Attenuated Aortic Vasodilation and Sympathetic Prejunctional Facilitation in Epinephrine-Deficient Mice: Selective Impairment of $\beta_2$-Adrenoceptor Responses

Mónica Moreira-Rodrigues, Ana L. Graça, Marlene Ferreira, Joana Afonso, Paula Serrão, Manuela Morato, Fátima Ferreirinha, Paulo Correia-de-Sá, Steven N. Ebert, and Daniel Moura

Laboratory of General Physiology (M.M.-R.) and Laboratory of Pharmacology and Neurobiology (F.F., P.C.), Unit for Multidisciplinary Investigation in Biomedicine, Institute of Biomedical Sciences Abel Salazar, University of Porto; Department of Pharmacology and Therapeutics, Faculty of Medicine, University of Porto (A.L.G., M.F., J.A., P.S., D.M.); Neuropharmacology, Institute of Molecular and Cellular Biology, University of Porto (M.M., D.M.); Center for Drug Discovery and Innovative Medicines, University of Porto (M.M.-R., A.L.G., M.F., J.A., P.S., M.M., F.F., P.C., D.M.); Laboratory of Pharmacology, Department of Drug Sciences, Faculty of Pharmacy, University of Porto and Rede de Química e Tecnologia (REQUIMTE), Porto, Portugal (M.M.); and Burnett School of Biomedical Sciences, College of Medicine, University of Central Florida, Orlando, Florida (S.N.E.).

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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 knockouts, thus potentially hindering its prejunctional facilitation in epinephrine-deficient mice.

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 isoprote- terenol 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 adrenal medulla. The work performed at the Advanced Microscopy and Imaging Center of Unit for Multidisciplinary Investigation in Biomedicine was supported by Fundação para a Ciência e a Tecnologia [Grants REEQ/1284/SAU/2005, PEst-OE/SAU/ UI0215/2011, and Pest-OE/SAU/UI0215/2014].

Abbreviations: CGP 20712 A, 1-[2-[(3-carbamoyl)-4-hydroxyphenoxyl]ethyl]aminol]-3-[4-(1-methyl-4-trifluoromethyl-2-imidazolyl)phenoxyl]-2-propanol dibydrochloride; Cl 316243, 5-[(2R)-2-chlorophenoxyl]-2-hydroxyethylamino]propyl]-1,3-benzodioxole-2,2-dicarboxylic acid; EP 410, maximal effect; ICI 118,551, (6S,7S)-[2S]-[(-)-erythro-(S',S')]-1-[2,3-(4-dihydro-7-methyl-1H-inden-4-yl)oxy]-3-[1-methylaminol]-2-butanoldihydrochloride; PBS, phosphate-buffered saline; PE50, negative logarithm of the molar concentration causing 50% of EP 410; Prnt, phenylethanolamine-$N$-methyltransferase; Prnt-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 \(\beta_1\)-adrenoceptors 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 \(\beta_1\)-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, chromogranin 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 \(\alpha\)-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. The breeding diet 4RF25/I; Ultragene, Porto, Portugal) and housed with 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. The breeding diet 4RF25/I; Ultragene, Porto, Portugal) and housed with

**Postjunctional Functional Studies.** Pnmt-KO and WT mice aortas were placed in Krebs-Henseleit solution bubbled with 95% O\(_2\) and 5% CO\(_2\), cut in rings (1–2 mm) and mounted in a myograph (DMT, Aarhus, Denmark). Each aorta ring was allowed to stabilize for 1 hour. Afterward, optimal resting tension was settled using a standardized normalization procedure (Mulvany and Halpern, 1977). Then the aortas were precontracted with phenylephrine (\(\alpha_1\)-adrenoceptor agonist) to about 60% of the maximum contraction, a level that has been shown to be optimal to obtain \(\beta\)-adrenoceptor–mediated relaxation (Guimarães, 1975). Phenylephrine was selected for preconstriction of vascular rings in our experiments because relaxation responses to \(\beta\)-adrenoceptor agonists in vivo occur under tonic constriction caused by \(\alpha\)-adrenoceptor stimulation. Both in animals and in humans, changes in the balance of \(\alpha\)- and \(\beta\)-adrenoceptor–mediated responses result in alterations of vascular tone in vivo (Landau et al., 2002). No differences were observed between the two experimental groups concerning the degree of contraction induced by the concentration of phenylephrine used (0.3 \(\mu\)M; data not shown). Finally, concentration-response curves to dobutamine (selective \(\beta_2\)-adrenoceptor agonist) and terbutaline (selective \(\beta_2\)-adrenoceptor agonist) were obtained in the absence or presence of CGP 20712 A (1-(2-(3-carbamoyl-4-hydroxy)phenoxy)ethylamino)-3-(4-(1-methyl-4-trifluoromethyl-2-imidazolyl)phenoxy)-2-propanol dihydrochloride; selective \(\beta_2\)-adrenoceptor antagonist; 40 \(\mu\)M) or IC 118,551 (\(\pm\)-erythro-(S\(^{+}\)*)-1-[2,3-(dihydro-7-methyl-1H-inden-4-yl)oxy]-3-[1-methylthylelaminio]-2-butanol dihydrochloride; selective \(\beta_2\)-adrenoceptor antagonist; 14 \(\mu\)M), respectively. The PK\(_G\) values of terbutaline for binding to \(\beta_2\) and \(\beta_1\)-adrenoceptors are around 5.82 and 9.82, respectively (Baker, 2005). Concentration-response curves to CL 316243 (5-[2R-(S)-4-[2-(3-chlorophenyl)-2-hydroxyethyl]amino]propyl)-1,3-benzodioxole-2,2-dicarboxylic acid; selective \(\beta_2\)-adrenoceptor agonist) were not obtained (although attempt was made). The force calibration process used weight and the transducer detected 1 g [9.81 millinewtons (mN) of force]. Isometric contractions or relaxations were recorded as changes in the millinewtons of tension.

