Increased baroreceptor response in mice deficient in monoamine oxidase A and B

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Holschneider, D. P., O. U. Scremin, K. P. Roos, D. R. Chialvo, K. Chen, and J. C. Shih. Increased baroreceptor response in mice deficient in monoamine oxidase A and B. Am J Physiol Heart Circ Physiol 282: H964–H972, 2002. First published November 1, 2001; 10.1152/ajpheart.00309.2001.—The recent development of mice doubly deficient for monoamine oxidase A and B (MAO-A/B, respectively) has raised questions about the impact of these mutations on cardiovascular function, in so far as these animals demonstrate increased tissue levels of the vasoactive amines serotonin, norepinephrine, dopamine, and phenylethylamine. We recorded femoral arterial pressures and electrocardiograms in adult MAO-A/B-deficient mice during halothane-nitrous oxide anesthesia as well as 30 min postoperatively. During both anesthesia and recovery, systolic, diastolic, and mean arterial pressures were 10–15 mmHg lower in MAO-A/B-deficient mice compared with normal controls (P < 0.01). Mutants also showed a greater baroreceptor-mediated reduction in heart rate in response to hypertension after intravenous pulses of phenylephrine or angiotensin II. Tachycardia elicited in response to hypotension after nitroprusside was greater in mutants than in controls. Heart rate responsiveness to changes in arterial pressure was abolished after administration of glycopyrrolate, with no differences in this phenomenon noted between genotypes. These data suggest that prevention of hypertension may occur in chronic states of catecholaminergic/indoleaminergic excess by increased gain of the baroreflex.

arterial baroreceptor reflex; serotonin; norepinephrine; phenylethylamine; dopamine; blood pressure; heart rate; sympathetic nervous system

SEROTONIN (5-HT), norepinephrine (NE), and dopamine (DA) play a major role in the control of arterial blood pressure and heart rate (HR). Most studies suggest that these neurotransmitters exert an acute pressor effect by acting on both the central and peripheral nervous system. The autonomic effects of chronic elevations of the catecholamines or indoleamines, however, remain controversial. On one hand, hypertension and tachycardia are frequent features of clinical conditions characterized by chronically elevated noradrenergic and serotonergic states. Such conditions include patients undergoing long-term treatment with monoamine oxidase (MAO) inhibitors (23) and patients suffering from pheochromocytoma (18, 28). On the other hand, a substantial number of such patients show a relatively normal resting blood pressure and additionally may suffer from orthostatic hypotension and wide blood pressure swings.

One mechanism that may help to reconcile such discrepancies is the occurrence of alterations in the baroreceptor reflex that serve to control blood pressure through a negative feedback system acting on cardiac output and peripheral vasomotor tone. Although evidence suggests that under normal physiological conditions the baroreflex plays little to no role in the long-term regulation of arterial blood pressure (25), recent studies suggest that arterial baroreceptors may be important in the long-term regulation of arterial pressure in pathological states characterized by a catecholamine excess (18, 28, 39), sodium overload (32), or hypertension (30). Desensitization of the adrenergic system associated with chronic catecholamine excess has been considered to be one of the determinants for the wide blood pressure fluctuations in patients with pheochromocytoma (18). Indeed, in these patients, a functional impairment of the baroreflex exists, which is reversible soon after normalization of catecholamine levels with removal of the tumor (28). Likewise, patients with a NE transporter deficiency show elevated levels of plasma NE as well as a decreased baroreceptor sensitivity (39). Improved understanding of the relationship between noradrenergic function and baroreceptor control may have implications also for clinical conditions such as heart failure, in which poor prognosis has been linked to sympathetic dysregula-
tion and attenuation of cardiac baroreceptor control (27).

