Attenuated Aortic Vasodilation and Sympathetic Prejunctional Facilitation in Epinephrine-Deficient Mice: Selective Impairment of $\beta$2-Adrenoceptor Responses

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ABSTRACT

It has been suggested that there is a link between epinephrine synthesis and the development of $\beta$2-adrenoceptor-mediated effects, but it remains to be determined whether this development is triggered by epinephrine. The aim of this study was to characterize $\beta$2-adrenoceptor-mediated relaxation and facilitation of norepinephrine release in the aorta of phenylethanolamine-N-methyltransferase–knockout (Pnmt-KO) mice. Catecholamines were quantified by reverse-phase high-performance liquid chromatography–electrochemical detection. Aortic rings were mounted in a myograph to determine concentration-response curves to selective $\beta_1$ or $\beta_2$-adrenoceptor agonists in the absence or presence of selective $\beta_1$ or $\beta_2$-adrenoceptor antagonists. Aortic rings were also preincubated with $[^3]$H]norepinephrine to measure tritium overflow elicited by electrical stimulation in the presence of increasing concentrations of non-selective $\beta$- or selective $\beta_2$-adrenoceptor agonists. $\beta_2$-Adrenoceptor protein density was evaluated by Western blotting and $\beta_2$-adrenoceptor localization by immunohistochemistry. Epinephrine is absent in Pnmt-KO mice. The potency and the maximal effect of the $\beta_2$-adrenoceptor agonist terbutaline were lower in Pnmt-KO than in wild-type (WT) mice. The selective $\beta_2$-adrenoceptor antagonist ICI 118,551 [(±)-erythro-(S',S')]-1-[2,3-(dihydro-7-methyl-1H-inden-4-yl)oxy]-3-[(1-methyl-1H-inden-4-yl)oxy]-2-butanol hydrochloride] antagonized the relaxation caused by terbutaline in WT but not in Pnmt-KO mice. Isoproterenol and terbutaline induced concentration-dependent increases in tritium overflow in WT mice only. $\beta_2$-Adrenoceptor protein density was decreased in membrane aorta homogenates of Pnmt-KO mice, and this finding was supported by immunofluorescence confocal microscopy. In conclusion, epinephrine is crucial for $\beta_2$-adrenoceptor-mediated vasodilation and facilitation of norepinephrine release. In the absence of epinephrine, $\beta_2$-adrenoceptor protein density was decreased in aorta cell membranes, thus potentially hindering its functional activity.

Introduction

In contrast to adults, human neonates have plasma concentrations of norepinephrine higher than those of epinephrine (Elliott et al., 1980). Accordingly, in the newborn canine adrenal medulla, norepinephrine is the predominant amine, whereas in adults, epinephrine predominates (Paiva et al., 1994). On the other hand, Gootman et al. (1981) showed that $\beta$-adrenoceptor–mediated vascular relaxation is immature in neonatal swine. Thies et al. (1986) also found a diminished response to the nonselective $\beta$-adrenoceptor agonist isoproterenol in human neonatal lymphocytes compared with adults. Paiva et al. (1994) also observed a parallel time course between the postnatal increase in the epinephrine content in the adrenal medulla and the development of $\beta_2$-adrenoceptor–mediated smooth muscle relaxation and facilitation of norepinephrine release by sympathetic nerve stimulation in the canine

