Bombesin-like receptor 3 regulates blood pressure and heart rate via a central sympathetic mechanism

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Lateef DM, Xiao C, Brychta RJ, Diedrich A, Schnermann J, Reitman ML. Bombesin-like receptor 3 regulates blood pressure and heart rate via a central sympathetic mechanism. Am J Physiol Heart Circ Physiol 310: H891–H898, 2016. First published January 22, 2016; doi:10.1152/ajpheart.00963.2015.—Bombesin-like receptor 3 (BRS-3) is an orphan G protein-coupled receptor that regulates energy expenditure, food intake, and body weight. We examined the effects of BRS-3 deletion and activation on blood pressure and heart rate. In free-living, telemetered Brs3 null mice the resting heart rate was 10% lower than wild-type controls, while the resting mean arterial pressure was unchanged. During physical activity, the heart rate and blood pressure increased more in Brs3 null mice, reaching a similar heart rate and higher mean arterial pressure than control mice. When sympathetic input was blocked with propranolol, the heart rate of Brs3 null mice was unchanged, while the heart rate in control mice was reduced to the level of the null mice. The intrinsic heart rate, measured after both sympathetic and parasympathetic blockade, was similar in Brs3 null and control mice. Intravenous infusion of the BRS-3 agonist MK-5046 increased mean arterial pressure and heart rate in wild-type but not in Brs3 null mice, and this increase was blocked by pretreatment with clonidine, a sympatholytic, centrally acting α2-adrenergic agonist. In anesthetized mice, hypothalamic infusion of MK-5046 also increased both mean arterial pressure and heart rate. Taken together, these data demonstrate that BRS-3 contributes to resting cardiac sympathetic tone, but is not required for activity-induced increases in heart rate and blood pressure. The data suggest that BRS-3 activation increases heart rate and blood pressure via a central sympathetic mechanism.

Bombesin-like receptor 3; blood pressure; heart rate; sympathetic nervous system; energy metabolism

NEW & NOTEWORTHY

MK-5046, a bombesin-like receptor 3 (BRS-3) agonist, increases heart rate and blood pressure via increased central sympathetic tone. Brs3 null mice have a reduced resting heart rate that increases disproportionately with physical activity. BRS-3 contributes to the central regulation of heart rate and blood pressure.

OBESITY is strongly associated with cardiovascular risk factors, including hypertension (6). However, the mechanisms linking obesity and hypertension are not fully understood (7, 44). A number of neuroendocrine systems controlling energy homeostasis also regulate blood pressure and heart rate, including leptin, the melanocortin system, and ghrelin (12, 36, 45, 48–50). The genetic and pharmacological manipulations of these systems that reduce adiposity generally also increase blood pressure, which is undesirable in a treatment for obesity.

Bombesin-like receptor 3 (BRS-3) is a G protein-coupled receptor for which the endogenous ligand is unidentified (4, 21, 29, 33). Despite the name, BRS-3 has a low affinity for bombesin and the related molecules, neuromedin B and gastrin-releasing peptide (34). BRS-3, like the leptin and melanocortin system, regulates energy metabolism and food intake. BRS-3 is located in several brain regions that regulate energy metabolism, including several hypothalamic nuclei and the caudal brain stem (14, 20, 31, 40, 53, 54). Brs3 knockout (Brs3−/−) mice exhibit hyperphagia, reduced metabolic rate, and obesity (27, 41). Treatment with a BRS-3 antagonist increased food intake and body weight in rats (14). Concordantly, BRS-3 agonists reduced food intake, increased metabolic rate, and reduced body weight in mice, rats, and dogs (14, 15).

Existing evidence for a role of BRS-3 in regulation of blood pressure is contradictory. One BRS-3 agonist, MK-5046, transiently increased blood pressure in rats, dogs, and humans (but has not been studied in mice) (15, 46). A different BRS-3 agonist also increased blood pressure, while a third agonist reduced it, making it unclear if the blood pressure effects are mediated via BRS-3 (15). Confusingly, Brs3−/− mice were reported to have increased tail-cuff blood pressure at 40 wk, but not at 16 wk of age (41). Thus the reported phenotype of the Brs3−/− mice is the opposite of that predicted from most pharmacological experiments.

