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Figure 1

Fig. 1

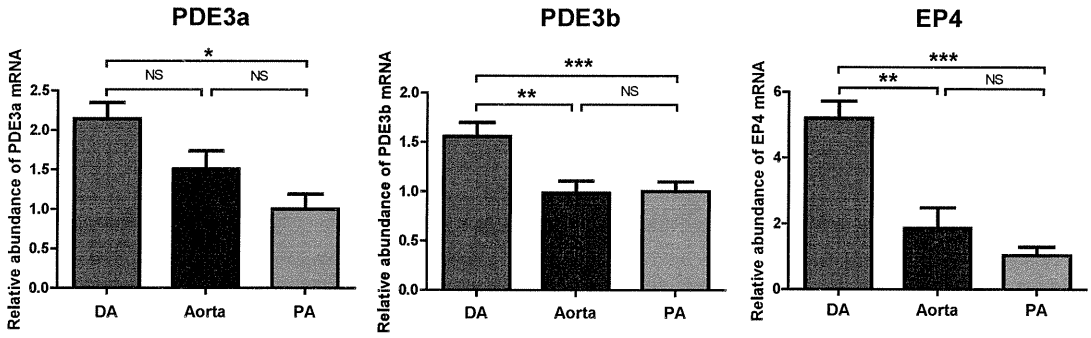


Figure 1
胎生 21 日ラット動脈管 (DA)、大動脈 (Aorta)、肺動脈(PA)における PDE3a、PDE3b、EP4 の定量 RT-PCR 解析。n = 4–5, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, NS indicates not significant.

Figure 2

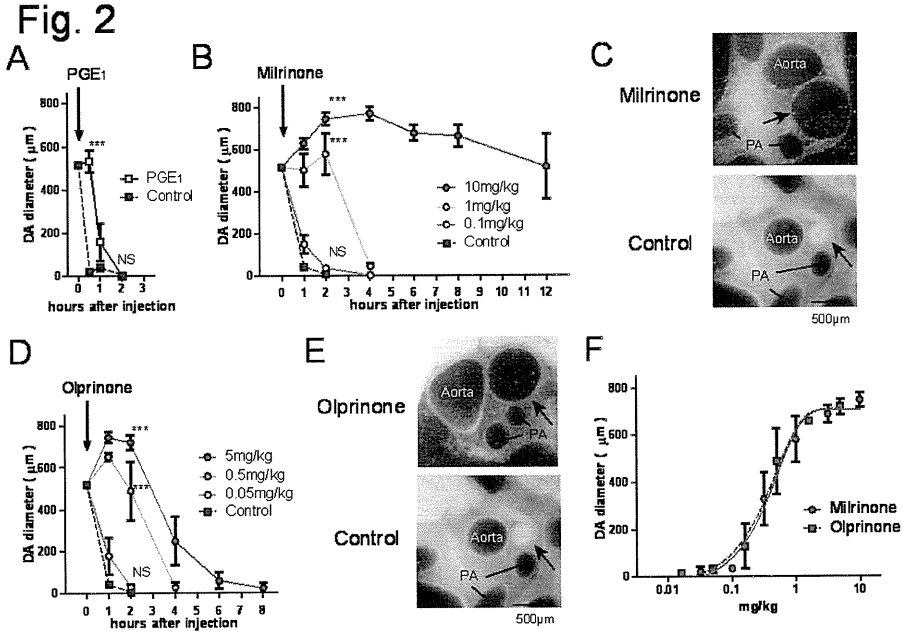
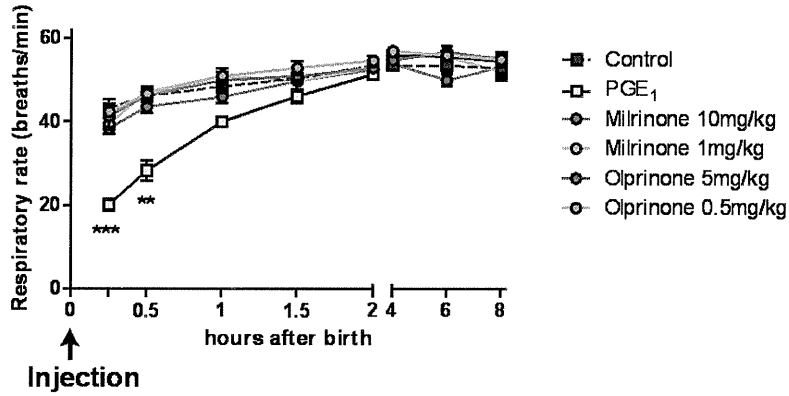