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ACKNOWLEDGMENTS: CGP 20712 A, 1-[2-[(3-carbamoyl)-4-hydroxyphenoxo]ethyl]amino]-3-[4-(1-methyl-4-trifluoromethyl-2-imidazolyl)phenoxo]-2-propanol dihydrochlorid; Cl 316243, 5-[2R,3S]-2-[3-chlorophenyl]-3-hydroxyethylaminopropyl]-1,3-benzodioxole-2,2-dicarboxylic acid; ERmax, maximal effect; ICI 118,551, (±)-erythro-(S',S')-1-[2,3-(dihydro-7-methyl-1H-inden-4-yl)oxy]-3-[(1-methyl-1H-inden-4-yl)oxy]-2-butanol hydrochloride; PBS, phosphate-buffered saline; pEC50, negative logarithm of the molar concentration causing 50% of ERmax; Pnmt, phenylethanolamine-N-methyltransferase; Pnmt-KO, phenylethanolamine-N-methyltransferase-knockout; RIPA, radioimmunoprecipitation assay; S1, evoked tritium overflow; TBS, Tris-buffered saline; WT, wild-type.
saphenous vein. In addition, epinephrine is the only biogenic catecholamine that has affinity for \( \beta_2 \)-adrenoceptors at physiologically relevant concentrations, whereas both epinephrine and norepinephrine are potent \( \beta_1 \)-adrenoceptor agonists (Lands et al., 1967a,b).

Thus, it was suggested that there is a link between epinephrine and the development of \( \beta_2 \)-adrenoceptor-mediated effects (Paiva et al., 1994; Guimarães and Moura, 2001). However, it remains to be proved whether the development of \( \beta_2 \)-adrenoceptor-mediated effects is triggered by epinephrine or by other causes that might simultaneously induce epinephrine production and the functional development of the responses to \( \beta_2 \)-adrenoceptor stimulation.

It has been difficult to decipher the role of epinephrine with the commonly used adrenal medullectomy because this procedure can damage the adrenal cortex, altering the release of corticosteroids, and it also removes the release of other adrenal amines and peptides, such as norepinephrine, chromatogamin A, catestatin, and neuropeptide Y (Harrison and Seaton, 1966). An alternative approach is the use of phenylethanolamine-N-methyltransferase (Pnmt) inhibitors to block epinephrine synthesis in vivo (Bondinell et al., 1983), but most of them also inhibit monoamine oxidase (Mefford et al., 1981) and \( \alpha \)-adrenoceptors (Feder et al., 1989). These drawbacks for the elucidation of the specific role of epinephrine in the development of \( \beta_2 \)-adrenoceptor subtype are avoided by doing experiments in an epinephrine-deficient animal model generated by knocking out the Pnmt gene (Ebert et al., 2004, 2008; Sharara-Chami et al., 2010). Therefore, the aim of this study was to characterize the role of epinephrine on \( \beta \)-adrenoceptor-mediated aorta relaxation and facilitation of norepinephrine release from sympathetic nerve endings using Pnmt-knockout (Pnmt-KO) and wild-type (WT) mice.

**Materials and Methods**

**Animals.** All animal care and experimental protocols were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the US National Institutes of Health and were approved by the Institute of Biomedical Sciences Abel Salazar, University of Porto, Portugal, ethics committee (project no. 020/2012). The Pnmt-KO mice (Pnmt \(-/-\) ) were produced by disruption of Pnmt locus by insertion of Cre-recombinase in exon 1 (Ebert et al., 2004). A couple of Pnmt-KO mice were kindly provided by S.N.E., and animals were bred in our conventional vivarium. All animal care and experimental protocols were carried out in accordance with the Guide for the Care and Use of Laboratory Animals (Paiva et al., 1994; Guimarães and Moura, 2001).

**Protein Quantification.** Three mice aortas in each sample were homogenized in radioimmunoprecipitation assay (RIPA) buffer (65 mM Tris-HCl, pH 7.4; 154 mM NaCl; 10 mM Na2EDTA; 1% IGEPAEL; 6 mM sodium deoxycholate; 1 \( \mu \)M phenylmethylsulfonyl fluoride; 1 \( \mu \)M NaF; 1 \( \mu \)M Na3VO4; 5 \( \mu \)g/ml leupeptin; 5 \( \mu \)g/ml aprotinin; 5 \( \mu \)g/ml pepstatin) and ultracentrifuged (4°C, 100,000 g, 15 minutes). The pellets were
suspended in RIPA buffer, sonicated, and then collected for protein quantification (membrane fraction of aorta). Protein concentration was determined using a protein assay kit (Bio-Rad Laboratories, Hercules, CA) using bovine serum albumin as standard.

