Inhibition of Phosphodiesterase Type 3 Dilates the Rat Ductus Arteriosus Without Inducing Intimal Thickening

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Background: Prostaglandin E₁ (PGE₁), via cAMP, dilates the ductus arteriosus (DA). For patients with DA-dependent congenital heart disease (CHD), PGE₁ is the sole DA dilator that is used until surgery, but PGE₁ has a short duration of action, and frequently induces apnea. Most importantly, PGE₁ increases hyaluronan (HA) production, leading to intimal thickening (IT) and eventually DA stenosis after long-term use. The purpose of this study was therefore to investigate potential DA dilators, such as phosphodiesterase 3 (PDE3) inhibitors, as alternatives to PGE₁.

Methods and Results: Expression of PDE3a and PDE3b mRNAs in rat DA tissue was higher than in the pulmonary artery. I.p. milrinone (10 or 1 mg/kg) or olprinone (5 or 0.5 mg/kg) induced maximal dilatation of the DA lasting for up to 2 h in rat neonates. In contrast, vasodilation induced by PGE₁ (10 μg/kg) was diminished within 2 h. No respiratory distress was observed with milrinone or olprinone. Most important, milrinone did not induce HA production, cell migration, or proliferation when applied to cultured rat DA smooth muscle cells. Further, high expression of both PDE3a and PDE3b was demonstrated in the human DA tissue of CHD patients.

Conclusions: Because PDE3 inhibitors induced longer-lasting vasodilation without causing apnea or HA-mediated IT, they may be good alternatives to PGE₁ for patients with DA-dependent CHD. (Circ J 2012; 76: 2456–2464)

Key Words: Congenital heart disease; Ductus arteriosus; Milrinone; Phosphodiesterase

The ductus arteriosus (DA), the fetal arterial connection between the pulmonary artery (PA) and the descending aorta, is essential to maintain fetal life in utero. The DA closes after birth by 2 different mechanisms: vasoconstriction and intimal thickening (IT). During the first few hours after birth, acute vasoconstriction occurs as a result of smooth muscle contraction in the DA. This is triggered by increased oxygen tension, due to the initiation of spontaneous breathing, and decreased circulating prostaglandin E₂ (PGE₂), due to disconnection from the placenta. This functional vasoconstriction, however, must be preceded by IT of the DA, because vascular remodeling, including IT, is critical for anatomical closure of the DA.

The IT of the DA is a result of many cellular processes, such as an increase in smooth muscle cell (SMC) migration and proliferation, the production of hyaluronan (HA) under the endothelial layer, and decreased elastin fiber assembly. We have previously demonstrated that PGEs promoted HA production via cAMP/protein kinase A and subsequent SMC migration, resulting in IT of the DA during the late gestational period. In patients with DA-dependent congenital heart disease (CHD), such as pulmonary atresia with intact ventricular septum or arch anomalies (coarctation of aorta or interruption of aortic arch), however, patent DA after birth is essential for survival.

PGE₁ is widely used to keep the DA open because it increases intracellular cAMP and thus dilates the DA. But PGE₁ induces HA-mediated IT and thus DA stenosis after prolonged use. The fact that it induces only a very short duration of vasodilation, together with its severe adverse effects, such as apnea, respiratory distress, and hypotension, present additional problems, making it difficult for some patients with CHD to...
continue the use of PGE$_1$ until surgery. As such, possible alternatives to PGE$_1$ need to be investigated.

Phosphodiesterases (PDEs), which catalyze the hydrolysis of cAMP/cGMP, constitute a superfamily of at least 11 gene families (PDE1–PDE11). The 2 PDE3 subfamilies, PDE3A and PDE3B, are encoded by closely related genes, and both hydrolyze cAMP. PDE3 inhibitors have been approved by the US Food and Drug Administration (FDA) for use as vasodilators as well as in heart failure. Two of these are milrinone and olprinone, which are widely used to treat heart failure and persistent pulmonary hypertension in neonates. Previous studies have shown that the PDE3 inhibitors milrinone, amrinone, and cilostazol counteract indomethacin-induced DA constriction. Thus, PDE3 inhibitors alone may be sufficient to dilate the DA. Nevertheless, it remains undetermined whether they induce IT, which is a major problem with PGE$_1$, via HA production, cell migration, or cell proliferation. In the current study, we investigated the role of PDE3 inhibitors in DA vascular remodeling and vasodilatation with a view to their potential use as alternatives to the current PGE$_1$ therapy.

