Published in final edited form as:

Anesth Analg. 2010 November ; 111(5): 1117–1121. doi:10.1213/ANE.0b013e3181f3351a.

Opioid-induced preconditioning is dependent on caveolin-3 expression

Yasuo M. Tsutsumi, MD PhD^{*}, Yoshitaka Kawaraguchi, MD[†], Ingrid R. Niesman, MS[†], Hemal H. Patel, PhD[†], and David M. Roth, MD PhD[†]

^{*}Department of Anesthesiology, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima, JAPAN

[†]Department of Anesthesiology, University of California, San Diego and VAMC San Diego, San Diego, CA USA

Abstract

We tested the hypothesis that caveolin-3 (Cav-3) is essential for opioid-induced preconditioning *in vivo*. Cav-3 overexpressing mice, Cav-3 knockout mice (Cav-3 KO), and controls were exposed to myocardial ischemia/reperfusion (I/R) in the presence of SNC-121 (SNC), a delta-selective opioid agonist, or naloxone, a non-selective opioid antagonist. Controls were protected from I/R injury by SNC. No protection was produced by SNC in Cav-3 KO mice. Cav-3 over-expressing mice showed innate protection from I/R compared with controls that was abolished by naloxone. Our results show opioid-induced preconditioning is dependent on Cav-3 expression and endogenous protection in Cav-3 over-expressing mice is opioid dependent.

Introduction

Myocardial ischemia/reperfusion (I/R) injury is a major cause of morbidity and mortality in the operative and non-operative settings. Experimentally, myocardial I/R injury can be attenuated using a variety of interventions (termed “preconditioning”) including brief episodes of ischemia, opioids, and volatile anesthetics.^{1,2} Caveolae are flask-like invaginations (~100 nm in diameter) of the sarcolemmal membrane that are enriched in lipids (e.g., cholesterol, and glycosphingolipids), structural proteins (caveolins), and signaling molecules.³ Recently, we have shown that caveolins are essential in myocardial preconditioning and that cardiac specific overexpression of caveolin-3 results in innate cardiac protection.^{4,5} Additionally, we identified that cardiac protection produced by opioid-induced preconditioning is absent when caveolae are disrupted *in vitro*.⁶ The impact of caveolin-3 (Cav-3) and caveolae on opioid-induced preconditioning *in vivo* and has not been investigated. In addition, the role of opioid receptors in the innate cardiac protection observed in Cav3 over-expressing mice is unknown. Therefore, we tested the hypothesis that expression of Cav-3 is a critical component of opioid-induced preconditioning *in vivo* and

Corresponding Author: Yasuo M. Tsutsumi, MD, PhD, Department of Anesthesiology, Institute of Health Biosciences, the University of Tokushima Graduate School, 3-18-15 Kuramoto, Tokushima, JAPAN 770-8503, TEL +81-88-633-7181 FAX +81-88-633-7182.

Disclaimers:

None

Reprints:

None requested

Conflict of Interest:

The authors have no conflicts to report

that the innate cardiac protection observed in Cav-3 over-expressing mice is opioid dependent.

Methods

All animals were treated in compliance with the *Guide for the Care and Use of Laboratory Animals*, and with animal use protocols approved by the VA San Diego Healthcare System Institutional Animal Care and Use Committee (San Diego, CA). Male C57BL/6 Cav-3 knockout (Cav-3 KO) mice and C57BL/6 transgenic mice with cardiac myocyte-specific over-expression of Cav-3 were generated as reported previously.^{4,7} Male C57BL/6 mice (Jackson Labs) served as controls.

In untreated hearts from all three experimental groups, left ventricular homogenates were used for immunoblotting for Cav-3 expression as described previously.⁴ Homogenates were separated by SDS-PAGE with 10% polyacrylamide precast gels and transferred by electroelution. Membranes were visualized using primary (Cav-3 and GAPDH) and secondary antibodies (anti-mouse). Additionally, electron microscopy was performed on myocardium as described previously⁴ to assess morphologic caveolae in all experimental groups.

Myocardial I/R was induced *in vivo* as previously described.⁴ Briefly, mice were anesthetized with pentobarbital and the lungs mechanically ventilated. Cardiac catheterization via the right carotid artery was performed with a microtip pressure transducer for the determination of hemodynamics. Ischemia was produced by occluding the left coronary artery with a snare occluder for 30 minutes. Hearts were reperfused for 2 hours.