**Western Blot.** Membrane fractions of aorta homogenates were diluted with RIPA buffer and then with 6:1 sample buffer (0.35 M Tris-HCl, pH 6.8; 4% SDS, 30% glycerol; 9.3% dithiothreitol; 0.01% bromophenol blue) and boiled at 95°C for 5 minutes. Samples (40 μg) were separated by SDS-PAGE with 10% polyacrylamide gel and then transferred onto nitrocellulose membranes (Bio-Rad Laboratories). Blots were blocked for 1 hour with 5% nonfat dry milk in Tris-buffered saline (TBS), incubated with a rabbit polyclonal anti-β2-adrenoceptor antibody (1:125; Santa Cruz Biotechnology, Dallas, TX) in 2.5% nonfat dry milk in TBS/Tween 20, overnight, at 4°C, then washed and incubated with an IRDye 800 goat anti-rabbit secondary antibody (1:10,000; Rockland, Gilbertsville, PA) for 1 hour at room temperature. Finally, they were washed and incubated with an Alexa Fluor 680 goat anti-mouse secondary antibody (1:10,000; Rockland, Gilbertsville, PA) for 1 hour at room temperature. The membranes were then washed and imaged by scanning at both 700 nm (for detection of western blot protein standard molecular weight; Precision Plus Protein Standard, Bio-Rad or NZY Color Protein Marker II; NZYtech, Lisbon, Portugal) and 800 nm (for IRDye 800 goat anti-rabbit secondary antibody detection) by fluorescence detection method, with an Odyssey Infrared Imaging System (LI-COR Biosciences, Lincoln, NE). Following this, the immunoblots were washed with mild stripping solution (to remove previous primary and secondary antibodies; 1% tween 20, 0.1% SDS, 200 mM glycine, pH 2.2), blocked overnight with 5% non-fat dry milk in TBS, and incubated with a mouse anti-β-actin antibody (1:10,000; Santa Cruz Biotechnology) in 2.5% nonfat dry milk in TBS/Tween 20 for 1 hour at room temperature. Finally, they were washed and incubated with an Alexa Fluor 680 goat anti-mouse secondary antibody (1:10,000; Invitrogen, Eugene, OR) for 1 hour, washed again, and imaged by scanning at 700 nm (for Western blot protein standard and Alexa Fluor 680 goat anti-mouse secondary antibody detection) (Moreira-Rodrigues et al., 2010).

**Immunohistochemistry.** Aortas were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) for 6 hours. Fixed tissue was washed and cryoprotected overnight with a solution containing 20% anhydrous glycerol (dissolved in 0.1 M phosphate buffer) and frozen. Then the tissue was sectioned (16 μm), and the tissue sections were incubated with a blocking buffer solution (10% fetal bovine serum, 1% bovine serum albumin, 0.3% Triton X-100, 0.025% NaN3 in PBS) for 60 minutes with constant stirring. Subsequently, samples were incubated overnight (4°C) with a rabbit polyclonal anti-β2-adrenoceptor antibody (1:50; Santa Cruz Biotechnology) diluted in dilution buffer solution (5% fetal bovine serum, 0.5% bovine serum albumin, 0.3% Triton X-100, 0.025% NaN3 in PBS; Tissue sections were then washed and incubated for 90 minutes (at room temperature in the dark) with an anti-rabbit secondary antibody Alexa Fluor 488 (1:1500; Molecular Probes, Eugene, OR) diluted in dilution buffer solution. Negative controls were performed using the secondary antibody alone. Finally, samples were washed and mounted on optical-quality glass slides. Vectashield with DAPI (1:1500; Molecular Probes, Eugene, OR) diluted in dilution buffer (10–10,000 nM) was used as mounting media (Vector Laboratories, Burlingame, CA). Observations and analyses were performed with a laser-scanning confocal microscope (FV1000; Olympus Fluoview, Tokyo, Japan) (Carneiro et al., 2014).