Figure 2 急速全身凍結法によって得られたミルリノンとオルプリノンの動脈管拡張効果。(A) PGE1 (10μg/kg) のラット動脈管拡張効果 (n = 4–6)。(B) ミルリノンのラット動脈管拡張効果。ラット新生児は腹腔内投与によってミルリノンを投与された (n = 4–6)。(C) 急速全身凍結法を用いて 10 mg/kgのミルリノン、コントロールを投与したときの写真。(矢印が動脈管) (D) オルプリノンのラット動脈管拡張効果。ラット新生児は腹腔内投与によってオルプリノンを投与された (n = 4–6)。(E) 急速全身凍結法を用いて 5 mg/kgのミルリノン、コントロールを投与したときの写真。(矢印が動脈管) (F) ミルリノン、オルプリノンともに濃度依存性に拡張作用を有する。血管拡張効果は PDE3 阻害薬を投与後 2 時間で計測した (n = 4–6)。*** $p < 0.001$ and NS vs. control. NS indicates not significant.

Figure 3

Fig. 3

A



B

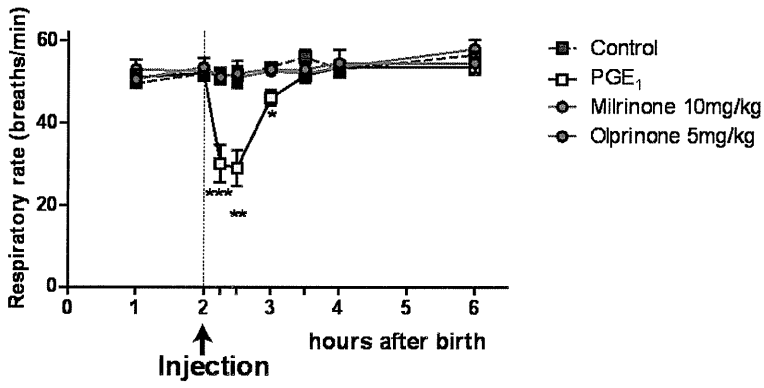


Figure 3　PDE3 阻害薬と PGE1 の呼吸抑制作用。(A) 生直後にそれぞれの薬剤を投与したときのラット新生児の呼吸回数。Figure 2 と同じタイミングで投与した (n = 6–9)。(B) 生後 2 時間にそれぞれの薬剤を投与したときのラット新生児の呼吸回数 (n = 4)。 $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$ vs. control. No mark indicates not significant vs. control.