Given this ambiguity, we dissected the interactions between BRS-3 and blood pressure and heart rate using both genetic (Brs3−/− mice) and pharmacological tools. From studying resting and active mice, we identified an interaction between BRS-3 and physical activity. We also explored the intrinsic heart rate in Brs3−/− mice and the contribution of sympathetic input to the increase in blood pressure and heart rate by the BRS-3 agonist, MK-5046.

MATERIALS AND METHODS

Compounds and Mice

MK-5046, (2S)-1,1,1-trifluoro-2-[[4-[(1H-pyrazol-1-yl)phenyl]-3-[4-[(1 trifluoromethyl)cyclopentyl][methyl]-1H-imidazol-2-yl]prop-2-ol, is a potent (mouse BRS-3 Ki = 1.6 nM), brain-penetrant and highly selective BRS-3 agonist (47) that was synthesized and generously provided by Merck Research Laboratories, Rahway, NJ. The parenteral MK-5046 doses (1 and 3 mg/kg ip) are selective, without activity in Brs3−/− mice (15, 28). Clonidine hydrochloride [40 μg/kg ip (3)], propranolol [5 mg/kg ip (23)], and atropine [1 mg/kg ip (42)]
were purchased from Sigma-Aldrich (St. Louis, MO), with dosing based on the indicated references.

C57BL/6J mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and bred in house, and Brs3−/− mice were provided by Dr. James Battey (27) and back-crossed at least eight generations onto a C57BL/6J background. Male mice were studied at 12–24 wk of age using littermate controls. Mice were housed in a temperature- and humidity-controlled environment with a 12:12-h light-dark cycle and with food and water available ad libitum. All animal studies were approved by the National Institute of Diabetes and Digestive and Kidney Diseases/National Institutes of Health Animal Care and Use Committee.

Hemodynamic Measurements in Conscious Mice

Blood pressure and heart rate were measured in conscious, unrestrained mice via radiotelemetry (DSI PhysioTel PA-C10 transmitter and Ponemah v4.80 software, Data Sciences International) with sampling at 500 Hz (24). Mice were allowed 7 days to recover from surgery and acclimatized to handling with vehicle intraperitoneal injections each day for the 5 days prior to dosing with drug.

Heart rate variability in the time domain was calculated as the pNN6, the percent of consecutive RR intervals differing by 6 ms (51), without excluding the RR intervals outside the 95.5% confidence intervals.

The baroreceptor reflex was investigated using spontaneous changes in blood pressure and heart rate in 1 h of light-phase data (0900–1000) by the sequence method (30) as described (17). Briefly, baroreceptor sensitivity (ms/mmHg) was calculated as the slope of the linear regression between SBP and the corresponding RR intervals using sequences defined as an episode of ≥3 heartbeats with changes of ≥0.01 mmHg SBP and ≥0.01 ms per beat in the same direction. The median of the slopes with an R squared value ≥0.85 was calculated for blood pressure upslopes (BRSup) and downslopes.

Fig. 1. Conscious Brs3−/− mice have a reduced resting heart rate and an activity-dependent increase in mean arterial pressure (MAP). A and B: light-phase MAP and heart rate from 13 to 24 wk of age in the same cohort of mice. Data at each age are from a weekday 2 h undisturbed interval early in the light phase. C and D: histograms of MAP and heart rate during 1-min intervals. For statistical analysis, the cumulative frequency percentiles for each mouse were compared (n = 5–6/group). MAPs in percentiles 57–80 were higher and all heart rate percentiles were lower (P < 0.05 by t-test without multiplicity correction). E and F: light-phase MAP and heart rate histograms for physically inactive and active intervals. In wild-type (WT) and Brs3−/− (KO) mice, 26% and 34% of intervals were active, respectively. G and H: dark-phase MAP and heart rate histograms for inactive and active intervals. Active intervals were 66% for both genotypes. In C–H, data are the same 48 h in 13-wk old mice as in Table 1. Histogram bins are 2.5 mmHg for MAP and 10 beats/min for heart rate.
Table 1. Blood pressure, heart rate, and activity in wild-type and Brs3$^{-/-}$ mice