A major catabolic pathway for bioamines is MAO, whose two isoforms (MAO-A and MAO-B) are distributed in virtually all mammalian cell types and serve to metabolize catecholamines as well as indoleamines. Our laboratory has recently identified a natural mutation of MAO-A (35) occurring in mice with targeted deletion of the MAO-B gene (17). These mice doubly deficient in both MAO isoforms [MAO-A/B knockout (KO)] demonstrate brain levels of 5-HT, NE, DA, and phenylethylamine (PEA) that are respectively increased 8.5–22.2, 1.7–15.7-fold above those noted in adult wild-type (WT) animals, with levels of the 5-HT metabolite 5-hydroxy-indoleacetic acid essentially undetectable in both the brain and urine. It is likely that MAO-A/B KO mice, with their elevated bioamine levels, exhibit altered autonomic control. This could be important in understanding the mechanisms contributing to the regulation of blood pressure in states characterized by excesses of the catecholaminergic and serotonergic tone. In this study, we compared autonomic function of MAO-A/B KO mice to that of their WT counterparts and evaluated the effects of differing genotypes on the baroreflex during anesthesia as well as during recovery. The surprising key findings are a decrease in basal blood pressure and an increase in the baroreceptor gain of MAO-A/B KO mice compared with their WT counterparts.

METHODS

Animals. Our laboratory has recently identified a natural mutation of MAO-A (35) in mice with targeted deletion of the MAO-B gene (17). MAO-A/B-deficient mice were originally identified by their marked decreased body size and behavioral hyperreactivity on handling or threat of being handled, a phenotype not seen in MAO-B KO mice. Breeding of these deficient mice were originally through MAO-A and MAO-B gene (17). MAO-A/B-deficient mice were reviewed and approved by the institutional animal care and use committee. At the end of the experiment, absence of the MAO-B gene was confirmed in MAO-A/B KO mice by a polymerase chain reaction of DNA prepared from tails (17) (data not shown) as well as by measurement of MAO-A enzymatic activity in the liver (MAO-A/B KO: 0.07 ± 0.02 nmol·20 min⁻¹·mg protein⁻¹, n = 8; WT: 5.51 ± 0.70 nmol·20 min⁻¹·mg protein⁻¹, n = 10) (20).

Surgery. Mice were anesthetized with halothane (2.0% induction, 1.2% maintenance) in 30% oxygen-70% nitrous oxide. Rectal temperature was maintained at 36.5°C with a BAT-12 thermocouple thermometer connected to a TCAT-1A temperature controller (Physitemp; Clifton, NJ), a heating pad set at 36.5°C, and a source of radiant heat. For recording of the electrocardiogram, two platinum needle electrodes were implanted in the subcutaneous tissue overlying the right scapula and the apex of the heart. The femoral artery and vein were cannulated with polypropylene and Silastic Fr-1.2 catheters, respectively, through a femoral cut down, which was closed with a 6-0 silk suture. Arterial blood pressure was continuously assessed from the arterial catheter, which was connected to a Statham strain-gauge pressure transducer. Data were digitized and recorded on HEM (version 3.3. Notocord; Croissy sur Seine, France), a computer software package for the recording of cardiovascular parameters, which derived HR on-line from the arterial pressure pulses.

Assessment of baroreflex. The first series of experiments (group 1) was undertaken in MAO-A/B KO mice (n = 3; age, 30.1 ± 0.8 wk; body weight, 24.1 ± 0.7 g) and WT mice (n = 3; age, 29.4 ± 0.4 wk; body weight, 31.5 ± 0.5 g). Baroreceptor function was examined using intravenous bolus injections of phenylephrine (PE; an α1-adrenergic agonist) and sodium nitroprusside (SNP; a nitric oxide donor) to test the responses of HR to transient hypertension and hypotension, respectively. All boluses were administered in a volume of 100 μl of 0.9% aqueous saline heparinized at 7 U/ml and injected over 7 s. Final drug concentrations were adjusted to account for the catheter dead volume of 50 μl. The following sequence of drug administrations was delivered, with 5-min periods between each bolus: saline → 5 μg/kg PE → 25 μg/kg PE → 70 μg/kg PE → saline → 5 μg/kg SNP → 15 μg/kg SNP → 30 μg/kg SNP → saline.