**Prescussional Functional Studies.** As previously described by Trendelenburg et al. (2000), the aortas were preincubated for 30 minutes with \(3^\text{H}\)norepinephrine and then placed in superfusion chambers between platinum electrodes. Perfusion started at \(t = 0\), and each preparation was challenged with seven periods of electrical stimulation (1 millisecond width, 80 mA, 120 pulses at 3 Hz). The first stimulation period (S0) was delivered at \(t = 30\) minutes of perfusion and was not used for determination of tritium overflow. The subsequent stimulation periods (S1–S6) were applied at \(t = 58, 76, 94, 112, 130,\) and 148 minutes. Cocaine (26 \(\mu\)M; inhibitor of norepinephrine reuptake) and phentolamine (1 \(\mu\)M; nonselective \(\alpha\)-antagonist) were present in the perfusion fluid throughout the experiment. Isoproterenol and terbutaline were added to the perfusion fluid at increasing concentrations (0.1 nM to 1 \(\mu\)M and 1 nM to 1 \(\mu\)M, respectively), 12 minutes before S2–S6. At the end of the perfusion period, tissues were placed in perchloric acid (0.2 M). Radioactivity was measured by liquid scintillation counting (liquid scintillation counter 1209 Rackbeta; LKB Wallac, Turku, Finland) in the perfusate or tissue extract after the addition of scintillation mixture (Optiphase HiSafe 3; LKB, Loughborough, Leics, UK). The spontaneous outflow of tritium was calculated as a fraction of the tritium content of the tissue at the onset of the respective collection period (fractional rate; \(\cdot\) /\(\cdot\)). The overflow elicited by electrical stimulation was expressed as a percentage of tritium content of the tissue at the time of stimulation. Percentage changes of Sn S1\(^{-1}\) ratios induced by drugs added after S1 were calculated. Finally, concentration-response curves to isoproterenol and terbutaline were obtained.

**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 Na\(_2\)EDTA; 1% IGEPA; 6 mM sodium deoxycholate; 1 \(\mu\)M phenylmethyl sulfonyl fluoride; 1 \(\mu\)M NaF; 1 \(\mu\)M Na\(_3\)VO\(_4\); 5 \(\mu\)g/ml leupeptin; 5 \(\mu\)g/ml aprotinin; 5 \(\mu\)g/ml peptatin) and ultracentrifuged (4°C, 100,000 \(g\), 65 minutes). The pellets were
suspected 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 6x1 sample buffer (0.35 M Tris-HCl, pH 6.8; 4% SDS; 30% glycerol; 9.3% diethanol; 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. 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% sucrose in phosphate-buffered saline (PBS) for 6 hours. Fixed tissue was 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.

**β2-Adrenoceptors in Pnmt-KO Mice**

**References**

**Table 1.**

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>Pnmt-KO</th>
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<tbody>
<tr>
<td>Adrenal gland</td>
<td>Epinephrine (nmol mg⁻¹)</td>
<td>2.72 ± 0.39</td>
</tr>
<tr>
<td></td>
<td>Norepinephrine (nmol mg⁻¹)</td>
<td>1.66 ± 0.23</td>
</tr>
<tr>
<td>Aorta</td>
<td>Epinephrine (pmol mg⁻¹)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Norepinephrine (pmol mg⁻¹)</td>
<td>20.06 ± 4.11</td>
</tr>
<tr>
<td>Plasma</td>
<td>Epinephrine (pmol ml⁻¹)</td>
<td>4.58 ± 1.17</td>
</tr>
<tr>
<td></td>
<td>Norepinephrine (pmol ml⁻¹)</td>
<td>6.00 ± 2.06</td>
</tr>
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ND, not detectable/below detectable limit.