**Methods**

**Animals and Materials**

Timed pregnant Wistar rats were purchased from Japan SLC (Hamamatsu, Japan). All animal studies were approved by the institutional animal care and use committees of Yokohama City University. Milrinone, platelet derived growth factor-BB (PDGF-BB), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), trichloroacetic acid, and 10% buffered formalin were obtained from Wako (Osaka, Japan). Olprinone, cilostazol, rolipram, PGE$_1$, PGE$_2$, elastase type II, trypsin inhibitor, bovine serum albumin V, poly-L-lysine, penicillin-streptomycin solution, acetic anhydride, triethylamine, Dulbecco’s modified Eagle’s medium (DMEM), and Hank’s balanced salt solution (HBSS) were purchased from Sigma-Aldrich (St Louis, MO, USA). Collagenase II was purchased from Worthington Biochemical (Lakewood, NJ, USA). Collagenase/dispase was purchased from Roche Diagnostics (Tokyo, Japan). Fetal bovine serum (FBS) was purchased from Equitech-Bio (Kerrville, TX, USA).

**Primary Culture of Rat SMCs**

Vascular SMCs in primary culture were obtained from the DA (DASMCs), the aorta (ASMCs), and the PAs (PASMCs) of Wistar rats on the 21st day of gestation. Isolation of DASMCs and ASMCs has been described previously. To obtain PASMCs, the branch extralobular PAs were dissected, cleaned from adherent tissue, and cut into small pieces. The tissues were transferred to a 1.5-ml centrifuge tube that contained 800 μl of collagenase-dispase enzyme mixture (1.5 mg/ml collagenase-dispase, 0.5 mg/ml of elastase type II A, 1 mg/ml of trypsin inhibitor type I S, and 2 mg/ml of bovine serum albumin fraction V in HBSS). Digestion was carried out at 37°C for 15 min. Cell suspensions were then centrifuged, and the medium was changed to a collagenase II enzyme mixture (1 mg/ml collagenase II, 0.3 mg/ml trypsin inhibitor type I S, and 2 mg/ml bovine serum albumin fraction V in HBSS). After 12 min of incubation at 37°C, cell suspensions were transferred to growth medium in 35-mm poly-l-lysine-coated dishes in a moist tissue culture incubator at 37°C in 5% CO$_2$-95% ambient mixed air. The growth medium contained DMEM with 10% FBS, 100 μg/ml penicillin, and 100 μg/ml streptomycin. We confirmed that >99% of cells were positive for α-smooth muscle actin and exhibited typical hill-and-valley morphology. Expression of PDE3, prostaglandin E receptor EP4, and prostacyclin (IP) receptor mRNAs in DASMCs, ASMCs, and PASMCs is shown in Figure S1.

**Human Tissue From CHD Patients**

We obtained 8 neonatal DAs and adjacent aortas during cardiac surgery in children between 0 days and 1 month of age. All excised tissue was fixed in 4% paraformaldehyde within 3 h. The DA tissues were obtained from the Yokohama City University Hospital and Kanagawa Children’s Medical Center. The study was approved by the human subject committees at both Yokohama City University and Kanagawa Children’s Medical Center. Detailed patient information is summarized in Table.

**RNA Isolation and Quantitative Reverse Transcription-Polymerase Chain Reaction (RT-PCR)**

Pooled vascular tissues were obtained from Wistar rats on the 21st day of gestation. After excision, tissues were frozen in liquid nitrogen and stored at –80°C. The total RNA was isolated from the tissues using an RNeasy Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer’s instructions and from the cultures using Trizol reagent (Invitrogen, Carlsbad, CA, USA). The primers were designed based on the rat nucleotide sequences of PDE3a (NM_017337) (5’-CGC CTG AGA AGA AGT TTG C-3’) and 5’-GCC AGA AAC TGA AGG AGG-3’), PDE3b (NM_017229.1) (5’-GTC AGG AAC TGA AGG AGG-3’), PDE4b (NM_007644.1) (5’-GGG CAC GAG AGG ATG AAG-3’), PDE7b (NM_00177644.1) (5’-GGG CAC GAG AGG ATG AAG-3’), and PDE11 (NM_009663.1) (5’-GGG CAC GAG AGG ATG AAG-3’). RT-PCR was performed using a PrimeScript RT reagent Kit (TaKaRa Bio, Tokyo, Japan) and real-time PCR was performed using SYBR Green (Applied Biosystems, Foster City, CA, USA). The abundance of each gene was determined relative to that in 18S ribosomal RNA.