Figure 4

Fig. 4

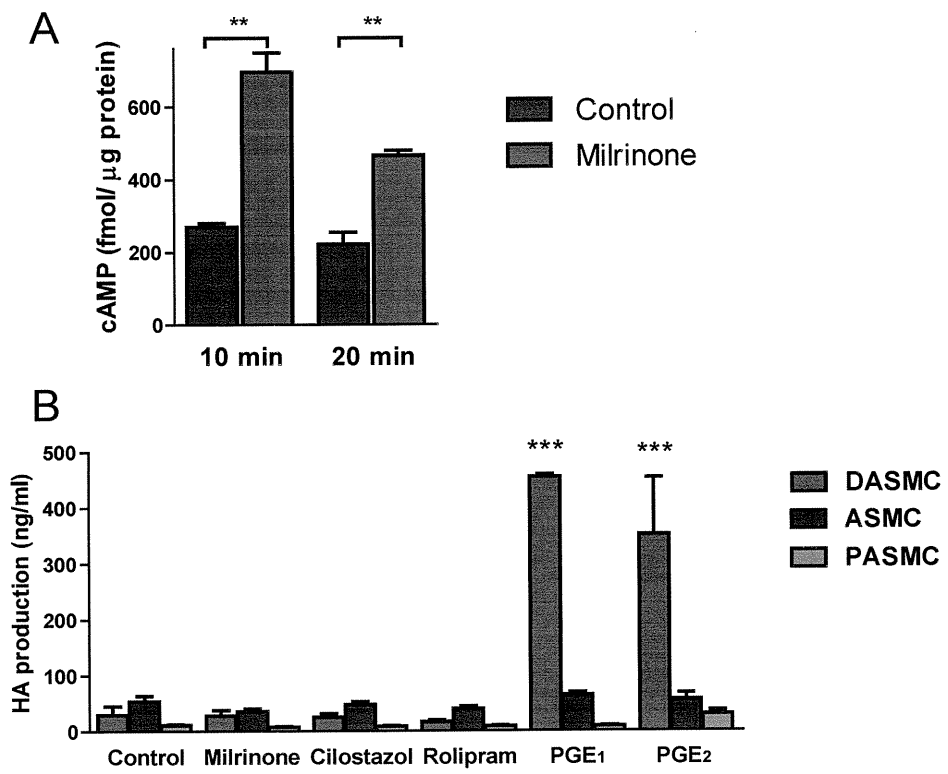


Figure 4　ミルリノンは cAMP の産生を増加させるが、ヒアルロン酸産生を誘導しない。(A) 10μM のミルリノンは動脈管平滑筋細胞において cAMP を有意に増加させる。(B) さまざまな薬剤で平滑筋細胞を刺激したときのヒアルロン酸の産生。milrinone (ミルリノン ; 10 μM), cilostazol (シロスタゾール ; 10 μM), rolipram (ロリプラム ; 10 μM), PGE_1 (1 μM), or PGE_2 (1 μM) (n = 4–6). Cilostazol: PDE3 inhibitor. Rolipram: PDE4 inhibitor. $^{**}p < 0.01$ and $^{***}p < 0.001$ vs. control. No mark indicates not significant vs. control.　DASMC:動脈管平滑筋細胞、ASMC:大動脈平滑筋細胞、PASMC:肺動脈平滑筋細胞。