**Drugs.** Isoflurane 100%, Isoflurane, was obtained from Abbott laboratories (Queenborough, UK). (-)-Epinephrine (+)-bitartrate salt, (-)-norepinephrine (+)-bitartrate salt monohydrate, isopropenol hydrochloride, phenolamine hydrochloride, dobutamine hydrochloride, terbutaline hemisulfate salt, CGP 20712A 12-methanesulfonate salt, ICI 118,551 hydrochloride, Phentolamine hydrochloride, and CL 316243 were purchased from Sigma-Aldrich (St. Louis, MO). Cocaine hydrochloride was obtained from Uquipa (Lisbon, Portugal), and (−)-7-P3Hinorepinephrine (specific activity 14.9 Ci mmol⁻¹, 1 μCi ml⁻¹) was purchased from PerkinElmer (Waltham, MA).

**Statistical Analysis.** Concentration-response curves were adjusted to data by nonlinear regression analysis using GraphPad Prism statistics software package (GraphPad Software Inc., La Jolla, CA). The E₁₀₀ (maximal effect), EC₅₀, and pEC₅₀ (negative logarithm of the molar concentration causing 50% of E_max) were estimated for each curve. Results are arithmetic means ± S.E.M. of values for the indicated number of determinations. Unless stated otherwise, statistical analysis was done by the two-tailed t test. Statistical analysis of the Sn S1⁻ ratios was done by the Mann-Whitney test. P < 0.05 was assumed to denote a significant difference.

**Results.**

**Epinephrine and Norepinephrine Content in Adrenal Glands.** Epinephrine content was baseline levels in the adrenal glands and plasma of Pnmt-KO mice when compared with WT mice, and it was below the detection level in the aorta samples of both groups (Table 1). In contrast, the norepinephrine content of the adrenal gland was higher in Pnmt-KO than in WT mice; however, norepinephrine levels in plasma and aorta were similar between the groups (Table 1).

**Postjunctional β-Adrenoceptor-Mediated Responses in Aorta Rings.** In aortas previously contracted with phenyl-epinephrine, terbutaline (Fig. 1A) and dobutamine (Fig. 1B) caused concentration-dependent relaxations in both Pnmt-KO and WT mice. The potency (EC₅₀, 29.00 ± 6.10 versus 5.20 ± 2.80 μM; n = 5–6) and maximal response (7.0 ± 2.4 versus 17.7 ± 3.3 mN·mg⁻¹; n = 5–6) to terbutaline were lower in Pnmt-KO than in WT mice (Fig. 1A). To further confirm the results obtained, we performed the concentration-response curve of terbutaline in the presence of a β2-adrenoceptor antagonist. ICI 118,551 antagonized the effect of terbutaline in WT (Fig. 2A) but not in Pnmt-KO mice (Fig. 2B). The potency (EC₅₀, 0.18 ± 0.05 versus 0.22 ± 0.07 μM; n = 6–8) and maximum response (13.8 ± 3.0 versus 20.0 ± 4.2 mN·mg⁻¹; n = 6–8) to dobutamine were not significantly different (P > 0.05) between experimental groups (Fig. 1B). CGP 20712A antagonized the effect of dobutamine in both WT (Fig. 2C) and Pnmt-KO (Fig. 2D) mice; no differences were observed in pA2 values between the two groups (8.1 ± 0.2 versus 8.0 ± 0.5, respectively). The β2-adrenoceptor agonist CL 316243 (10–10,000 nM) failed to cause vasorelaxation of mice aortas (data not shown).