After this initial series of injections, anesthesia was discontinued, and the animal was allowed to recover breathing only room air. During this time, animals remained in the supine position and their movements were limited by soft paper restraints. This state, beginning 30 min after discontinuation of the anesthesia, is subsequently referred to as the “recovery” state.

During the recovery state, animals were administered the following sequence of pharmacological agents, again with 5-min delays between each bolus: 25 μg/kg PE → saline → 30 μg/kg SNP → saline. Animals were not manipulated in any way and would rest quietly during the drug administration, which occurred at a distance through the immobilized catheters. Subsequently, in the same animals, the effects of cholinergic or β-adrenergic blockade on the baroreceptor reflex were examined, respectively, using glycopyrrolate (a muscarinic antagonist without central effects) and propranolol (a nonselective β-adrenergic antagonist). These were administered under the following protocol using single doses: 75 μg/kg glycopyrrolate → 25 μg/kg PE → saline → 750 μg/kg propranolol → 30 μg/kg SNP → saline. At the end of this last sequence, animals were euthanized by a high level of halothane (5%) until respiration ceased, followed by cardiotomy.

To examine the effects of a pressor agent that, unlike PE, is independent of MAO catalysis, we repeated the above experiments using human angiotensin II (ANG II; Sigma). In a separate group of mice, the baroreceptor reflex was examined using a drug administration sequence identical to that described above except that ANG II (0.5, 1.0, and 4.0 μg/kg) replaced the low, medium, and high doses of PE. Experiments (group 2) were again performed in the anesthetized animals and 30 min after recovery in the MAO-A/B KO mice (n = 5; age, 24.7 ± 1.5 wk; body weight, 25.7 ± 1.0 g) and their WT counterparts (n = 7; age, 29.3 ± 2.5 wk; body weight, 30.7 ± 1.3 g).

Data analysis. Basal levels of HR, mean arterial pressure (MAP), systolic pressure (SBP), and diastolic pressure (DBP)
were examined 1) during anesthesia before intravenous drug administration and 2) 30 min after discontinuation of the anesthesia (before acute drug intervention). Differences between MAO-A/B KO (n = 8) and WT mice (n = 10) were examined using t-tests (two-tailed, P < 0.05).

To assess the baroreceptor gain, we plotted the peak HR as a function of the peak SBP before and after each drug administration. Analysis included the saline flushes that followed drug delivery and cleared the catheter of any residual drug. A regression of peak HR on peak SBP was calculated using the least-squares method. Because baroreceptor gain is reflected in the relative decrease/increase in HR in response to either hypertension/hypotension, our analysis for group 1 for the observed changes in HR versus SBP pooled the responses to PE and SNP of all animals under study. Likewise, in group 2, changes in HR versus SBP after ANG II were pooled with those after SNP. Analysis was performed separately for each genotype and separately for the anesthetized or recovery state. Significance between the regression lines was assessed by calculating the ratio of the regression sum of squares over groups by the residual sum of squares within groups (7). This approach, although powerful, does not provide information of whether the difference resides in the slope, the intercept, or both. A second approach was used to obtain this information. Regressions of peak HR on peak SBP were obtained as described above for every animal. These individual values of slope and intercept were analyzed by ANOVA with the factors genotype (KO or WT), drugs (PE or ANG II), and anesthesia (anesthetized or recovering).

The effects of cholinergic blockade on attenuating the baroreceptor response was examined by statistical comparison of the regression of peak HR on peak SBP during the response to PE or ANG II before and after administration of glycopyrrolate. Similarly, the effects of β-adrenergic blockade on attenuating the baroreceptor response were examined by statistical comparison of the regression of peak HR on peak SBP during the response to SNP before and after administration of propanolol. Measurements of the time delay between the occurrence of the peak of the SBP and the trough of the HR after administration of PE (25 μg/kg) or ANG II (1.0 μg/kg) were determined for each animal during recovery using the HEM software. The time delay between the SBP trough and the HR peak after administration of SNP (30 μg/kg) was similarly determined, with comparison between the genotypes made using t-tests (2-tailed, P < 0.05).

