Diminished baroreflex control of heart rate responses in otoconia-deficient C57BL/6JEi head tilt mice

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Xue, Baojian, Karl Skala, Timothy A. Jones, and Meredith Hay. Diminished baroreflex control of heart rate responses in otoconia-deficient C57BL/6JEi head tilt mice. Am J Physiol Heart Circ Physiol 287: H741–H747, 2004. First published April 1, 2004; 10.1152/ajpheart.01023.2003.—The maintenance of stable blood pressure during postural changes is known to involve integration of vestibular and cardiovascular central regulatory mechanisms. Sensory activity in the vestibular system plays an important role in cardiovascular regulation. The purpose of this study was to determine the role of vestibular gravity receptors in normal baroreflex function. Baroreflex heart rate (HR) responses to changes in blood pressure (BP) in otoconia-deficient head tilt (het) mice (n = 8) were compared with their wild-type littermates (n = 12). The study was carried out in conscious male mice chronically implanted with arterial and venous catheters for recording BP and HR and for the infusion of vasoactive drugs. Resting HR was higher in the het mice (661 ± 13 beats/min) than in the wild-type mice (579 ± 20 beats/min). BP was comparable in the het mice (113 ± 4 mmHg) and wild-type mice (104 ± 4 mmHg). The slopes of reflex decreases in HR in response to phenylephrine (PE) were blunted in the het mice (−5.5 ± 1.5 beats·min⁻¹·mmHg⁻¹) compared with the wild-type mice (−8.5 ± 0.9 beats·min⁻¹·mmHg⁻¹). Likewise, reflex tachycardic responses to decreases in BP with sodium nitroprusside (SNP) were significantly blunted in the het mice (−0.8 ± 0.3 beats·min⁻¹·mmHg⁻¹) versus the wild-type mice (−2.2 ± 0.6 beats·min⁻¹·mmHg⁻¹). Frequency-domain analysis of the HR variability suggests that under resting conditions, parasympathetic contribution was lower in the het versus wild-type mice. Mapping of the expression of immediate-early gene product, c-Fos, in forebrain and brain stem nuclei in response to a BP challenge showed no differences between the wild-type and het mice. These results suggest that tonic activity of gravity receptors modulates and is required for normal function of the cardiac baroreflexes.

otoconia receptors; baroreflex; c-Fos expression

THE MAINTENANCE OF STABLE BLOOD PRESSURE (BP) during postural changes is known to involve integration of vestibular and cardiovascular central regulatory mechanisms (10, 36, 54). This integration is manifested as the vestibulosympathetic reflex (VSR). Activation of the otoconial organs, the peripheral vestibular end-organs, results in an increase in sympathetic nerve activity that is believed to assist in maintaining arterial pressure during an orthostatic challenge (5, 30, 38, 44). Likewise, the arterial baroreflex mechanisms are also important for the overall regulation of BP during orthostatic stress. Under orthostasis, the baroreflex mediates tachycardia and peripheral vasoconstriction to lessen gravity-induced fluid shift and to preserve arterial BP (11, 12, 24, 28, 40). Impairment of the baroreflex would produce inadequate homeostatic responses to the changing posture that lead to orthostatic hypotension (6, 15, 25). Therefore, neural interaction between VSR and baroreflexes are important in understanding the hemodynamic changes that occur during postural changes.

Previous neuroanatomical and electrophysiological studies have shown direct projections from vestibular nuclei to brain stem nuclei regulating cardiovascular functions including nucleus tractus solitarius (NTS), dorsal motor vagal nucleus, nucleus ambiguous, caudal ventrolateral medulla (CVLm), and rostral ventrolateral medulla (RVLM) (2, 45, 51–53, 55). Direct stimulation of the vestibular nerves in animals results in increased sympathetic activity to a number of vascular beds (5, 23, 32, 54). However, the importance and role of vestibular inputs in baroreflex regulation of heart rate (HR) have been largely unrecognized, despite the abundant anatomic evidence for synaptic connections from the vestibular nuclear complex to numerous brain stem autonomic nuclei.