Figure 5

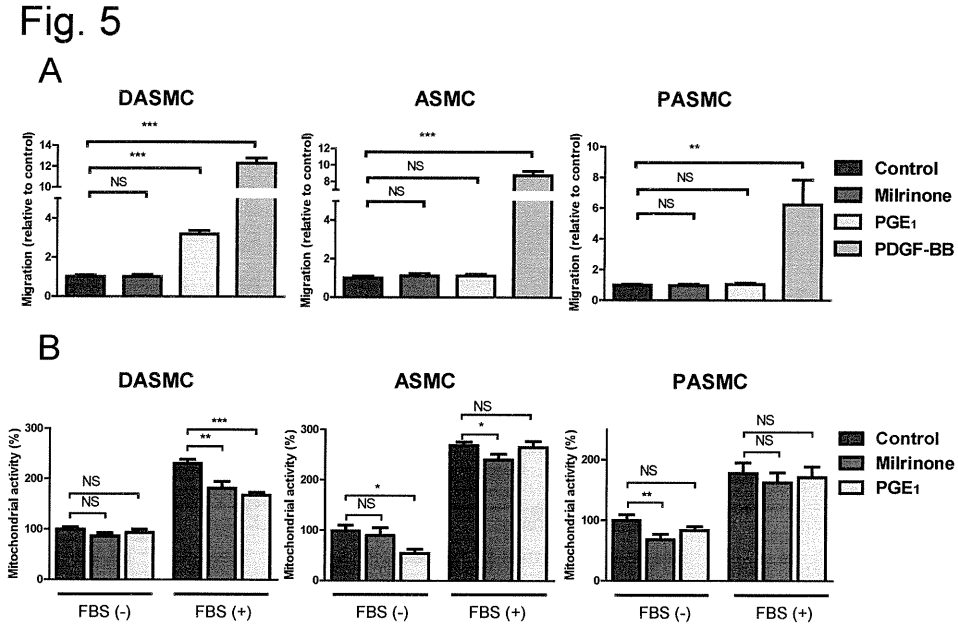


Figure 5 ミルリノンは平滑筋細胞において細胞遊走能、細胞増殖能を促進しない。(A) ボイデンチャンバー法を用いてミルリノン (10 μM), PGE_1 (1 μM), PDGF-BB (10 ng/ml)で刺激したときの血管平滑筋の細胞遊走能 (n = 4–5)。(B) MTT アッセイでミルリノン (10 μM), PGE_1 (1 μM) で刺激したときの細胞増殖能 (n = 5–9)。 $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$. NS indicates not significant. DASMC:動脈管平滑筋細胞、ASMC:大動脈平滑筋細胞、PASMC:肺動脈平滑筋細胞。