**RESULTS**

**Basal state.** We examined the “basal” autonomic state of the mice I) during anesthesia before intravenous drug administration and 2) 30 min after discontinuation of the anesthesia. During both conditions, MAO-A/B KO compared with WT mice showed significantly lower MAP, SBP, and DBP, with differences ranging from 10 to 15 mmHg for each parameter (Table 1). HR were lower in MAO-A/B KO mice compared with WT mice both during the recovery state as well as during anesthesia, although differences were not statistically significant.

MAP, SBP, and DBP increased 14–17 mmHg during recovery compared with the baseline 30 min earlier during anesthesia, with no significant difference in this effect between genotypes (Table 1). HR did not differ between anesthesia and the recovery state for both genotypes.

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<tr>
<th>Table 1. HR and blood pressure in MAO-A/B KO and WT mice</th>
<th>Recovery</th>
<th>Anesthesia</th>
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<tr>
<td>MAO-A/B KO</td>
<td>WT</td>
<td>MAO-A/B KO</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>80.8 ± 2.3*</td>
<td>93.2 ± 3.0</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>91.1 ± 2.0†</td>
<td>101.6 ± 3.0</td>
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<tr>
<td>DBP, mmHg</td>
<td>70.0 ± 2.8*</td>
<td>82.7 ± 2.4</td>
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<td>HR, beats/min</td>
<td>467 ± 18</td>
<td>497 ± 13</td>
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Values are means ± SE; n = 8 monoamine oxidase A and B (MAO-A/B, respectively) knockout (KO) mice and 10 wild-type (WT) mice. Shown are the basal mean arterial pressure (MAP), systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) in MAO-A/B KO and WT mice during anesthesia and 30 min after discontinuation of anesthesia (recovery). Data were pooled from all animals used in the phenylephrine (PE)-sodium nitroprusside (SNP) and angiotensin II (ANG II)-SNP experiments. *P < 0.005 and †P < 0.01, MAO-A/B KO vs. WT mice in the recovery or anesthetized states; ‡P < 0.005 and §P < 0.01, recovery vs. anesthetized states in mice of the same genotype.

**Baroreflex response to PE and SNP.** Responses of blood pressure to drug injections were well developed and were followed after a short delay by changes in HR (Fig. 1). In the recovery state, ANOVA of regression demonstrated a statistically significant dependence of HR on blood pressure for MAO-A/B KO and WT mice (Table 2 and Fig. 2A). For the statistical analysis in which data from all observations were pooled, slopes were more negative for MAO-A/B KO mice than WT mice, with significant differences between genotypes (Table 2). Results in anesthetized animals also showed a statistically significant dependence of HR on blood pressure for MAO-A/B KO and WT mice (Table 2). Comparison across genotypes demonstrated a significant difference, with a more negative slope in MAO-A/B KO mice than in WT mice.

When regression slopes and intercepts were calculated separately for every animal, the factors genotype (P < 0.001) and anesthesia (P < 0.00001) were found significant, but not the factor drugs (P = 0.9) in the case of slopes. In the case of intercepts, only the factor anesthesia was significant (P < 0.00001). When the same analysis was conducted separately for the anesthesia condition, it was found that the average of the slopes of MAO-A/B KO mice was significantly different from WT mice in the recovery condition (MAO-A/B KO: −4.14 ± 0.16; WT: −3.275899 ± 0.14, P = 0.003). The same analysis performed in animals under anesthesia indicated no significance between the genotypes (MAO-A/B KO: −1.93 ± 0.18; WT: −1.48 ± 0.17). These results confirmed the conclusions of the initial statistical analysis in which data from all observations were pooled and indicated, in addition, that those differences resided (for the animals recovering from anesthesia) in the slopes only.