Clarifying the potential roles of the respective vestibular sensors in baroreflex function is difficult in part due to the complexity of the labyrinthine organs themselves. Isolating the influence of gravity receptor input from the input of ampullar organs, for example, is not easily accomplished experimentally. However, a new approach to this problem has been described recently, which capitalizes on the complete absence of otoconia in the C57BL/6JEi head tilt (het) mouse (17, 26). These mice, originally described by Sweet (46), carry a spontaneous mutation that has been linked to chromosome 17 and results in complete otoconial agensis (3). This mutation produces a selective loss of gravity receptor function, as shown by the absence of responses to linear acceleration stimuli (26). The specificity of the functional loss in these animals provides an opportunity to better assess the influence of the vestibular otoconial organs on baroreflex function. We hypothesize that the activity of gravity receptors enhances the sensitivity of the cardiac baroreflex, thus facilitating homeostasis during changes in posture. To critically test this, we characterized cardiac baroreflex in normal and het mice. The immediate-early genes are considered useful neurobiological tools for mapping brain functional activity (43). Thus the expression and regional distribution of c-Fos protein in brain stem and forebrain nuclei including the vestibular nuclear complex were also compared between normal and het mice to assess the functional activity of brain structures during BP challenges.
METHODS

All experiments were carried out in sibling normal C57BL/6J wild-type (het/het) and C57BL/6D/Ei-het (het/het) otoconia-deficient head tilt male mice. The source stock for these animals was obtained from the Jackson Laboratory. Het animals are homozygous for a spontaneous mutation located on chromosome 17 (3). The labyrinth and both auditory and vestibular sensory organs are normal in these animals except that the macular gravity receptors are devoid of otoconia (3). Gravity receptors are dysfunctional, but ampullar receptors in the semicircular canals that respond normally to rotational head motion are functional. Vestibular gravity receptor responses to linear rotation are normal in these spontaneous mutation located on chromosome 17 (3). The labyrinth from the Jackson Laboratory.

head tilt male mice. The source stock for these animals was obtained in their own cages 4–5 days after surgery. On the day of the experiment, after the arterial and venous catheters were connected to the pressure transducer and drug infusion lines, the mice were allowed to recover for 45–60 min before the infusion of the next drug was started. While baroreflexes were tested, we took precautions to ensure the mice were resting quietly in their cages and the resting BPs and HRs were stable for ~45–60 min before beginning the experiment. If at any point during the infusion the mice moved, the data set was discarded and the baroreflex test repeated after an appropriate amount of time (45–60 min).

We also measured BP in wild-type and het mice in the presence of the ganglionic blocker hexamethonium (10 mg/kg iv). The mice were allowed to stabilize for at least 60 min, after which arterial pressure was recorded 20 min before hexamethonium infusion and for 20 min after the treatment.

METHODS

H742 VESTIBULAR GRAVITY RECEPTORS AND BAROREFLEX REGULATION

RESULTS

Resting BP and HR in conscious mice. Resting values for BP were not significantly different (P = 0.126, unpaired t-test, power value = 0.6) between the wild-type (n = 12, 104 ± 4 mmHg) and het mice (n = 8, 113 ± 4 mmHg). In contrast, resting HRs were significantly higher in het mice (661 ± 13 beats/min, P < 0.05) than in wild-type mice (579 ± 20

Statistical analysis. All the data are presented as means ± SE. Statistically significant differences between groups were determined using an unpaired t-test and factorial ANOVA where applicable. Statistical significance was accepted when P < 0.05.
Previous studies have shown that mean activity levels of resting HRs were due to differences in activity levels. Although we did not measure activity levels, there is no compelling reason to suspect that differences in resting HRs were due to differences in activity levels. Previous studies have shown that mean activity level of het and normal animals did not differ significantly (17), and the experimental design in the present study strictly controlled for movement artifacts.

Frequency-domain indexes of variation of HR in conscious mice. Consistent with previously reported studies in mice (27), we saw two peaks corresponding to a LF (0.1–1.75 Hz) and HF (1.75–5.0 Hz) range. The HF range is indicative of parasympathetic contribution to the HR fluctuations, whereas the LF peak is not representative of purely sympathetic activity (27). Under resting condition, the het mice had a significantly higher LF component (74.9 ± 7.1 vs. 53.7 ± 6.8, P < 0.05) and lower HF component (26.4 ± 6.9 vs. 46.9 ± 6.0, P < 0.05) than the wild-type mice (Fig. 2).