Figure 6

Fig. 6

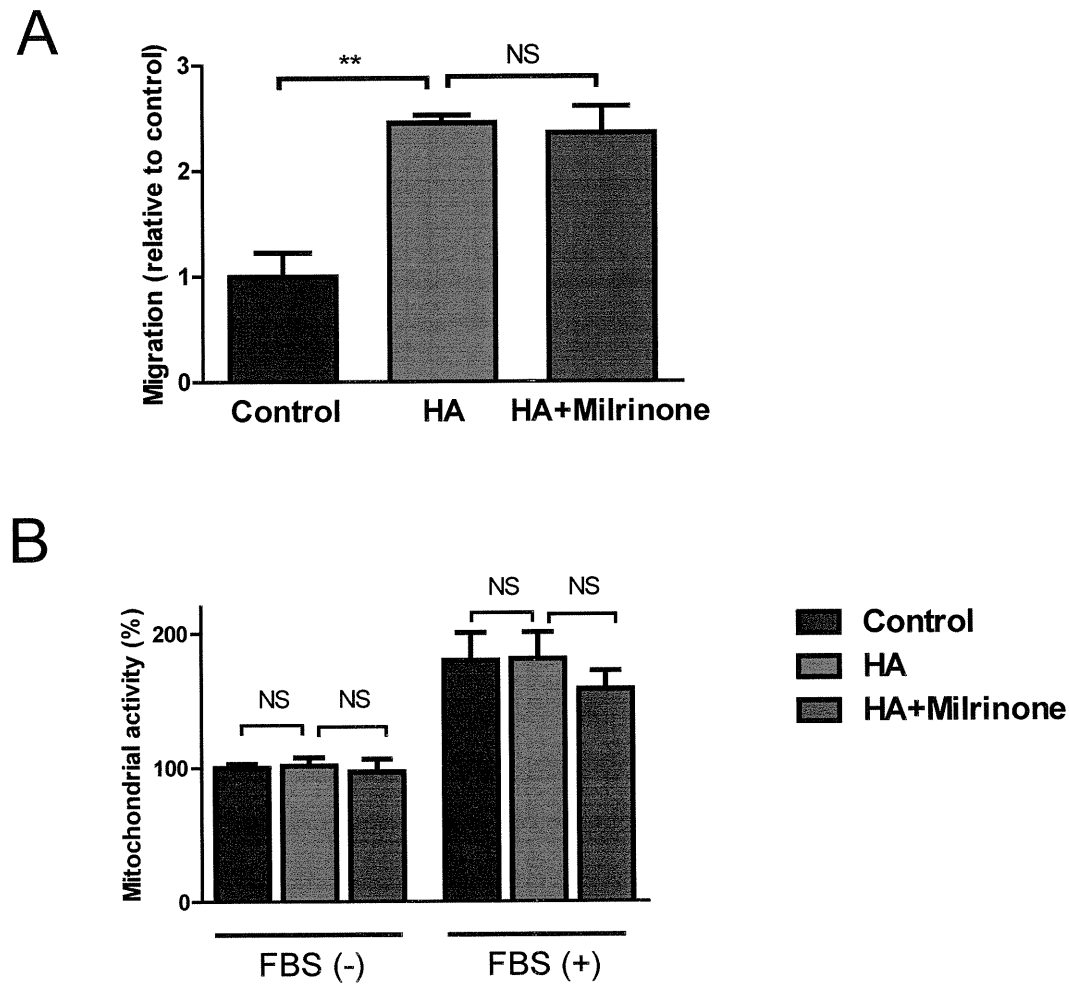


Figure 6　動脈管平滑筋細胞においてミルリノンとヒアルロン酸を両方投与したときの細胞遊走能および細胞増殖能。(A) ボイデンチャンバー法を用いてミルリノン (10 μM)＋ヒアルロン酸 (200ng/ml) で刺激したときの血管平滑筋の細胞遊走能 (n = 4)。(B) MTT アッセイでミルリノン (10 μM) ＋ヒアルロン酸 (200ng/ml) で刺激したときの細胞増殖能 (n = 8)。**$p < 0.01$. NS indicates not significant.