There was no significant genotypic difference in the delay between the peak SBP and the occurrence of the lowest HR value after administration of PE (MAO-A/B KO: 2.44 ± 1.77 s; WT: 2.62 ± 1.21 s, P > 0.05). Similarly, there was no significant genotypic difference
in the delay between the lowest SBP and the occurrence of the highest HR value after administration of SNP (MAO-A/B KO: 4.16 ± 1.28 s; WT: 2.41 ± 0.78 s, P > 0.05).

**Baroreflex response to ANG II and SNP.** The results obtained with ANG II-SNP were similar to those obtained using PE-SNP. In the recovery state, ANOVA of regression in the pooled data analysis demonstrated a statistically significant dependence of HR on blood pressure for MAO-A/B KO and WT mice (Table 2 and Fig. 2B). Slopes were more negative for MAO-A/B KO mice than for WT mice, with significant differences between genotypes. Results in anesthetized animals also showed a statistically significant dependence of HR on blood pressure for MAO-A/B KO and WT mice (Table 2). Comparison across genotypes demonstrated a significant difference, with a more negative slope in MAO-A/B KO mice than in WT mice.

MAO-A/B KO mice showed a significantly shortened delay between the peak SBP and the occurrence of the lowest HR value after administration of ANG II (MAO-A/B KO: 7.58 ± 2.42 s; WT: 23.13 ± 3.81 s, P < 0.007). There was no significant genotypic difference in the delay between the lowest SBP and the occurrence of the highest HR value after administration of SNP (MAO-A/B KO: 4.16 ± 1.28 s; WT: 2.41 ± 0.78 s, P > 0.05).

**Effect of peripheral cholinergic blockade on the baroreflex response.** Muscarinic blockade with glycopyrrolate effectively blocked the cardiac effects of the baroreflex response to PE in both strains. Comparison of the regression slopes of pre- versus postglycopyrrolate treatment showed significant changes for MAO-A/B KO mice (F = 88.09, P < 0.00005) and WT mice (F = 102.16, P < 0.00005) (data not shown). Muscarinic blockade with glycopyrrolate also effectively blocked the occurrence of the baroreflex response to ANG II in MAO-A/B KO mice (F = 96.61, P < 0.00005) and WT mice (F = 136.64, P < 0.00005) (Fig. 3). The blockade increased the slope of the graph of HR on SBP from negative to near zero (Table 3). There was no significant genotypic difference in the effect of glycopyrrolate on the baroreflex to PE or ANG II.

**Effect of β-adrenergic blockade on the baroreflex response.** β-Adrenergic blockade with propranolol did not significantly alter the baroreflex response to SNP in either MAO-A/B KO mice (F = 1.38, P > 0.26) or WT mice (F = 0.02, P > 0.98), with minimal changes noted in the slope of the graph of HR on SBP (Fig. 4 and Table 3).