Baroreflexes sensitivity. Mean HR baroreflex responses to intravenous infusions of PE in wild-type and het mice are shown in Fig. 3. Slopes of the regression lines relating absolute BPs to absolute HRs were significantly blunted in the het mice (−4.9 ± 0.9 beats·min⁻¹·mmHg⁻¹, n = 8, P < 0.05) compared with the wild-type mice (−9.9 ± 1.6 beats·min⁻¹·mmHg⁻¹, n = 12; Fig. 3A). The resting BPs and HRs were different between the wild-type and het mice. To observe true differences in baroreflex sensitivity independent of the starting values, the slope values for regression lines relating changes in HR to changes in BP during PE infusion were also calculated. The average changes in HR for a given change in mean arterial pressure are shown in Fig. 3B. The het mice had significantly smaller slope values (−5.5 ± 1.5 beats·min⁻¹·mmHg⁻¹, P < 0.05) compared with the wild-type mice (−8.5 ± 0.9 beats·min⁻¹·mmHg⁻¹).

Figure 4 illustrates the linear regression analysis of the HR baroreflex responses to intravenous SNP in wild-type and het mice. The slope of the line relating absolute BPs to absolute HRs for SNP in het mice (−0.7 ± 0.3 beats·min⁻¹·mmHg⁻¹, P < 0.05) was less than that observed in wild-type mice (−2.1 ± 0.4 beats·min⁻¹·mmHg⁻¹; Fig. 4A). Likewise, in het mice, the slope of the line relating changes in HR to changes in BP during SNP infusion was −0.8 ± 0.3 beats·min⁻¹·mmHg⁻¹, which was also significantly less than that observed in the wild-type animals (−2.2 ± 0.6 beats·min⁻¹·mmHg⁻¹, P < 0.05; Fig. 4B).

Fig. 1. Resting mean arterial blood pressure (MAP; in mmHg) and heart rate (HR; in beats/min) in wild-type (WT; n = 12) or het (n = 8) mice. Resting values for HR were significantly higher in het than WT mice. *Significant difference compared with WT mice (P < 0.05).

Fig. 2. A and B: frequency spectra for one WT (A) and one het mouse (B) are compared. The frequency bands that were analyzed are 0.1–1.75 Hz [low frequency (LF)], and 1.75–5.0 Hz [high frequency (HF)]. C and D: frequency analysis of HR on LF (C) and HF (D) components compared between WT and het mice. n.u., Normalized units. *Significant difference compared with WT mice (P < 0.05).
Responses of BP to autonomic blockade. Ganglionic blockade with hexamethonium produced similar decreases in BP in wild-type (n = 5, 30.4 ± 0.4 mmHg, P < 0.05) and het mice (n = 5, 29.2 ± 4.1 mmHg).

c-Fos expression in forebrain and brain stem nuclei involved in cardiovascular regulation. Intravenous administration of saline at the same volume and rate as the infused PE or SNP did not induce significant c-Fos expression in forebrain and brain stem nuclei in wild-type (n = 2) and het mice (n = 2). Acute hypertension induced a significant increase in c-Fos expression in the area postrema (AP), NTS, and CVLM. Acute hypotension also produced a significant increase in c-Fos expression in the above regions as well as in the RVLH, paraventricular nucleus (PVN), and supraoptic nucleus (SON). However, there were no differences in c-Fos expression in these forebrain and brain stem nuclei between the wild-type (n = 6) and het mice (n = 6) (Fig. 5). Moreover, c-Fos-like immunoreactive neurons were not found in the vestibular nuclei after the BP changes in both mice (Table 1).

DISCUSSION

The present study evaluates baroreflex control of HR during increases in arterial pressure with PE or decreases in arterial pressure with SNP in animals with (wild-type) or without (het) functional gravity receptors. The principal findings of the present study are that, first, otoconia-deficient het mice had significantly higher resting HRs than the wild-type mice. However, the resting BPs were not significantly different. Second, baroreflex HR responses to increases in BP with PE or decreases in BP with SNP were significantly blunted in het mice compared with wild-type mice.

There is considerable experimental evidence in animals and humans to suggest an interaction between the VSR and the baroreceptor reflex in maintaining stable BPs during postural changes (20, 36–40). Although the level at which this interaction occurs is unclear, it is generally accepted that the VSR and baroreflexes share common neural pathways (2, 45, 51–53, 55). An orthostatic challenge or stimulation of vestibular nuclei is known to cause increases in muscle or renal sympathetic nerve activity (10, 36, 38, 54), and in the presence of intact baroreflexes these responses are augmented (37, 38, 40). However, very little is known about the influence of vestibular inputs or more specifically the lack of these inputs on cardiac baroreflexes.