**Rapid Whole-Body Freezing Method**

To study the in situ morphology and inner diameter of the neonatal DA, a rapid whole-body freezing method was used as previously described. Fetuses on the 21st day of gestation were delivered by cesarean section, and immediately after birth i.p. injections of milrinone (10 mg/kg, 1 mg/kg, 0.1 mg/kg), olprinone (5 mg/kg, 0.5 mg/kg, 0.05 mg/kg), or PGE$_1$ (10 μg/kg) were given. The rat pups were frozen in liquid nitrogen at 0,
Figure 1. Quantitative reverse transcription-polymerase chain reaction (RT-PCR) of PDE3a, PDE3b, and EP4 in rat ductus arteriosus (DA), aorta, and pulmonary artery (PA) tissue on the 21st day of gestation. n=4–5. *P<0.05; **P<0.01; ***P<0.001. PDE, phosphodiesterase; NS, not significant.

Figure 2. Effects of milrinone and olprinone on vasodilation of the ductus arteriosus (DA) as observed using the rapid whole-body freezing method. (A) Prostaglandin E1 (PGE1; 10μg/kg)-induced dilation of rat DA (n=4–6). (B) Vasodilatory effect of milrinone on rat DA. Rat neonates were injected i.p. with milrinone (n=4–6). (C) Representative images of rat DAs treated with 10mg/kg of milrinone or saline (control) for 2h using the whole-body freezing method (arrow). (D) Vasodilatory effect of milrinone on rat DA. Rat neonates were injected i.p. with olprinone (n=4–6). (E) Representative images of rat DAs treated with 5mg/kg of olprinone or control for 2h using the whole-body freezing method (arrow). (F) Milrinone or olprinone dilated DA in a dose-dependent manner. Vasodilatory effects of phosphodiesterase 3 (PDE3) inhibitors were examined 2h after injection (n=4–6). ***P<0.001 and NS vs. control. NS, not significant.
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Figure 3. Effects of phosphodiesterase 3 (PDE3) inhibitors and prostaglandin E1 (PGE1) on respiratory distress. (A) Respiratory rate of rat neonates given each drug immediately after birth, the same as in Figure 2 (n=6–9). (B) Respiratory rate of rat neonates given each drug 2 h after birth (n=4). *P<0.05, **P<0.01 and ***P<0.001 vs. control. No mark, not significant vs. control.

Figure 4. Milrinone increased cAMP production, but it did not induce hyaluronan (HA) production. (A) Milrinone (10 μmol/L) significantly increased cAMP accumulation in ductus arteriosus smooth muscle cells (DASMCs; n=4). (B) HA production in SMCs treated with milrinone (10 μmol/L), cilostazol (10 μmol/L), rolipram (10 μmol/L), prostaglandin (PG)E1 (1 μmol/L), or PGE2 (1 μmol/L; n=4–6). Cilostazol, phosphodiesterase 3 (PDE3); Rolipram, PDE4 inhibitor. **P<0.01 and ***P<0.001 vs. control. No mark, not significant vs. control. ASMCs, aortic smooth muscle cells; PASMCs, pulmonary artery smooth muscle cells.
0.5, 1, 2, 4, 6, 8, and 12 h after injection. The frozen thoraxes were then cut on a microtome, and the inner diameter of each DA was measured.

**Determination of Respiratory Rate**
Fetuses on the 21st day of gestation were delivered by cesarean section, and at 0 or 2 h after birth i.p. injections of milrinone (10 mg/kg, 1 mg/kg), olprinone (5 mg/kg, 0.5 mg/kg), or PGE1 (10 μg/kg) were given. We measured the respiratory rate by counting the movements of the rat thorax.

**Quantification of HA**
The amount of HA in the cell culture supernatant was measured according to the latex agglutination method as previously described.

**SMC Migration Assay**
The migration assay was performed using 24-well transwell culture inserts with polycarbonate membranes (8-μm pores; Corning, Corning, NY, USA) as previously described. Cells were stimulated with milrinone (10 μmol/L), PGE1 (1 μmol/L), PDEF-BB (10 ng/ml), HA (200 ng/ml), or milrinone + HA for 3 days.