### Table 2. Baroreflex response to PE-SNP or ANG II-SNP

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<th>PE-SNP</th>
<th>ANG II-SNP</th>
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<tr>
<td></td>
<td>Slope</td>
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<td>Recovery</td>
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<tr>
<td>WT</td>
<td>-3.08</td>
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<tr>
<td>MAO-A/B KO</td>
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<tr>
<td>WT</td>
<td>-1.48</td>
<td>71.7</td>
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<tr>
<td>MAO-A/B KO</td>
<td>-2.17*</td>
<td>61.6</td>
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Values are means ± SE. Data were obtained during anesthesia and 30 min after discontinuation of anesthesia (recovery) for WT (n = 3) and MAO-A/B KO (n = 3) in response to PE (intravenous) or SNP (intravenous) as well as for WT (n = 7) and MAO-A/B KO mice (n = 5) in response to ANG II (intravenous) or SNP (intravenous). Shown are the slopes, F values of regression, and their significance for the plots of peak HR vs. peak SBP. Significance for the across-genotype comparison: *P < 0.002 (recovery) and †P < 0.05 (anesthesia); ‡P < 0.00005 (recovery) and §P < 0.0125 (anesthesia), ANG II-SNP.
The following findings were revealed by our study: First, the basal autonomic state of MAO-A/B KO mice was characterized by significantly lower blood pressures (MAP, SBP, and DBP) than those observed in WT mice; these findings were seen during anesthesia and to a greater extent after the 30-min postsurgical recovery period. Second, MAO-A/B KO mice compared with WT mice demonstrated a significantly increased gain of the baroreflex, with exaggerated responses to both PE-SNP as well as ANG II-SNP noted during the recovery state as well as during anesthesia. Third, the response after administration of ANG II between occurrence of the SBP peak and subsequent trough of the HR was significantly more rapid in the MAO-A/B KO mice than in the WT mice. Finally, cholinergic blockade with glycopyrrolate effectively blocked the cardiac effects of the baroreflex activation in both MAO-A/B KO and WT mice, with no significant difference between genotypes. The baroreflex recovered partially, minutes after glycopyrrolate treatment, but was not affected after this by propranolol treatment in both strains.

**DISCUSSION**

The following findings were revealed by our study: First, the basal autonomic state of MAO-A/B KO mice was characterized by significantly lower blood pressures (MAP, SBP, and DBP) than those observed in WT mice; these findings were seen during anesthesia and to a greater extent after the 30-min postsurgical recovery period. Second, MAO-A/B KO mice compared with WT mice demonstrated a significantly increased gain of the baroreflex, with exaggerated responses to both PE-SNP as well as ANG II-SNP noted during the recovery state as well as during anesthesia. Third, the response after administration of ANG II between occurrence of the SBP peak and subsequent trough of the HR was significantly more rapid in the MAO-A/B KO mice than in the WT mice. Finally, cholinergic blockade with glycopyrrolate effectively blocked the cardiac effects of the baroreflex activation in both MAO-A/B KO and WT mice, with no significant difference between genotypes. The baroreflex recovered partially, minutes after glycopyrrolate treatment, but was not affected after this by propranolol treatment in both strains.
A trend toward lower blood pressure in MAO-deficient mice has been previously reported by our group in mice singly deficient for either the MAO-A or MAO-B gene (21, 37). In addition, our findings are consistent with anecdotal reports of altered peripheral autonomic function and resting hypotension in patients with Norrie disease, an X-linked recessive disorder encompassing deletions in the genes for MAO-A and/or MAO-B (29, 41). The decreased blood pressure and trend toward bradycardia in the present MAO-A/B KO mice appears counterintuitive based on the known sympathetic effects of NE, 5-HT, and DA. One possible explanation for the lower basal blood pressure of MAO-A/B KO mice examined in the present study is the occurrence of an increased gain of the baroreflex. Baroreceptor input, even under normotensive conditions, tonically inhibits sympathetic effects on blood vessels and the heart. An increased gain of this reflex could serve to lower blood pressure in response to the excessive basal levels of pressor amines. Results of the present study are consistent with this claim, although we cannot rule out the possibility that lower basal blood pressure may be determined by an altered set point, acting possibly through serotonergic mechanisms felt to be independent of the baroreceptor reflex (31, 44).

Resetting of the baroreflex is well known in animal and human subjects with chronic hypertension. Typically, in these subjects, a desensitization of the reflex has been reported, with differences more apparent in younger than in older animals (30). Evidence suggests that changes in sensitivity may be a result rather than a cause of the increased pressures, in so far as baroreflex sensitivity modulate each other, identifying causation becomes difficult. A similar challenge is presented in interpreting the findings of the current study.