In the present study, this issue was addressed by using the het mouse model with selective gravity receptor dysfunction.
In the absence of any gravity receptor vestibular inputs, baroreflex HR responses to changes in BP were significantly blunted in het mice compared with wild-type mice. The blunted responses in het animals occurred despite the fact that in both groups, BP challenges clearly were effective in eliciting similar c-Fos expression in brain regions normally mediating baroreflex function. The latter finding is important because it shows that normal afferent activation of baroreceptors occurred in both groups and the blunted baroreflex HR responses observed in the het mice were not due to an impairment of baroreceptor function. The baroreflex test in the present study involved no movement of the animals during the test; thus the reflex was exclusively baroreceptive in nature and could not invoke a vestibular reflex in either the normal or het mice. The data suggest that the absence of otoconia in itself leads to disfacilitation of the baroreflexes in het mice. It is reasonable to conclude that there is a general tonic facilitation of cardiac baroreflexes by gravity receptors even in the absence of tilt-evoked orthostatic challenge.

An absence of tonic gravity receptor inputs may also have contributed to the higher resting HRs in the het mice. In a recent study, rats with lesions of the vestibular nuclei were reported to have high basal HRs but similar BPs compared with intact rats (20). It is well accepted that labryrinthectomy induces compensatory changes in the neuronal architecture and function of the vestibular nuclei (7, 9, 49). In view of the evidence showing neural projections from the vestibular nuclei to major brain stem nuclei involved in autonomic control of HR (34, 42), it is conceivable that altered neuronal function or activity in the vestibular nuclear complex of the het mice has downstream effects on the autonomic regulation of HR.

An elevated sympathetic outflow or low parasympathetic outflow to the heart or both could contribute to a higher HR in the het mice. In the present study, frequency-domain analysis of the HR variability under resting conditions suggests that the parasympathetic contribution (HF) to the HR was lower in the het mice compared with the wild-type mice. It is also possible that the sympathetic contribution to HR is elevated in the het mice; however, a definitive conclusion is difficult because the LF peak is not representative of purely sympathetic activity (27). High sympathetic tone under resting conditions may limit the extent to which the het mice can increase the sympathetic outflow to the heart in response to lowering of BP. Consequently, the blunted reflex tachycardic responses to SNP in the het mice could be due to their inability to increase sympathetic outflow any further. However, ganglionic blockade with hexamethonium produced similar decreases in BP in both groups, suggesting that overall sympathetic outflow in wild-type and het mice are not different.

In humans, activation of the vestibular reflexes after unloading of the baroreceptors with application of lower body negative pressure (LBNP) had an additive effect with regard to increases in sympathetic nerve activity but had no effect on increases in HR (37, 39, 40). This is in contrast to the observations in the present study, where unloading of baroreceptors in the het mice with SNP led to blunted reflex tachycardic responses. One possibility is species differences in autonomic regulation of HR between humans and rodents. In

Table 1. Mean number of Fos-like immunoreactive neurons in the brain stem and forebrain nuclei 2 h after increases or decreases in blood pressure in WT and het mice

<table>
<thead>
<tr>
<th></th>
<th>AP</th>
<th>NTS</th>
<th>VLM</th>
<th>SON</th>
<th>PVN</th>
<th>VN</th>
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<tbody>
<tr>
<td>Control</td>
<td>5.4±1.6</td>
<td>12.4±2.9</td>
<td>5.4±0.8</td>
<td>4.8±1.7</td>
<td>4.0±0.6</td>
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</tr>
<tr>
<td>WT + PE</td>
<td>73±7.9*</td>
<td>98±5.2*</td>
<td>31±2.1*</td>
<td>27.7±2.6</td>
<td>49.4±5.8</td>
<td>NL</td>
</tr>
<tr>
<td>het + PE</td>
<td>57±13*</td>
<td>92±3.7*</td>
<td>34±1.9*</td>
<td>32.6±2.7</td>
<td>56.5±10.5</td>
<td>NL</td>
</tr>
<tr>
<td>WT + SNP</td>
<td>68.5±6.8*</td>
<td>95±6.7*</td>
<td>30.2±1.7</td>
<td>29.5±1.9</td>
<td>60.4±3.9</td>
<td>NL</td>
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<tr>
<td>het + SNP</td>
<td>61.4±9.1*</td>
<td>102±11.2*</td>
<td>35.1±2.4*</td>
<td>26.7±1.5*</td>
<td>67.5±5.4*</td>
<td>NL</td>
</tr>
</tbody>
</table>