**Cell Proliferation Assay**
SMCs were cultured on 24-well plates at 1×10^5 cells per well in DMEM supplemented with 10% FBS. After various treatments over 3 days, 500 μl of 1 mg/ml MTT solution was added to each well and incubated for 2 h. The supernatants were aspirated, and the formazan crystals in each well were solubilized with 0.05 mol/L HCl (500 μl). Each solution (100 μl) was placed in a 96-well plate. SMC proliferation was measured based on absorbance at 570 nm using a microplate reader.

**Immunohistochemistry**
Immunohistochemical analysis was performed as previously described. Rabbit polyclonal anti-PDE3A antibody (sc-20792) and goat polyclonal anti-PDE3b antibody (sc-11835) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). A color extraction method using BIOREVO bz-9000 (KEYENCE, Osaka, Japan) was performed to quantify the expression of PDE3s in the DAs and the aortas of patients 1, 4, 5, and 8. Eighteen fields in the smooth muscle layer of the DA and the aorta respectively were examined in 4 cases. On diaminobenzidine staining, PDE3a-positive or PDE3b-positive areas were extracted and counted on the screen.

**Radioimmunoassay Measurement of Cyclic AMP Production**
Measurement of cAMP accumulation in DASMCs was performed as previously described. Briefly, DASMCs grown on 24-well plates were serum-starved for 24 h and assayed for cAMP production after a 10- or 20-min period of incubation with 10 μmol/L of milrinone. Reactions were terminated by aspiration of the media and the addition of 300 μl of ice-cold trichloroacetic acid (7.5%) to each well. Forty microliters of each sample were acetylated and incubated with 125I-cAMP (Perkin Elmer, Waltham, MA, USA) and 50 μl of rabbit anti-cAMP antibody (diluted 1:3,000, Millipore, Billerica, MA, USA) overnight at 4°C. Each mixture was then incubated with...
50μl of goat anti-rabbit antibody with magnetic beads (Qia-gen) for 1h. Separation of bound antibodies from free antibodies was achieved by filtration, and bound radioactivity was counted. Production of cAMP was normalized to the amount of protein per sample.

**Statistical Analysis**

Data are presented as mean±SEM of independent experiments. Statistical analysis was performed between 2 groups using unpaired 2-tailed Student’s t-test or unpaired t-test with Welch’s correction, and among multiple groups using 1-way analysis of variance followed by Tukey’s multiple comparison test. *P<0.05 was considered significant.

**Results**

**Messenger RNA of PDE3 Isoforms Highly Expressed in Rat DA**

We first examined whether the target molecule of PDE3 inhibitors is highly expressed in the DA. We measured the mRNA expression of PDE3s using quantitative RT-PCR in the rat DA, aorta, and PA on the 21st day of gestation (Figure 1). Expression of PDE3a mRNA was higher in the DA than in the PA. Expression of PDE3b mRNA was higher in the DA than in the aorta or the PA. We also confirmed that EP4 mRNA was more highly expressed in the DA than in the aorta or the PA. Thus, PDE3 isoforms were abundantly expressed in the DA relative to the PA.

**Vasodilatory Effects of PDE3 Inhibitors on Rat DA In Vivo**

PDE3 inhibitors are widely used in neonates and children with low cardiac output following myocarditis and cardiovascular surgery for CHD. We examined whether milrinone or olprinone dilated the DA using the rapid whole-body freezing method in rat neonates. Neonates were injected with 1 of these drugs immediately after birth to mimic the vasodilatory treatment currently used in DA-dependent CHD.

I.p. injection of PGE2 (10μg/kg, the amount that is given i. v. daily as a clinical maintenance dose) induced maximum dilatation of the DA for 30min, but this effect was completely lost within 2h after injection (Figure 2A). A single i.p. injection of 10mg/kg of milrinone maintained maximum dilatation of the DA for up to 12h (Figures 2B,C); 1mg/kg of milrinone, the amount that is given i.v. daily as a clinical maintenance dose, maintained maximum dilatation for 2h, after which DA closure occurred at 4h after injection. And 0.1mg/kg of milrinone did not affect DA tone. Both 5mg/kg and 0.5mg/kg of olprinone, the latter of which is suitable for daily i.v. use as a clinical maintenance dose, induced maximum dilatation for 1h after injection (Figures 2D,E). A dose of 0.05mg/kg olprinone did not dilate the DA. Thus, both milrinone and olprinone produced dose-dependent vasodilatory effects (Figure 2F), but those of milrinone lasted longer.