<table>
<thead>
<tr>
<th>Phase</th>
<th>Wild Type</th>
<th>Brs3$^{-/-}$</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>24.3 ± 0.8</td>
<td>29.3 ± 1.0</td>
<td>0.004</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>Light</td>
<td>96.3 ± 0.3</td>
<td>99.3 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Dark</td>
<td>110.3 ± 1.5</td>
<td>112.8 ± 1.4</td>
</tr>
<tr>
<td>Systolic pressure, mmHg</td>
<td>Light</td>
<td>109.5 ± 0.6</td>
<td>112.5 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>Dark</td>
<td>124.2 ± 2.1</td>
<td>127.2 ± 1.7</td>
</tr>
<tr>
<td>Diastolic pressure, mmHg</td>
<td>Light</td>
<td>81.9 ± 0.5</td>
<td>85.3 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>Dark</td>
<td>96.0 ± 1.7</td>
<td>98.0 ± 1.7</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>Light</td>
<td>542 ± 11</td>
<td>513 ± 9</td>
</tr>
<tr>
<td></td>
<td>Dark</td>
<td>609 ± 7</td>
<td>569 ± 5</td>
</tr>
<tr>
<td>Activity, AU</td>
<td>Light</td>
<td>2.8 ± 0.4</td>
<td>4.5 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>Dark</td>
<td>15.8 ± 0.8</td>
<td>13.9 ± 1.6</td>
</tr>
</tbody>
</table>

Data are means ± SE of the individual means; n = 5–6 mice/group. Hemodynamic parameters were measured by radiotelemetry in 13-wk-old conscious mice as 1-min averages for 48 continuous hours during the weekend, a quiet period in the vivarium. MAP, mean arterial pressure; AU, arbitrary units.

(BRSdown) and an R-R response delay of 0, 1, or 2 heartbeats. Data containing movements and other artifacts in the SBP or RR interval series were excluded. All baroreceptor sensitivity analysis was performed using customized software (PHYSIOWAVE BRSSpectra) programmed (by A.D. and R.J.B) using MatLab.

Hemodynamic Measurements during Anesthesia

Mice were anesthetized with pentobarbital sodium (80 mg/kg ip) and catheterized via the jugular vein using PE-10 tubing. A high-fidelity 1.4-F Millar microtip catheter (ADInstruments, Colorado Springs, CO) was inserted via the left carotid artery as described (18). Anesthetized mice were kept on a small-animal operating table set at Springs, CO) was inserted via the left carotid artery as described (18). Hemodynamic parameters were measured by radiotelemetry in 13-wk-old mice and catheterized via the jugular vein using PE-10 tubing. A high-fidelity 1.4-F Millar microtip catheter (ADInstruments, Colorado Springs, CO) was inserted via the left carotid artery as described (18).

Intrahypothalamic Infusions

Mice were anesthetized with ketamine/xylazine (80/10 mg/kg ip). Sterile guide cannulas (5.25 mm, 26 gauge. Plastics One, Roanoke, VA) were stereotaxically implanted bilaterally into the anterior hypothalamus [1.34 mm posterior, ±0.75 mm lateral to bregma, 4.75 mm below the surface of the skull (9)] and fixed with dental cement (Parkell, Edgewood, NY). MK-5046 or vehicle (saline) was infused (1 µl per side over 60 s) through a 33-gauge cannula protruding 0.5 mm past the tip of the guide cannula using PE-10 tubing fitted to a 5-µl syringe (Hamilton, Reno, NV) and dual-syringe pump (KD Scientific, Holliston, MA). Cannula positions in the anterior hypothalamus were verified by postmortem histology. The mouse hypothalamus is ~10 mm$^3$ (1); the large infusion volume was used so that drug would reach Brs3 in adjacent brain nuclei (14, 20, 31, 43, 44).

Statistics

Results are shown as means ± SE. Two-way ANOVA with or without repeated measures followed by Holm-Sidak’s post test was used for comparing genotypes vs. the treatment groups. Student’s t-test was used when two groups were compared. Statistical analyses used two-tailed tests using P < 0.05 as statistically significant. Least-squares model analysis was performed using JMP 10.0.2 (SAS Institute, Cary, NC).