Values are means ± SD. WT, wild-type mice; het, otoconia-deficient head tilt mice; PE, phenylephrine; SNP, sodium nitroprusside; AP, area postrema; NTS, nucleus tractus solitarii; VLM, ventrolateral medulla; PVN, paraventricular nucleus; SON, supraoptic nucleus; VN, vestibular nuclei; NL, no labeled neurons. *Significant difference compared with control (P < 0.05).
most mammals, resting HRs are predominantly under parasympathetic control and reflex increases in HR in response to unloading of baroreceptors is due to withdrawal of vagal tone and sympathetic activation (4, 18, 33). Compared with humans, mice have a lower parasympathetic drive under resting conditions (8), and it may be the case that the high resting HRs in mice are the result of greater sympathetic drive (22). However, there are also several studies in the literature that suggest that in mice vagal innervation accounts for more of the total range of HR changes than sympathetic innervation (19, 48). This is not unlike the autonomic regulation of HR in humans except in mice the baroreflexes operate around an even higher set point. In response to unloading of baroreceptors, the mice may be unable to withdraw vagal tone or increase sympathetic tone to the heart as much as humans. Although this accounts for the smaller reflex tachycardic responses in mice in general, it does not completely account for the discrepancy between the observation in the study by Ray (37) and the present study. A more likely explanation for these differences is the experimental conditions under which the interaction was studied. In the study by Ray (37), vestibular reflexes are being activated on top of the baroreflex responses evoked by LBNP. Any facilitatory effects of vestibular inputs on HR are probably being masked by significant vagal withdrawal, which has already occurred in response to LBNP. It is possible that the absence of any inputs from the gravity receptors is far more effective in unmasking the influence of vestibular inputs on baroreflex control of HR. Our conclusions are supported by a recent study in rats by Gotoh et al. (20). In this study, activation of vestibular reflexes in response to an increase in gravity resulted in significant bradycardia, which was completely abolished in rats with vestibular lesions. Bradycardia was also attenuated in sinoaortic denervated rats with vestibular lesions despite augmented increases in BP and sympathetic activity. The authors conclude that vestibular inputs can cause bradycardia independent of changes in arterial BP. In light of these observations, the significantly attenuated baroreflex HR responses in the het mice suggest not only that gravity receptor inputs play a significant role in the modulation of cardiac baroreflexes but also that this influence may be, in part, tonic in nature.

The immediate-early genes are considered useful neurobiological tools for mapping brain functional activity (43). Several experimental studies have demonstrated that hypertension and hypotension elicits the expression of c-Fos protein in several forebrain and brain stem nuclei affecting cardiovascular function (29, 31, 35). In the present study, the significant increases in c-Fos expression occur in the area postrema, NTS, VLM, supraoptic nucleus, and paraventricular nucleus after acute BP changes (increase or decrease) in wild-type and het mice, which are consistent with those of previous observations (29, 31). However, the immunohistochemical results also indicate that BP manipulations alone do not produce differences in c-Fos expression in the vestibular nuclei of either group. Recently, Kim et al. (29) reported that c-Fos-like immunoreactive neurons were expressed bilaterally in vestibular nuclei after acute hypotension in normal but not bilaterally labyrinthectomized, anesthetized rats. On the basis of these findings, the authors suggested that peripheral vestibular receptors are essential for neuronal activation in vestibular nuclei after acute changes in BP. Het mice lack otoconia in the two gravity-sensing organs of their inner ear, the utricle and saccule, preventing them from experiencing gravity or linear acceleration (3, 26). Therefore, in the present study, based on the findings of Kim et al. (29), one would expect c-Fos labeling in the vestibular nuclei of wild-type mice but not in het mice after BP manipulations. However, our results do not confirm this expectation. The reason for the discrepancy between the two studies is unknown, but it may reflect differences in experimental conditions, in particular, the use of anesthetized animals by Kim and co-workers. Erickson and Millhorn (13) reported that hypoxic stimulation of the carotid sinus induced c-Fos expression in the bilateral medial vestibular nuclei of urethane-chloralose-anesthetized rats but not in conscious rats. It is known that anesthetic agents such as urethane tend to increase c-Fos expression in brain nuclei regulating cardiovascular function (41, 47). Thus the contrasting c-Fos results of the two studies may be due to differences associated with the use of unanesthetized versus anesthetized animals.

In summary, cardiac baroreflex responses to systemic increases (PE infusion) and decreases (SNP infusion) in BP were significantly blunted in otoconia-deficient (het) mice. These results suggest that tonic activity of gravity receptors modulates and is required for normal function of the cardiac baroreflexes.

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