**PDE3 Inhibitors Do Not Induce Respiratory Distress**

Given that respiratory distress is a major adverse effect of PGE2, we examined whether PDE3 inhibitors cause respiratory distress. We measured the respiratory rate of rat neonates given milrinone, olprinone, or PGE2. When rat neonates were given each drug immediately after birth, PGE2 significantly reduced the respiratory rate at 15 or 30min after injection, whereas milrinone (1 and 10mg/kg) and olprinone (0.5 and 5mg/kg) did not induce respiratory distress up to 8h after injection compared to the saline control (Figure 3A). To exclude the possibility that neonates given PGE2 had a congenital respiratory problem, we examined the effect of drugs using a different injection timing. We confirmed that all rat neonates had established normal breathing 1h after birth, and then each drug was given. PGE2 significantly reduced the respiratory rate up to 1h after injection. In contrast, milrinone (10mg/kg) and olprinone (5mg/kg) did not affect the respiratory rate compared to the control (Figure 3B). These data suggest that PDE3 inhibitors did not cause respiratory distress.

**Milrinone Do Not Promote HA Production or SMC Migration and Proliferation**

Although it was previously suggested that PDE3 inhibitors induced vasodilation of the DA, it remained unknown whether they also induced IT formation, a key process in the anatomical closure of the DA. It is known that PGEs stimulate HA production along with increased DASMC migration through the action of HA as a potent trigger of cell migration. This is the major mechanism underlying the increase in IT induced by PGEs.

We thus examined whether a PDE3 inhibitor, milrinone,
regulated HA production or SMC migration. First, we confirmed cAMP production in the presence of milrinone. Milrinone significantly increased cAMP accumulation in DASMCs at a dosage of 10 μmol/L, which also induced marked dilatation of DA explants (Figure 4A).

But the same dosage of milrinone (10 μmol/L) did not induce HA production in DASMCs (Figure 4B). We also confirmed that the PDE3 inhibitor cilostazol did not induce HA production in DASMCs. Similarly, PGE1 (1 μmol/L) induced DASMC migration; but milrinone did not increase DASMC migration, as determined using the Boyden chamber method (Figure 5A). The cells used for these tests were sufficiently stimulated with PGE1 to induce HA production and with PDGF-BB to induce migration. Next, we examined the effects of a PDE3 inhibitor on SMC proliferation, because SMC proliferation plays a role in IT formation of the DA.

Milrinone and PGE1 did not increase DASMC proliferation, as determined on MTT assay, in the presence of 0 or 10% FBS (Figure 5B). Moreover, we found that milrinone did not enhance HA-mediated migration in DASMCs (Figure 6A). Milrinone also did not affect proliferation in HA-treated DASMCs (Figure 6B). Similarly, in ASMCs and PASMCs, neither milrinone nor PGE1 increased HA production or cell migration and proliferation (Figures 4B, 5). These findings suggest that PDE3 inhibitors do not promote HA production or cell migration or proliferation, although they do produce cAMP and dilate the DA.

**PDE3a and PDE3b Highly Expressed in the Smooth Muscle Layer in Human DA Tissue**

The expression pattern of PDE3s in human DA remains unknown. We examined PDE3a and PDE3b protein expression in the DA of 8 patients with CHD, such as interruption of the aortic arch, complex aortic coarctation, hypoplastic left ventricle, and asplenia. The DA of all patients had a strong immunoreaction for both PDE3a and PDE3b (Figure 7A). It has been demonstrated that PDE3a and PDE3b are abundantly expressed in the rat and human aorta.25,26 The expression of PDE3a and PDE3b in the DAs was equivalent to that in the adjacent aortas (Figure 7B). This demonstrates that PDE3s are abundantly expressed in human patients with CHD of the type that may require long-term vasodilatotherapy prior to surgery.