**Presynaptic β-Adrenoceptor-Mediated Responses in Aorta Rings.** No differences were found between Pnmt-KO and WT mice in basal conditions: tissue accumulation of tritium (430.50 ± 44.95 versus 404.19 ± 26.22, μg g⁻¹; n = 7 to 8), fractional rate of loss (4.26 × 10⁻³ ± 0.50 × 10⁻³ versus 3.89 × 10⁻³ ± 0.13 × 10⁻³, min⁻¹; n = 7–8) or evoked

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<th>TABLE 1</th>
<th>Epinephrine and norepinephrine content in the adrenal gland, aorta, and plasma in Pnmt-KO and WT mice</th>
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<td>Adrenal gland</td>
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*Significantly different from correspondent values in WT mouse (P < 0.05).
overflow of tritium induced by the control stimulation (S1, 0.43 ± 0.04 versus 0.32 ± 0.04, % of tritium content; n = 7–8). In the aorta of WT mice, both isoproterenol (Fig. 3A) and terbutaline (Fig. 3B) increased the overflow of tritium elicited by electrical stimulation in a concentration-dependent manner, with a pEC50 of 9.36 ± 0.55 (n = 7–8) and a maximal effect of 167.3 ± 37.5% (n = 7–8) for isoproterenol and a pEC50 of 7.65 ± 0.94 (n = 6) and a maximal effect of 173.3 ± 31.3% (n = 6) for terbutaline. This facilitatory effect of isoproterenol and terbutaline on norepinephrine release was absent in Pnmt-KO mice (Fig. 3). In Pnmt-KO mice, curves failed to converge to a sigmoidal equation (Fig. 3).

Quantification and Visualization of β2-Adrenoceptors in Aorta. The protein density of β2-adrenoceptors (~61 kDa) in membrane aorta homogenates was significantly (P < 0.05) lower in Pnmt-KO compared with WT mice (Fig. 4). This finding was also evidenced by immunofluorescence confocal microscopy (Fig. 5). In WT mouse aorta rings, the β2-adrenoceptor immunofluorescent labeling is concentrated mainly in the media layer (where smooth muscle cells are the most abundant cell type) but also in the endothelium. β2-Adrenoceptor immunoreactivity was decreased in aorta rings from Pnmt-KO mice (341 ± 37 versus 190 ± 10, arbitrary fluorescence intensity units; n = 3; Fig. 5). The residual fluorescence labeling observed in the absence of the primary antibody (negative control) is due to autofluorescent elastic fibers (Fig. 5).

Discussion

Our results show that epinephrine-deficient mice do not develop aorta β2-adrenoceptor-mediated responses both at a postjunctional level (β2-adrenoceptor-dependent relaxation) and at a prejunctional level (facilitation of norepinephrine release from sympathetic nerve endings), strengthening the hypothesis that epinephrine is critical for the functional development of β2-adrenoceptor-mediated responses.

The Pnmt-KO mouse is an epinephrine-deficient mouse model generated by knocking out the Pnmt gene (Ebert et al., 2004, 2008; Sharara-Chami et al., 2010). The absence of Pnmt mRNA expression alters epinephrine biosynthesis. Accordingly, we found only baseline levels of epinephrine in the adrenal medulla and plasma of Pnmt-KO mice. Our results agree with those of Sun et al. (2008) showing that epinephrine is absent from the adrenal gland and plasma of Pnmt-KO mice, whereas the norepinephrine content of the adrenal glands is significantly increased. This result might be due to an upstream accumulation of norepinephrine that would normally be methylated to epinephrine.
In conduit arteries (thoracic aorta and carotid artery) of mice, both \(\beta_1\)- and \(\beta_2\)-adrenoceptors mediate smooth muscle relaxation (Chruscinski et al., 1999, 2001; Rohrer et al., 1999). We chose the mouse aorta because 1) it contains postjunctional relaxing \(\beta_2\)-adrenoceptors, 2) it is easy to mount in the myograph to evaluate \(\beta_2\)-adrenoceptor-mediated relaxation, and 3) it has enough nerve terminals to evaluate prejunctional \(\beta\)-adrenoceptor-mediated responses.