*Significantly different from correspondent values in WT mice (P < 0.05).
overflow of tritium induced by the control stimulation \( (S_1, 0.43 \pm 0.04 \text{ versus } 0.32 \pm 0.04, \% \text{ of tritium content}; n = 7–8) \). 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 \( pEC_{50} \) of \( 9.36 \pm 0.55 \) (\( n = 7–8 \)) and a maximal effect of \( 167.3 \pm 37.5\% \) (\( n = 7–8 \)) for isoproterenol and a \( pEC_{50} \) of \( 7.65 \pm 0.94 \) (\( n = 6 \)) and a maximal effect of \( 173.3 \pm 31.3\% \) (\( n = 6 \)) 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 \( \beta_2 \)-Adrenoceptors in Aorta.** The protein density of \( \beta_2 \)-adrenoceptors (\( \sim 61 \) 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 \( \beta_2 \)-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. \( \beta_2 \)-Adrenoceptor immunoreactivity was decreased in aorta rings from Pnmt-KO mice (341 \pm 37 versus 190 \pm 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 \( \beta_2 \)-adrenoceptor–mediated responses both at a postjunctional level (\( \beta_2 \)-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 \( \beta_2 \)-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.

![Image of Fig. 1](image1.png)

**Fig. 1.** (A) Terbutaline (\( \beta_2 \)-adrenoceptor agonist) and (B) dobutamine (\( \beta_1 \)-adrenoceptor agonist) concentration–response curves (relaxation in percentage of maximal relaxation) of phenylephrine (\( \alpha_1 \)-adrenoceptor agonist) precontracted aortas in Pnmt-KO and WT mice. maxR, maximum relaxation. Each curve point represents the mean of 5–8 experiments per group and error bars represent S.E.M. *Significantly different from correspondent values in WT mice (\( P < 0.05 \)).

![Image of Fig. 2](image2.png)

**Fig. 2.** (A and B) Terbutaline (\( \beta_2 \)-adrenoceptor agonist) concentration-response curves in the absence or presence of ICI 118,551 (\( \beta_2 \)-adrenoceptor antagonist) and (C and D) dobutamine (\( \beta_1 \)-adrenoceptor agonist) concentration–response curves in the absence or presence of CGP 20712 A (\( \beta_1 \)-adrenoceptor antagonist), in (A and C) WT and (B and D) Pnmt-KO mice, of phenylephrine (\( \alpha_1 \)-adrenoceptor agonist) precontracted aortas, maxR, maximum relaxation. Each curve point represents the mean of 5 experiments per group and error bars represent S.E.M. *Significantly different from correspondent values in respective mice group (\( P < 0.05 \)).
In conduit arteries (thoracic aorta and carotid artery) of mice, both β₁- and β₂-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 β₂-adrenoceptors, 2) it is easy to mount in the myograph to evaluate β₂-adrenoceptor-mediated relaxation, and 3) it has enough nerve terminals to evaluate prejunctional β-adrenoceptor-mediated responses.

Our results showed that dobutamine (β₁-adrenoceptor agonist) and terbutaline (β₂-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 β₂-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 β₂-adrenoceptor stimulation by terbutaline were lower in Pnmt-KO mice than in WT mice. In addition, the β₂-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 β₂-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 β-adrenoceptor complexes was observed (Davies et al., 1981), and after adrenal demedullation, a decrease in β-adrenoceptor cardiac density was observed in rats (Tumer et al., 1990).

On the other hand, no differences were observed in β₁-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 β₁-adrenoceptor antagonist) antagonized the effect of dobutamine with similar potency in both groups. Thus, our results suggest that both β₁- and β₂-adrenoceptors mediate aortic relaxation in WT mice but that only β₁-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, β₁-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 β₂-adrenoceptor-mediated vasodilator response is blunted, and then blood pressure rises. In agreement with this hypothesis is the fact that β₂-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 β₂-adrenoceptor-mediated vasorelaxation induced by CL 316243 in WT mice, which is in agreement with the fact that in aortas from β₁β₂-adrenoceptor double-knockout mice, the β₁-agonist isoproterenol does not cause any relaxation (Chruscinski et al., 2001). These findings suggest that β₂-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 β-adrenoceptors, in particular of the β₂ subtype, are present in the mouse aorta. In addition, we report here the absence of β₂-adrenoceptor-mediated effect on norepinephrine release in Pnmt-KO mice. In agreement with
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 β2-subtype seems rather unlikely because we did not observe any β-adrenoceptor induced effect in the release of norepinephrine using isoproterenol (a nonselective β-adrenergic 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 which may justify impairments of vasorelaxation and of coupling to stimulatory G protein and lower cAMP levels, aorta of Pnmt-KO mice may be correlated with decreased

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 (Kudlace 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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