Genotypic differences in the baroreceptor response became increasingly apparent during postsurgical recovery from anesthesia, consistent with the known attenuation of the baroreflex by halothane (5). Although the possibility of lingering effects of halothane on the baroreflex 30 min after its discontinuation cannot be fully ruled out, at least in human subjects, a rapid return of the baroreflex sensitivity to normal within 5 min has been reported (9). The genotypic differences in the baroreflex are unlikely to be explained simply by a differential effect of postoperative stress in the KO and WT mice. We believe this to be the case, because the increase in the baroresponsiveness of KO compared with WT mice was observed not only after discontinuation of the halothane-nitrous oxide but also

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<th>Table 3. Baroreflex response after cholinergic or β-adrenergic blockade</th>
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<td>Phenyl</td>
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<td>Postpropanol</td>
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Values are means ± SE. Shown are the responses of WT (n = 10) and MAO-A/B KO mice (n = 8) to 1) PE or ANG II before or after administration of glycopyrrolate and 2) SNP before or after administration of propranolol.

Fig. 4. Effect of propranolol on attenuating the reflex tachycardia resulting after nitroprusside-induced hypotension. Depicted are the data for the response to nitroprusside of WT mice (A; n = 7) and MAO-A/B KO mice (B; n = 5) before (gray squares) and after (open circles) propranolol. Baseline starting values (means ± SD) before nitroprusside challenge are represented for the propranolol (solid squares) and postpropranolol (solid circles) treatment states.
during anesthesia, when effects of behavioral stress would presumably be absent. Furthermore, there was no significant genotypic difference in HR during anesthesia or recovery.

The time to reach a minimum HR in response to PE was nearly ninefold more rapid than that after administration of ANG II. Given the near instantaneous response (2–4 s of lag) to PE, no genotypic differences were detected in the delay between the peak SBP and HR trough. In the slower response to ANG II, however, it was apparent that MAO-A/B KO mice had not only a greater gain in the baroreflex but also a significantly more rapid response. The reason for the exaggerated delay in the baroreflex response using ANG II compared with PE remains a matter for speculation. PE effects immediate vasoconstriction at the vascular level through direct adrenergic agonism at α1-receptor sites, whereas ANG II, likely acting through AT1 receptor sites, secondarily results in vasoconstriction through an indirect adrenergic mechanism. Furthermore, ANG II may in fact increase HR through a β-adrenergic receptor mechanism. Such increases do not appear to play an important role in mice in mediating pressor responses to ANG II, because these are largely buffered by a concomitant decrease in HR mediated by the baroreflex (6). However, they might counteract, hence delay, the time to reach minimum HR after the initial pressor effect of ANG II.

Changes in the baroreflex gain might occur at several levels: the central nervous system, the peripheral nervous system, or else the associated vasculature, the sinoatrial node, and the heart. It is possible that MAO-A/B KO mice, due to their chronically elevated levels of vasoactive amines, compensate by altering the sensitivity of receptors and signal transduction pathways important in maintaining vascular tone. Although such mechanisms were not directly measured in the current study, our group has previously reported presence of a downregulation in these animals of postsynaptic 5-HT1A, 5-HT2A, and 5-HT2C receptors in the central nervous system (8, 40), which, as described below, may play a role in regulation of the baroreflex.