**Discussion**

The present study has demonstrated that the PDE3 inhibitors milrinone and olprinone dilate the DA without causing apnea and have a longer duration of action than PGE1. These findings are expected to apply to human patients, given that PDE3s are abundantly expressed in the DA tissue of infants.
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with CHD. Importantly, this study has shown for the first time that these PDE3 inhibitors do not promote HA production, cell migration, and cell proliferation in the DASM C, processes that potently induce IT and thus DA closure. The PDE3 inhibitors are very unlikely to produce these unfavorable effects when used as DA dilators. Furthermore, these PDE3 inhibitors are already used in humans for other purposes,4,10,13,14 Accordingly, they may serve as useful alternatives to PGE1, the current means of keeping the DA patent. PGE1 increases the production of cAMP by activating G protein and adenylyl cyclase.1,2,27 In contrast, milrinone increases the concentration of cAMP by inhibiting its breakdown.7 Although both drugs increase cAMP and dilate the DA, PGE1 induces HA production and subsequent migration in DASM C while milrinone does not. We do not know the molecular mechanism underlying this difference between PGE1 and the PDE inhibitors. It can be tentatively speculated, however, that they differ in terms of intracellular localization and thus in terms of coupling with other molecules, as recent studies have suggested.28 Regardless of the mechanisms involved, it is known that PGE1 and PGE2 both increase cAMP production and induce HA production via increased expression of HA synthase 2,7,15 and we found that a PDE4 inhibitor, rolipra m, did not induce HA production (Figure 4B). Alternatively, increases in cGMP, which is also induced by milrinone, may play a role. These issues need to be further investigated in future studies.

Previous studies effectively demonstrated the vasodilatory effects of the PDE3 inhibitors milrinone, amrinone and cilio stazol on the rat or sheep DA that underwent contraction by indomethacin.15,16 In contrast, we have evaluated the effects of PDE3 inhibitors in the absence of indomethacin to examine the effects of PDE3 inhibitors in more relevant clinical settings. We also found, for the first time, that olprinone, a relatively new PDE3 inhibitor, dilates the DA. The present finding that these PDE3 inhibitors do not increase HA production is also novel, because this question had not been investigated previously.

The present study shows that milrinone does not induce SMC migration and proliferation in the DA (Figures 5,6). The present findings are, at least in part, consistent with those obtained using vascular SMCs from non-DA vessels. PDE3 inhibitors have elsewhere been shown to reduce proliferation and migration of vascular SMCs and to decrease the accumulation of synthetic/activated vascular SMCs in the intimal layers of damaged blood vessels.7,29,30 Similarly, in peripheral PAs, PDE3 and PDE4 inhibition do not promote PASMC migration.31 Furthermore, PDE3a deficiency caused G0/G1 cell cycle arrest in PDE3a knockout mice.8 PGE1 is currently the sole DA dilator, but PGE1-induced apneic or respiratory distress was noted in 18% of patients with CHD.12 Respiratory depression was particularly common in infants weighing <2.0 kg at birth who received PGE1 therapy (42%).22 The present study showed that milrinone and olprinone did not induce respiratory distress in rat neonates (Figure 3). Furthermore, no occurrence of apnea or respiratory distress due to PDE3 inhibitors has been previously reported.9,10,13,14 Therefore, the PDE3 inhibitors are very unlikely to produce an unfavorable effect on respiration when used as DA dilators. It should be noted that PDE3 inhibitors have adverse effects, such as arrhythmia or hypotension.33 Milrinone reduces the risk of low cardiac output syndrome for some pediatric patients after congenital heart surgery, but milrinone use is an independent risk factor for clinically significant tachyarrhythmias.34 Although it was not feasible to examine arrhythmias and change in blood pressure in rat neonates in this study, careful further study is warranted to examine adverse effects.

It should be emphasized that both the PDE3a protein and the PDE3b protein were abundantly detected in the smooth muscle layer and the IT layer in all human DA samples tested, regardless of diagnosis or patient age at the time of operation (Figure 7). A previous study demonstrated that PDE3 inhibitors prevented DA closure in premature infants with persistent pulmonary hypertension.15,16 Together with these findings, those of the present study suggest that PDE3 inhibitors can dilate the DA without inducing IT, and that they may serve as alternatives to PGE1, the current DA vasodilator used for patients with DA-dependent CHD.

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Disclosure

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References


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Supplementary Files

Supplementary File 1

Figure S1. Quantitative RT-PCR of PDE3a, PDE3b, EP4 and IP in the DASMCs, ASMCs, PASMCs.

Please find supplementary file(s): http://dx.doi.org/10.1253/circj.CJ-12-0215