RESULTS

Studies in Brs-3 Deficient Mice

Blood pressure and heart rate in conscious Brs3$^{-/-}$ mice. In telemetered mice studied from 13 to 24 wk of age, body weight increased more in Brs3$^{-/-}$ (from 24.3 ± 1.0 to 23.9 ± 0.9 g) than control mice (from 24.3 ± 0.8 to 23.6 ± 0.8 g; P = 0.002 for genotype effect on body weight gain). Despite the weight changes, mean arterial pressure (MAP) and heart rate were stable within each genotype from 13 to 24 wk of age (Fig. 1, A and B).

Free-living telemetered Brs3$^{-/-}$ mice had an elevated (~3 mmHg) systolic, diastolic, and MAP in the light phase, with no difference from controls during the dark phase (Table 1). Heart

Table 2. Models of MAP and heart rate

<table>
<thead>
<tr>
<th>Variable</th>
<th>MAP Estimate (SE)</th>
<th>t Ratio</th>
<th>Prob &gt; t</th>
<th>Heart Rate Estimate (SE)</th>
<th>t Ratio</th>
<th>Prob &gt; t</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light phase</td>
<td>97.10 (0.09)</td>
<td>1127</td>
<td>&lt;0.0001</td>
<td>523.3 (0.5)</td>
<td>982</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Activity</td>
<td>11.92 (0.15)</td>
<td>80</td>
<td>&lt;0.0001</td>
<td>60.8 (0.9)</td>
<td>66</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Genotype</td>
<td>0.67 (0.09)</td>
<td>8</td>
<td>&lt;0.0001</td>
<td>-19.1 (0.5)</td>
<td>-36</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Genotype × activity</td>
<td>0.60 (0.15)</td>
<td>4</td>
<td>&lt;0.0001</td>
<td>14.1 (0.9)</td>
<td>15</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Dark phase</td>
<td>106.64 (0.10)</td>
<td>1055</td>
<td>&lt;0.0001</td>
<td>561.1 (0.6)</td>
<td>890</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Activity</td>
<td>7.84 (0.10)</td>
<td>78</td>
<td>&lt;0.0001</td>
<td>44.0 (0.6)</td>
<td>71</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Genotype</td>
<td>1.35 (0.08)</td>
<td>17</td>
<td>&lt;0.0001</td>
<td>-19.3 (0.5)</td>
<td>-39</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Genotype × activity</td>
<td>1.88 (0.10)</td>
<td>19</td>
<td>&lt;0.0001</td>
<td>7.3 (0.6)</td>
<td>12</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Standard least-squares models for MAP and heart rate were constructed separately for the light and dark phases, with the activity [as log(activity + 0.5)] and genotype (wild type vs. Brs3$^{-/-}$) and their interaction as fixed effects, using the same data as in Table 1. The adjusted $r^2$ are 0.32 (light, MAP), 0.31 (dark, MAP), 0.28 (light, heart rate), and 0.30 (dark, heart rate). SE, standard error.

Table 3. Cardiovascular baroreceptor reflex sensitivity

<table>
<thead>
<tr>
<th>Delay, heartbeats</th>
<th>Wild Type, mmHg</th>
<th>Brs3$^{-/-}$, mmHg</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRSup 0</td>
<td>3.1 ± 0.8</td>
<td>2.8 ± 0.6</td>
<td>0.50</td>
</tr>
<tr>
<td>1</td>
<td>2.5 ± 0.6</td>
<td>2.3 ± 0.5</td>
<td>0.44</td>
</tr>
<tr>
<td>2</td>
<td>2.4 ± 0.8</td>
<td>2.1 ± 0.3</td>
<td>0.31</td>
</tr>
<tr>
<td>BRSdown 0</td>
<td>2.6 ± 0.9</td>
<td>2.6 ± 0.6</td>
<td>0.92</td>
</tr>
<tr>
<td>1</td>
<td>2.9 ± 0.7</td>
<td>2.7 ± 0.8</td>
<td>0.79</td>
</tr>
<tr>
<td>2</td>
<td>2.9 ± 0.8</td>
<td>2.6 ± 0.6</td>
<td>0.52</td>
</tr>
</tbody>
</table>

Baroreceptor sensitivity (mmHg) was measured in 1 h of the light phase by sequence analysis of episodes with increasing (BRSup) and decreasing (BRSdown) blood pressure as detailed in MATERIALS AND METHODS. Delay is of 0, 1, or 2 heartbeats using intervals with increasing or decreasing blood pressure and RR interval, as indicated. n = 5–6/group.
rate was lower in $Brs3^{-/-}$ mice in both the dark ($\sim 40$ beats/min) and light ($\sim 29$ beats/min) phases. Physical activity in $Brs3^{-/-}$ mice was greater than wild-type mice only during the light phase.