Our results showed that dobutamine (\(\beta_1\)-adrenoceptor agonist) and terbutaline (\(\beta_2\)-adrenoceptor agonist) caused concentration-dependent relaxation of aorta rings precontracted with phenylephrine in both WT and Pnmt-KO mice. In WT mice, terbutaline caused aorta relaxation at \(\beta_2\)-selective concentrations, whereas in Pnmt-KO mice, very high (non-selective) concentrations of terbutaline were required to produce relaxation. Actually, the potency and the maximal response to \(\beta_2\)-adrenoceptor stimulation by terbutaline were lower in Pnmt-KO mice than in WT mice. In addition, the \(\beta_2\)-adrenoceptor antagonist ICI 118,551 failed to modify the relaxing effect of terbutaline in Pnmt-KO mice, contrary to that observed in WT mice. Overall, these results suggest a loss of \(\beta_2\)-adrenoceptor–mediated relaxation in the aorta of Pnmt-KO compared with WT mice. In agreement with these results is the fact that after adrenalectomy impaired formation of high-affinity myocardial \(\beta\)-adrenoceptor complexes was observed (Davies et al., 1981), and after adrenal demedullation, a decrease in \(\beta\)-adrenoceptor cardiac density was observed in rats (Tumer et al., 1990).

On the other hand, no differences were observed in \(\beta_1\)-adrenoceptor–mediated aorta relaxation to dobutamine between the two groups since the potency and the maximal response to dobutamine were similar. In addition, CGP 20712 A (a \(\beta_1\)-adrenoceptor antagonist) antagonized the effect of dobutamine with similar potency in both groups. Thus, our results suggest that both \(\beta_1\)- and \(\beta_2\)-adrenoceptors mediate aortic relaxation in WT mice but that only \(\beta_1\)-adrenoceptors are operative in the absence of epinephrine, as happens in Pnmt-KO mice. This might explain why Pnmt-KO mice show normal basal blood pressure values, but blood pressure dramatically increases during treadmill exercise compared with WT mice (Bao et al., 2007). In Pnmt-KO mice at rest, \(\beta_1\)-adrenoceptors may be sufficient to produce vasodilation and control blood pressure. One can speculate that under stressful conditions, this mechanism might not be enough because \(\beta_2\)-adrenoceptor–mediated vasodilator response is blunted, and then blood pressure rises. In agreement with this hypothesis is the fact that \(\beta_2\)-adrenoceptor KO mice also have a normal basal resting blood pressure and become hypertensive during exercise (Chruscinski et al., 1999).

Conversely, we did not observe \(\beta_2\)-adrenoceptor–mediated vasorelaxation induced by CL 316243 in WT mice, which is in agreement with the fact that in aortas from \(\beta_1\)\(\beta_2\)-adrenoceptor double-knockout mice, the \(\beta\)-agonist isoproterenol does not cause any relaxation (Chruscinski et al., 2001). These findings suggest that \(\beta_2\)-adrenoceptors do not contribute to the adrenoceptor-mediated vasodilation in mice aorta.

In the aorta of WT mice, the facilitatory effect of isoproterenol on norepinephrine release was similar to that found by other authors in mice spleen and atria (Trendelenburg et al., 2000). To our knowledge, data from this study show for the first time that prejunctional \(\beta\)-adrenoceptors, in particular of the \(\beta_2\) subtype, are present in the mouse aorta. In addition, we report here the absence of \(\beta_2\)-adrenoceptor–mediated effect on norepinephrine release in Pnmt-KO mice. In agreement with

![Fig. 3.](image-url)
our results, it had been previously shown that carteolol (nonselective β-adrenoceptor antagonist) does not inhibit [3H]norepinephrine release in a concentration-dependent manner induced by nerve stimulation from pulmonary arteries in guinea pigs subjected to adrenalectomy or adrenomedullation, contrary to sham-operated animals (Misu et al., 1989). On the other hand, putative prejunctional β1-adrenoceptor–mediated effects were not evaluated because in all tissues tested so far, presynaptic β1-adrenoceptors are of the β2 subtype (Kahan and Hjemdahl, 1987; Molderings et al., 1988; Trendelenburg et al., 2000; Todorov et al., 2001). Although compensatory changes may occur, the presence of presynaptic β1- or β2-adrenoceptors seems rather unlikely because we did not observe any β-adrenoceptor induced effect in the release of norepinephrine using isoproterenol (a nonselective β-agonist).

