh(−/−) mice, we were able to test directly whether Epi and NE are required for such a cardiovascular change. We found that the mean blood pressure of Dbh(−/−) mice did not drop significantly upon caloric restriction (Fig. 2). This was also true for systolic, diastolic, and pulse pressures (data not shown). It is possible that the blood pressures of the Dbh(−/−) mice were already so low before the caloric restriction that they could not drop further and still maintain adequate perfusion pressure, somewhat of a “basement” effect. It is of interest that the drop in blood pressure in Dbh(+/−) mice associated with caloric restriction (Fig. 2, 13–14 mmHg) is roughly the same as that associated with the difference between the dark and light cycle (Table 1, also 13–14 mmHg).

In addition, the lack of a prominent cardiovascular circadian rhythm and lack of caloric restriction-induced hypotension in Dbh(−/−) mice provides circumstantial evidence that the circadian rhythm and diet-induced low pressure utilize the same mechanism, and that mechanism requires Epi and NE for realization. The bradycardia that is associated with caloric restriction in mice (Fig. 2 and Refs. 26, 38, and 39) was blunted in the Dbh(−/−) mice, suggesting that Epi and/or NE only partially mediate the heart rate effect of caloric restriction. Presumably the remainder of the heart rate response is mediated by enhanced parasympathetic nervous system (PNS) activity, but this remains to be tested.

To further analyze the role of Epi and NE in caloric restriction-induced changes, we also calculated the SDIBI. The SDIBI is a measure that describes heart rate variability on a beat-to-beat basis. Others have suggested that SDIBI measurements are a reasonable estimator of the relative contribution of the SNS and PNS in the cardiovascular system, with an increase in SDIBI suggesting an increase in PNS input relative to SNS input (24, 28, 40). Our data from ad libitum-fed Dbh(−/−) mice support this notion. The SDIBI for Dbh(−/−) mice in the dark cycle was more than two times that of Dbh(+/−) mice (Table 1) and 50% greater in the light cycle. The lack of SNS input in cardiovascular control is consistent with the higher SDIBI in Dbh(−/−) mice. In the dark cycle, it is very likely that the increase in SDIBI activity of littermate controls during caloric restriction reflects withdrawal of SNS activity, since this increase in SDIBI was not found during caloric restriction in Dbh(−/−) mice (Table 1). SDIBI did increase in Dbh(−/−) mice in the light cycle during caloric restriction. This provides evidence that PNS activity to the heart likely increased in these mice during the light cycle. Hence, these SDIBI data suggest that both the PNS and SNS are altered in the normal mouse in response to a bout of caloric restriction.

Although stress tests have been used extensively in rodents to assess animal behavior and test anxiolytic drugs, only a few have actually measured cardiovascular parameters in an open-field test (32–34), and none have used mice as subjects. Therefore, we used the mice to 1) characterize the cardiovascular response of the mouse to an open field and 2) to examine the impact on the cardiovascular response in mice missing Epi and NE. Mean blood pressure and heart rate in Dbh(+/−) mice were elevated 29 mmHg (representing a 29% increase over baseline) and 235 beats/min (representing a 43% increase), respectively, within only a few minutes of exposure to the open field. The magnitude of these changes is consistent with the cardiovascular response of the rat (32–34). However, exposure of the Dbh(−/−) mouse to the open field had no effect on heart rate and elevated blood pressure 12 mmHg (representing a 20% increase), also consistent with pharmacological studies within the rat (1, 33). The fact that blood pressure is elevated in Dbh(−/−) mice in the open field is perplexing given the absence of sympathoadrenergic catecholamines and the absence of any tachycardic response. One explanation may be the fourfold elevation in activity observed within the open field (Fig. 4). It is possible that muscle pump action that occurs with activity increased venous pressure, leading to an increase in stroke volume, cardiac output, and blood pressure. However, Janssen and Smits (14) previously reported that stroke volume and cardiac output in mice fall with large movements. Another explanation for the elevated blood pressure is an increase in resistance in the arterial tree. In the absence of Epi and NE, a likely candidate to mediate an increase in resistance is neuropeptide Y (NPY). NPY is released from sympathetic nerve endings upon activation of those nerves and produces potent vasoconstrictor activity (reviewed in Ref. 44). The physiological role of peripheral NPY in these mice, however, remains to be tested. It may be argued that the Dbh(−/−) mice cannot detect the stressful open-field situation, since these mice have ptosis of the eyelids (29). This is unlikely, though, given the large increase in activity observed in these animals when exposed to the open field (Figs. 3 and 4).

In conclusion, we show here the requirement of Epi and/or NE in the adaptive response of the cardiovascular system to caloric restriction. These sympathoadrenergic catecholamines are required for the full 13- to 14-mmHg change in blood pressure and a portion of the bradycardia that occurs in mice during a 7-day bout of caloric restriction at 29°C. Furthermore, data from the Dbh(−/−) mice also implicate a role of the PNS for the cardiovascular response to dieting. These data include 1) the attenuated, but not abolished, bradycardia in the Dbh(−/−) mice; and 2) the rise in SDIBI during caloric restriction in the light cycle in Dbh(−/−) mice. Collectively, caloric restriction of Dbh(−/−) mice suggests a primary role of Epi and NE and a secondary role of the PNS in the cardiovascular response to dieting.

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