The MAP and heart rate frequency distributions were each bimodal, with a larger span in the $Brs3^{-/-}$ mice (Fig. 1, C and D). To understand this variation, the light/dark phases and physically active/inactive intervals were analyzed separately (Fig. 1, E–H). The resting MAP was similar in $Brs3^{-/-}$ and control mice, but increased more with activity in $Brs3^{-/-}$ mice. The resting heart rate was lower in $Brs3^{-/-}$ mice and also increased more with activity, reaching close to the same level as wild-type mice. Statistical models demonstrated that both genotype and genotype activity interaction (both $P < 0.0001$) contribute to predicting both MAP and heart rate (Table 2). Consistent with the wider range in heart rate, the standard deviation of the RR intervals was greater in $Brs3^{-/-}$ mice ($17.4 \pm 1.7$ vs. $11.8 \pm 2.4$ ms, $P = 0.001$). However, the percentage of consecutive RR intervals differing by $>6$ ms (pNN6), a measure of cardiac parasympathetic activity (51), was the same in wild-type and $Brs3^{-/-}$ mice ($17.2 \pm 3.0$ vs. $18.9 \pm 4.8\%$, respectively).

Baroreceptor sensitivity (17, 30) was investigated using the sequence method during a 1-h light phase interval and was not
different in wild-type vs. Brs3\(^{-/-}\) mice under a variety of analysis parameters (Table 3).

Intrinsic heart rate. The heart rate of the Brs3\(^{-/-}\) mice increased more than control mice when parasympathetic input was inhibited with the muscarinic receptor antagonist atropine, with both groups reaching similar posttreatment levels (Fig. 2, 31–45 min after dosing; 16–30 min after dosing was quantitatively similar, not shown). Sympathetic inhibition with the \(\beta\)-adrenergic antagonist propranolol reduced the higher heart rate of the control mice to the level of the Brs3\(^{-/-}\) mice and had no effect in Brs3\(^{-/-}\) mice. The intrinsic heart rate, measured after blockade of both sympathetic and parasympathetic signals with propranolol and atropine, was similar in Brs3\(^{-/-}\) and control mice. Taken together, these data suggest that the lower heart rate in Brs3\(^{-/-}\) mice is due to reduced sympathetic (and/or increased parasympathetic) drive to the heart.

Cardiovascular Effects of the BRS-3 Agonist MK-5046

Studies in conscious mice. The effects of single intraperitoneal injections of the BRS-3 agonist MK-5046 on MAP and heart rate of conscious mice are presented in Fig. 3. The increase in MAP and heart rate caused by handling was intact in the Brs3\(^{-/-}\) mice, being similar to controls (Fig. 4, A, B, D, and E). Whereas the handling masked any early effects of MK-5046, in the second hour after treatment BRS-3 activation was associated with significantly increased MAP (by \(9 \pm 3\) mmHg) and heart rate (by \(69 \pm 21\) beats/min) in wild-type mice (Fig. 3, A, C, D, and F). In contrast, MK-5046 had no effect on MAP or heart rate in Brs3\(^{-/-}\) mice, demonstrating that the increase in MAP and heart rate is mediated by MK-5046 acting on its intended target, BRS-3 (Fig. 3, B, E, and F).