Figure 7

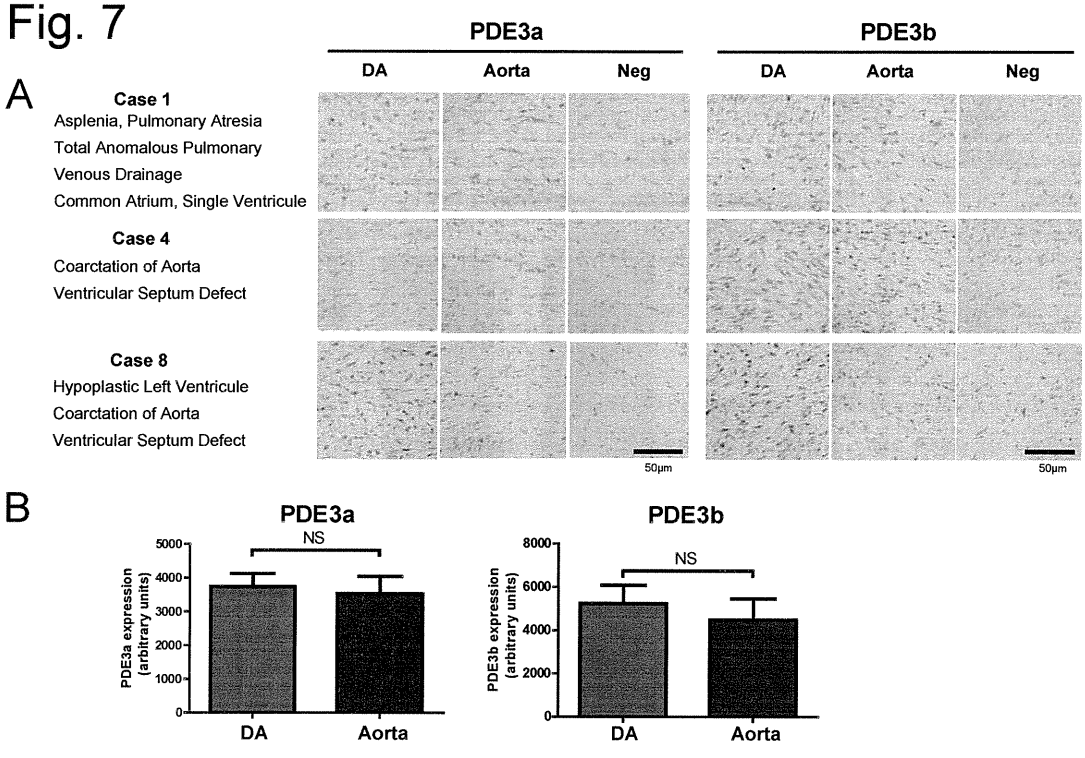


Figure 7　(A) さまざまな先天性心疾患における PDE3a と PDE3b の免疫染色。写真は動脈管、大動脈の平滑筋層である。PDE3a、PDE3b ともに一次抗体を使用しないと免疫反応は観察されない (Neg)。(B) 色抽出法を使用して、動脈管と大動脈の PDE3a と PDE3b の発現量を定量化した (n = 4)。NS indicates not significant.

Table 1　ヒト動脈管検体のプロフィール

Table 1.

Summary of patient characteristics

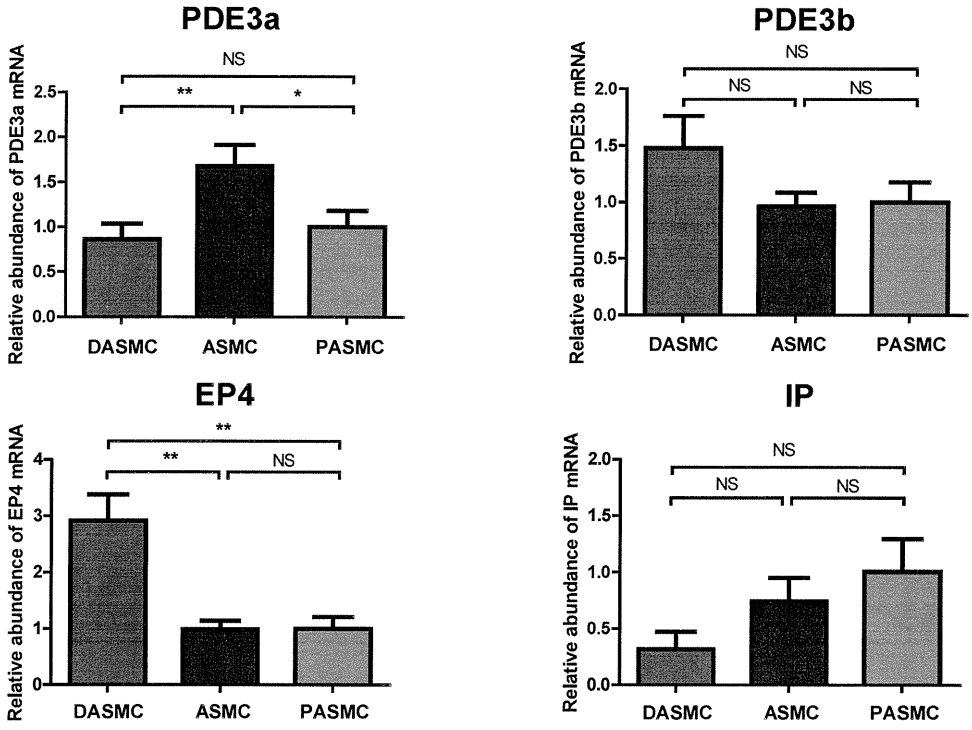
Case No.	Age at Operation	Diagnosis
1	0 days	Asplenia, PA, TAPVD, CA, SV
2	1 day	Asplenia, CoA, CA, SV
3	2 days	IAA, Aorticopulmonary window
4	2 days	CoA, VSD
5	3 days	TGA, CoA
6	4 days	CoA, VSD
7	13 days	CoA, VSD
8	1 month	hypoLV, CoA, VSD

PA: Pulmonary Atresia, TAPVD: Total Anomalous Pulmonary Venous Drainage,
CA: Common Atrium, SV: Single Ventricule,
CoA: Coarctation of Aorta, IAA: Interruption of Aortic Arch,
VSD: Ventricular Septum Defect, TGA: Transposition of the Great Arteries,
hypoLV: Hypoplastic Left Ventricule