Numerous studies have shown that several neuroactive substances, in particular 5-HT, NE, and DA, are implicated in the reflex regulation of arterial blood pressure at the level of the nucleus tractus solitarius (NTS). Experiments using direct injection of agonists and antagonists into the NTS have suggested dose-dependent effects on blood pressure mediated via α2-adrenergic receptors (42, 49) and serotoninergic 5-HT1A, 5-HT2, and 5-HT3 receptors (2, 4, 12, 45) and possibly dopaminergic D2 receptors (26). Of relevance to the issue of changes in baroreflex gain is the observation that when NE is increased acutely (15) or chronically (14, 48) after peripheral administration, or if NE is directly administered into the NTS (11), the result is an increased gain of the baroreceptor reflex. Clonidine and guanfacine (both central α2-adrenergic agonists) increase the sensitivity of the baroreflex (16), whereas prazosin (an α-adrenergic blocker) diminishes it (36). The dopamine D2/D3 agonist quinpirole after intravenous administration also results in profound increases in the baroreflex gain (47). That 5-HT does not directly affect baroreflex gain is suggested by reports that neither acute nor chronic administration of fluoxetine (a selective 5-HT reuptake inhibitor) nor administration of ketanserin (a 5-HT2 receptor antagonist) nor intracerebral injection of 5-HT change the sensitivity of the reflex arc (1, 13, 31, 33, 43). The mechanism of action of PEA in effecting autonomic changes remains unknown, although an inhibition of the reuptake, as well as effects on direct release of DA, NE, and possibly 5-HT, has been suggested (3, 24, 34).

Another means of altering baroreceptor sensitivity is through changes in vagal tone (10). It has been suggested that activation of presynaptic 5-HT receptors may increase neurotransmitter release from vagal afferent neurons and thus may modulate the cardiac reflex responses through mechanisms linked to increases in vagal tone (19, 38). Within the NTS, there have been reported D2 receptors both pre- and postsynaptic to vagal terminals (26), and DA has also been shown to facilitate baroreceptor discharges of the carotid sinus in vitro (50). In our study, muscarinic peripheral cholinergic blockade with glycopyrrolate effectively blocked the occurrence of the baroreflex of the MAO-A/B KO mice, suggesting an intact vagal component to the reflex response at the level of the heart. However, because of the possibility that the dose (75 μg/kg) chosen in our study may have allowed for the occurrence of a maximal response (“ceiling effect”), absence of a genotypic difference in response to glycopyrrolate does not rule out alterations within the cholinergic system of MAO-A/B KO mice.

β-Adrenergic blockade with propranolol demonstrated little effect on the baroreflex of both MAO-A/B KO and WT mice. Our experiment was designed to examine the effect of β-adrenergic blockade after cholinergic blockade. Because of the robust response to glycopyrrolate that was equivalent in both genotypes, the subsequent baseline HR was high, making it likely that presence of a ceiling effect may have masked the attenuation in the HR response expected to occur after administration of propranolol. The doses employed were equivalent to those used by other investigators (6, 46), although the possibility must be considered that higher doses in our mouse strain (129 Sv) might have elicited a response.

MAO-A/B KO mice have been shown to demonstrate elevated brain levels of catecholamines, 5-HT and PEA, and presumably similar findings are to be found in blood. The increase in these animals of the baroreceptor response should be contrasted with the decreased baroreceptor response noted in patients with pheochromocytoma. In these patients, desensitization of the adrenergic system associated with chronic catecholamine excess has been considered to be one of the determinants for the decreases in baroreceptor gain. In addition, patients with a NE transporter deficiency show elevated levels of plasma NE as well as a decreased baroreceptor sensitivity (39). These findings suggest that increases in the baroreflex noted in our

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MAO-A/B KO mice may not relate directly to increases noted in these animals in levels of NE. Alternatively, decreases in baroreceptor gain noted in the above-mentioned clinical disorders may relate to factors outside of the noradrenergic system. Future studies will be needed to evaluate which of the neurotransmitters that show elevated tissue concentrations in MAO-A/B KO mice is responsible for the observed changes in HR and blood pressure regulation and whether such effects take place within the central nervous system or in the periphery.

In conclusion, our results demonstrate a decreased basal blood pressure and an increased baroreceptor gain in MAO-A/B KO mice compared with their WT counterparts. Such a physiological overcompensation that resets the blood pressure of MAO-A/B KO mice to a lower basal level may function to attenuate the physiological overcompensation in MAO-A/B KO mice compared with their WT counterparts. Such a physiological overcompensation may be needed to evaluate which of the neurotransmitters may be involved in the regulation of blood pressure and baroreceptor function.

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REFERENCES