Studies in anesthetized mice. We next explored hemodynamics in pentobarbital sodium-anesthetized mice to assess the acute effects of BRS-3 activation on blood pressure and heart rate, which were partially masked in conscious mice due to the confounding effects of activity and handling. Similar to the results in conscious mice, intravenous MK-5046 (1 mg/kg) increased MAP by \(8 \pm 2\) mmHg and heart rate by \(49 \pm 14\) beats/min in wild-type mice (Fig. 4, B and E). The increases in MAP and heart rate were short-lived, lasting \(\sim 5\) min. Again, MK-5046 had no significant effect on MAP or heart rate in Brs3\(^{-/-}\) mice, demonstrating that the effects in control mice are mediated by BRS-3. In contrast to the results in conscious mice, anesthetized Brs3\(^{-/-}\) mice had a 7 mmHg lower MAP than controls (60.6 ± 3.5 vs. 70.3 ± 2.2 mmHg, \(P = 0.02\))
The effects of clonidine, a centrally acting sympatholytic drug, on cardiovascular changes caused by MK-5046, we assessed the possible role of the sympathetic nervous system in the cardiovascular regulation of BRS-3 

D

0.26) (Fig. 4). However, clonidine pretreatment completely abolished the effect of MK-5046 to increase MAP and heart rate, consistent with a sympathetic mechanism.

Central infusion of MK-5046. To test directly if the MAP and heart rate effects of MK-5046 occur through the central nervous system, we bilaterally implanted cannulas into the anterior hypothalamus. This region has a high level of BRS-3 expression and ligand binding (14), and several nuclei in this region are implicated in cardiovascular regulation (5, 39). Infusion of MK-5046 increased both MAP and heart rate significantly more than vehicle in wild-type mice, while central infusion of MK-5046 had no effect in Brs3−/− mice (Fig. 5).

DISCUSSION

BRS-3 regulates energy homeostasis, but its role in blood pressure and heart rate control has been unclear. We show that despite mild obesity, Brs3−/− mice are not hypertensive in the resting state and exhibit a normal intrinsic heart rate. We also establish for the first time that BRS-3 activation with a selective, brain-penetrant agonist causes an on-target, BRS-3 mechanism-based increase in MAP and heart rate.

Previously, Brs3−/− mice were reported to have an unchanged heart rate (of ~700 beats/min) and an increased MAP (by 19 mmHg) in old, but not young, mice (41). In contrast, using telemetry in free-living mice, we demonstrate a reduced resting heart rate and an exaggerated increase with physical activity that reaches a maximum approaching that of controls. Similarly, the apparently elevated blood pressure in the Brs3−/− mice is attributed to an exaggerated MAP increase with physical activity and not an inherently high resting blood pressure. The MAP and heart rate did not change with age. Thus the prior results measured by tail cuff (41), which can increase heart rate and MAP (13, 26, 55), match our data during physical activity, and our analysis demonstrates a significant genotype × activity interaction. A model to explain these results is that a primary reduction in heart rate in Brs3−/− mice elicits compensatory mechanisms to defend blood pressure, presumably including the intact baroreceptor reflex. Activity-induced increases in heart rate and blood pressure are intact in Brs3−/− mice, indicating that BRS-3 does not have a role in these processes.

The cardiovascular system is regulated by both parasympathetic and sympathetic tone (10). In conscious Brs3−/− mice, the reduced resting heart rate and attenuated reduction by...
propranolol suggest that the sympathetic tone to the heart is reduced. An increase in parasympathetic tone seems less likely from the effect of the sympatholytic drug clonidine on MK-5046 action in wild-type mice. In addition, we have shown that MK-5046 increases temperature in brown adipose tissue, likely reflecting a role for BRS-3 in sympathetic activation of thermogenesis (28). Despite the reduced basal sympathetic tone, the response to physical activity in *Brs3*+/− mice is exaggerated, suggesting that BRS-3 contributes to setting the resting sympathetic tone but not the activity-induced increase in heart rate and MAP.

BRS-3 action is likely mediated via a central mechanism since delivery of MK-5046 in the brain increases MAP and heart rate, and the effects of peripheral MK-5046 are abolished by clonidine, a centrally acting sympatholytic. BRS-3 is present in brain regions that regulate blood pressure and heart rate (2, 11, 16), including the medial preoptic, posterior hypothalamic, dorsomedial hypothalamus, periaqueductal gray, dorsal raphe, parabrachial nucleus, and nucleus of the solitary tract (14, 31, 54). Modulation of other central antiobesity mechanisms (leptin, melanocortin, and ghrelin) can also increase blood pressure. For example, MC4R-deficient mice and humans are obese in the absence of hypertension (12, 50), and the central MC4R agonist, α-MSH, increases blood pressure and heart rate (8, 35). Recently, a central role for the leptin pathway was demonstrated in obesity-associated hypertension (49). At this time it is unknown how or if BRS-3 interacts with these mechanisms. Similarly, it is also unknown how or if BRS-3 interacts with other cardiovascular regulators, such as vasopressin and the renin and adrenocorticotropic hormone cascade.