PA:肺動脈閉鎖、TAPVD:総肺静脈還流異常症、CA:単心房、SV:単心室、CoA:大動脈縮窄、IAA:大動脈離断、VSD:心室中隔欠損症、TGA:完全大血管転移、hypoLV:左室低形成

補足 Figure 1

Supplemental Fig. S1



Supplemental Fig. S1

Quantitative RT-PCR of PDE3a, PDE3b, EP4 and IP in the DASMCs, ASMCs, PASMCs. All SMCs are used at passage 5 to 8. **P*<0.05, ***P*<0.01. NS: not significant. n=8.

ラット動脈管平滑筋細胞（DASMC）、大動脈平滑筋細胞（ASMC）、肺動脈平滑筋細胞(PASMC)における PDE3a、PDE3b、EP4 の定量 RT-PCR 解析。

[III]

研究成果の刊行に関する一覧表

研究成果の刊行

別紙４

書籍

著者 氏名	論文 タイトル名	書籍全体の 編集者名	書　籍　名	出版社名	出版地	出版年	ページ
		総監訳　泉井亮 監訳　河南洋、 久保川　学 訳　青木史暁、 赤池紀生、石川義弘、 石山延吉、上田陽一、 上野伸哉、大野忠雄、 河原克雅、河南洋、 北村竜一、久保川学、 桑木共之、小島至、 佐々木和彦、 高瀬堅吉、槌田成紀、 照井直人、中村晃、 花森隆充、	ボロン・プルーペ　生理学	西村書店	東京	2011	14-24章

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Ichikawa Y, Yokoyama U, Okumura S, Sato M, Iwasaki S, Iwamoto M, Yokota S, Masuda M, Aso T, Ishikawa Y	Inhibition of Phosphodiesterase Type 3 Dilates the Rat Ductus Arteriosus without Forming Intimal Thickening.	Circ J.	in press		2012
Yokota T, Aida T, Ichikawa Y, Fujita T, Yokoyama U, Minamisawa S.	Low-dose Thromboxane A2 Receptor Stimulation Promotes Closure of the Rat Ductus Arteriosus with Minimal Adverse Effects.	Pediatr Res.	in press		2012

Yokoyama U*, Ishiwata R, Jin MH, Kato Y, Suzuki O, Jin H, Ichikawa Y, Kumagaya S, Katayama Y, Fujita T, Okumura S, Sato M, Sugimoto Y, Aoki H, Suzuki S, Masuda M, Minamisawa S, Ishikawa Y. *corresponding author	Inhibition of EP4 signaling Attenuates Aortic Aneurysm Formation.	PLoS ONE,	7(5)	e36724	2012
Jiao Q, Takeshima H, Ishikawa Y,and Minamisawa S.	Sarcalumenin plays a critical role in age-related cardiac dysfunction due to decreases in SERCA2a expression and activity.	Cell Calcium.	51	31-39	2012
Iwatsubo K, Bravo C, Uechi M, Baljinnyam E, Nakamura T, Umemura M, Lai L, Gao S, Yan L, Park M, Qiu H, Okumura S, Iwatwsubo M, Vatner DE, Vatner SF, and Ishikawa Y.	Prevention of heart failure in mice by an antiviral agent that inhibits type 5 cardiac adenylyl cyclase.	Am J Physiol Heart Circ Physiol.	62	251-257	2012
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Kobayashi Y, Hirawa N, Tabara Y, Muraoka H, Fujita M, Miyazaki N, Fujiwara A, Ichikawa Y, Yamamoto Y, Ichihara N, Saka S, Wakui H, Yoshida S, Yatsu K, Toya Y, Yasuda G, Kohara K, Kita Y, Takei K, Goshima Y, Ishikawa Y,	Mice Lacking Hypertension Candidate Gene ATP2B1 in Vascular Smooth Muscle Cells showSignificant Blood Pressure Elevation.	Hypertension	59	854-860	2012
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Kurotani R, Okumura S, Matsubara T, Yokoyama U, Buckley JR, Tomita T, Kezuka K, Nagano T, Esposito D, Taylor TE, Gillette WK, Ishikawa Y, Abe H, Ward JM, and Kimura S.	Secretoglobin 3A2 supresses bleomycin-inducedpulmonary fibrosis by transforming growth factor signaling down-regulation.	J.Biol Chem.	286	19682-19692	2011
Jin MH, Yokoyama U, Sato Y, Shioda A, Jiao Q, Ishikawa Y and Minamisawa S	DNA microarray profiling identified a new role of growth hormone in vascular remodeling of rat ductus arteriosus.	J. Physiol. Sci.	61	167-179	2011
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Fujita T and Ishikawa Y	Apoptosis in heart failure.The role of the beta-adrenergic receptor-mediated signaling pathway and p53-mediated signaling pathway in the apoptosis of cardiomyocytes.	Circ J.	75	1811-1818,	2011
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Hokosaki T, Mori M, Nishizawa T, Nakamura T, Imagawa T, Iwamoto M, and Yokota S:	Long-term efficacy of plasma exchange treatment for refractory Kawasaki disease.	Pediatric International.	54	99-103	2012
Ichikawa Y , Iwamoto M , Yanagi S, and Masuda M	Intrapericardinal and Retrocardial Implantation of Implantable Cardioverter Defibrillator Lead in a Child with Type 3 Long QT Syndrome.	Pediatric Cardiology.	32	1048-1052	2011