The lower β2-adrenoceptor protein density observed in aorta of Pnmt-KO mice may be correlated with decreased coupling to stimulatory G protein and lower cAMP levels, which may justify impairments of vasorelaxation and of norepinephrine release facilitation on β2-adrenoceptor activation (Guimaraes and Moura, 2001). Signaling complexes are located in specialized membrane compartments and are enriched in components of the signal transduction cascade, including G proteins and effector molecules. Thus, these complexes may act as a scaffold promoting the interaction of specific signaling proteins (Galbiati et al., 2001). In Pnmt-KO mice, one possible explanation for the presence of reduced β2-adrenoceptor protein density (41.7 ± 8.3%) and the absence of β2-adrenoceptor function could be a differential association of β2-adrenoceptors to the signaling complexes in the membrane, which could alter the spatial relationship of β2-adrenoceptors with the associated signaling proteins, thereby leading to an abrupt decrease in activation of the protein effectors.

The highest β2-adrenoceptor immunofluorescent labeling found in aorta rings was in the media layer, where smooth muscle cells are most abundant. Immunolocalization studies also revealed the presence of β2-adrenoceptors in the endothelial lining of mice aortic rings. Given that β2-adrenoceptors located in vascular endothelial cells may regulate NO release (Conti et al., 2013), one cannot exclude that relaxation of mouse aortic rings produced by β2-adrenoceptor agonists involves this mechanism.

The results from this study agree with the fact that the time course of postnatal increase in the epinephrine content of the adrenal medulla positively correlates with the development of β2-adrenoceptor–mediated effects, as previously described in the canine saphenous vein (Paiva et al., 1994). This view is supported by the diminished response of β-adrenoceptors in lymphocytes from human newborns compared with adults (Thies et al., 1986), and lymphocytes almost exclusively express adrenoceptors of the β2 subtype (Sanders, 2012). Interestingly, it was found that treatment of newborn rats with β2-adrenoceptor agonists caused a surprising sensitization of β-adrenoceptors instead of evoking desensitization (Kudlacz and Slotkin, 1990). Also, a single exposure of newborn rats to terbutaline induces a strong sensitization of the β2-adrenoceptor–mediated stimulation of adenyl cyclase activity in the brainstem and cerebellum (Slotkin and Seidler, 2006). Epinephrine is the only biogenic catecholamine that has a good affinity for β2-adrenoceptors (Lands et al., 1967a,b), and it is conceivable that it can act similarly, causing sensitization of β2-adrenoceptors. Because Pnmt-KO mice were never exposed to epinephrine, β2-adrenoceptor function does not appear to develop efficiently.

One limitation of our experimental design is the limited selectivity of terbutaline and dobutamine at β2- and β1-adrenoceptors, respectively (Ruffolo et al., 1984; Young et al., 2002; Baker, 2005). This prevents us from using higher nonselective concentrations of those agonists because the mouse aorta relaxes to activation of both subtypes of β-adrenoceptors.

In conclusion, epinephrine is crucial for the development of β2-adrenoceptors and associated β2-adrenoceptor–mediated aorta vasodilation and facilitation of norepinephrine release by sympathetic nerves. In the absence of epinephrine, β2-adrenoceptor protein density was decreased in aorta cell membranes, thus potentially hindering its functional activity.

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Authorship Contributions

Participated in research design: Moreira-Rodrigues, Moura.
Conducted experiments: Moreira-Rodrigues, Graça, Ferreira, Afonso, Serrão, Morato, Ferreirinha, Moura.
Performed data analysis: Moreira-Rodrigues, Graça, Ferreira, Ferreirinha, Correia-de-Sá, Moura.
Wrote or contributed to the writing of the manuscript: Moreira-Rodrigues, Graça, Morato, Correia-de-Sá, Ebert, Moura.

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