We did not directly measure cardiac output or peripheral vascular resistance. However, cardiac output can be estimated from the heart rate (43), since in very small animals the upper end of the heart rate range is limited, restricting changes in stroke volume (26). Making these assumptions, the 13% increase in heart rate caused by MK-5046 in conscious wild-type mice is calculated (25) to produce a 12% increase in cardiac output. Since the measured MAP increased in proportion to the calculated cardiac output, this suggests that no major changes in peripheral vascular resistance occurred. This hypothesis requires confirmation by direct experiment.

The on-target effects of MK-5046 in mice suggest that clinical adverse events, including transiently increased blood pressure, erections, and feeling hot, cold, and/or jittery (46), may be mechanism-based (i.e., due to BRS-3 activation) and result from sympathetic stimulation. It is unclear why no heart rate increase was observed clinically; possibilities include sympathetic stimulation. It is unclear why no heart rate increase was observed clinically; possibilities include sympathetic stimulation. It is unclear why no heart rate increase was observed clinically; possibilities include sympathetic stimulation. It is unclear why no heart rate increase was observed clinically; possibilities include sympathetic stimulation. It is unclear why no heart rate increase was observed clinically; possibilities include sympathetic stimulation. It is unclear why no heart rate increase was observed clinically; possibilities include sympathetic stimulation. It is unclear why no heart rate increase was observed clinically; possibilities include sympathetic stimulation. It is unclear why no heart rate increase was observed clinically; possibilities include sympathetic stimulation. It is unclear why no heart rate increase was observed clinically; possibilities include sympathetic stimulation. It is unclear why no heart rate increase was observed clinically; possibilities include sympathetic stimulation. It is unclear why no heart rate increase was observed clinically; possibilities include sympathetic stimulation. It is unclear why no heart rate increase was observed clinically; possibilities include sympathetic stimulation. It is unclear why no heart rate increase was observed clinically; possibilities include sympathetic stimulation. It is unclear why no heart rate increase was observed clinically; possibilities include sympathetic stimulation. It is unclear why no heart rate increase was observed clinically; possibilities include sympathetic stimulation. It is unclear why no heart rate increase was observed clinically; possibilities include sympathetic stimulation. It is unclear why no heart rate increase was observed clinically; possibilities include sympathetic stimulation. It is unclear why no heart rate increase was observed clinically; possibilities include sympathetic stimulation. It is unclear why no heart rate increase was observed clinically; possibilities include sympathetic stimulation. It is unclear why no heart rate increase was observed clinically; possibilities include sympathetic stimulation. It is unclear why no heart rate increase was observed clinically; possibilities include sympathetic stimulation. It is unclear why no heart rate increase was observed clinically; possibilities include sympathetic stimulation. It is unclear why no heart rate increase was observed clinically; possibilities include sympathetic stimulation.

with anti-obesity but not cardiovascular activities would be welcome.

ACKNOWLEDGMENTS

We thank Oksana Gavrilova, Xiongce Zhao, and Yuning George Huang, of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), for advice and suggestions and Danielle Springer, Audrey Noguchi, and Michelle Allen of the National Heart, Lung, and Blood Institute (NHLBI) Murine Phenotyping Core for collaboration on the conscious telemetry.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

Author contributions: D.M.L., J.S., and M.L.R. conception and design of research; D.M.L. and C.X. performed experiments; D.M.L., R.J.B., A.D., and M.L.R. analyzed data; D.M.L., R.J.B., and M.L.R. interpreted results of experiments; D.M.L. and M.L.R. prepared figures; D.M.L. and M.L.R. drafted manuscript; D.M.L., C.X., R.J.B., A.D., J.S., and M.L.R. approved final version of manuscript; C.X., A.D., J.S., and M.L.R. edited and revised manuscript.

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