[IV]

研究成果の刊行物・別刷

Circulation Journal

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

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Manuscript ID:	CJ-12-0215.R2
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Date Submitted by the Author:	n/a
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Category:	Congenital heart desease/Child cardiovascular disease

Complete List of Authors:
Ichikawa, Yasuhiro; Yokohama City University, Cardiovascular Research Institute
Yokoyama, Utako; Yokohama City University, Cardiovascular Research Institute
Iwamoto, Mari; Yokohama City University, Department of Pediatrics
Oshikawa, Jin; Yokohama City University, Department of Medical Science and Cardiorenal Medicine
Okumura, Satoshi; Yokohama City University, Cardiovascular Research Institute
Sato, Motohiko; Yokohama City University, Cardiovascular Research Institute
Yokota, Syumpei; Yokohama City University, Department of Pediatrics
Masuda, Munetaka; Yokohama City University, Department of Surgery
Asou, Toshihide; Kanagawa Children's Medical Center, Department of Cardiovascular Surgery
Ishikawa, Yoshihiro; Yokohama City University, Cardiovascular Research Institute

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

Yasuhiro Ichikawa MD [1,2], Utako Yokoyama MD, PhD [1], Mari Iwamoto MD, PhD [2], Jin Oshikawa MD, PhD [3], Satoshi Okumura MD, PhD [1], Motohiko Sato MD, PhD [1], Shumpei Yokota MD, PhD [2], Munetaka Masuda MD, PhD [4], Toshihide Asou MD, PhD [5], Yoshihiro Ishikawa MD, PhD [1]

1) Cardiovascular Research Institute, Yokohama City University
2) Department of Pediatrics, Yokohama City University
3) Department of Medical Science and Cardiorenal Medicine, Yokohama City University
4) Department of Surgery, Yokohama City University
5) Department of Cardiovascular Surgery, Kanagawa Children's Medical Center

Short title: PDE3 Inhibitors Dilate the DA Without Remodeling.

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Correspondence to: Utako Yokoyama or Yoshihiro Ishikawa, Cardiovascular Research Institute, Yokohama City University, 3-9 Fukuura, Kanazawa-ku, Yokohama, Kanagawa 236-0004, Japan
Fax:+81-45-787-1470
Telephone:+81-45-787-2575
Email: utako@yokohama-cu.ac.jp or yishikaw@med.yokohama-cu.ac.jp
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