Cav-3 KO and control mice were randomly assigned to receive the δ -opioid receptor agonist, SNC-121 (10 mg/kg)⁸, 15 minutes before I/R to initiate opioid-induced preconditioning (Fig. 1). A subset of Cav-3 over-expressing mice were randomly treated with naloxone (a non-selective opioid receptor antagonist; 3.0 mg/kg i.v.)⁹ 10 minutes before ischemia (Fig. 1). After reperfusion, the area at risk (AAR) and the myocardial infarct size were determined as described before.⁴ Cardiac troponin-I in serum was measured with a high-sensitivity mouse cardiac troponin-I ELISA kit.

Sample size was determined for the primary endpoint of myocardial infarct size. The standard deviation in measurement of infarct size was determined from historic control mice of similar strain undergoing a similar ischemia-reperfusion protocol (SD=6%). We determined the sample size needed would be at least 6 mice per experimental group assuming two-tailed α of 0.05 at 90% power with a hypothetical difference of 15%. Statistical analyses were performed by one-way ANOVA, followed by Bonferroni *post-hoc* test or unpaired Student's *t*-test. All data are expressed as mean $\pm$ SD. Statistical significance was defined as $P<0.05$.

Results

We first assessed Cav-3 expression in the three treatment groups by immunoblot and caveolae by electron microscopy. Immunoblots of left ventricular homogenates revealed expression of Cav-3 in the both control mice and Cav-3 over-expressing mice, and the absence of Cav-3 protein in Cav-3 KO mice (Fig. 2A). The relative expression of Cav-3 protein was greater in Cav-3 over-expressing mice compared with control mice. Electron microscopy revealed caveolae formation in control and Cav-3 overexpressing mice, however, no caveolae were observed in Cav-3 KO mice (Fig. 2B-D).

We next assessed hemodynamics and myocardial infarction in the experimental groups with opioid receptor agonism or antagonism. There were no differences in pre-occlusion baseline hemodynamics (Table 1) or in the cardiac AAR among groups (Fig. 3A). Mice pre-administrated SNC-121 had a reduction in myocardial infarct size compared with control mice ($30\pm 2\%$ versus $41\pm 2\%$, $P<0.05$, respectively; Fig. 3B). This cardiac protective effect of opioid-induced preconditioning was abolished in Cav-3 KO mice ($40\pm 4\%$). Additionally, we confirmed the previous findings of an innate preconditioning-like cardiac protection in Cav-3 over-expressing mice. The innate protective effect of Cav-3 over-expression was eliminated by pretreatment with naloxone ($25\pm 4\%$ versus $41\pm 2\%$ of AAR, $P<0.05$, respectively; Fig. 3B). The myocardial infarct sparing effects of opioid-induced preconditioning and Cav-3 overexpression were confirmed by cardiac troponin-I measurements (Fig. 3C).

Discussion

The signal transduction pathways described for myocardial preconditioning are complex. It is thought that preconditioning involves activation of G-protein coupled receptors. Specific receptors that have been identified in producing preconditioning include A_1 and A_3 adenosine,¹⁰⁻¹² adrenergic,¹³ M_2 muscarinic,^{14,15} B_2 bradykinin,^{16,17} δ opioid,¹⁸ and cannabinoid¹⁹ receptors. Opioid receptor activation produces preconditioning and cardiac protection from myocardial I/R injury in experimental animals and in patients.^{20,21} Opioid peptides are expressed in the heart.²² Further, it has been demonstrated that the delta opioid receptor, the dominant opioid receptor in the heart, facilitates preconditioning and cardiac protection.²³

Caveolae are cholesterol and sphingolipid enriched invaginations of the plasma membrane and caveolins, especially Cav-3, are the structural proteins essential for caveolae formation in myocytes. Caveolins function as chaperones and scaffolds recruiting signaling molecules to caveolae to provide direct temporal and spatial regulation of signal transduction.³ Specifically, many G-protein coupled receptors including opioid receptors localize to caveolae and co-immunoprecipitate with caveolins.²⁴ Previous work in isolated-adult cardiac myocytes from our laboratory showed that caveolae were essential for protection from hypoxia/reoxygenation *in vitro* and immunohistochemistry showed Cav-3 organized opioid receptors in caveolae.^{6,24} Our previous work suggested that caveolae and the expression of Cav-3 might be essential for opioid-induced preconditioning *in vivo*. The current study confirmed this notion by showing that Cav-3 deficient mice, in which no caveolae were observed, were resistant to opioid-induced preconditioning. We showed also that the endogenous cardiac protection in Cav-3 over-expressing mice could be abolished by pretreatment with naloxone, a non-specific opioid receptor antagonist. The mechanism involving opioid dependence of the innate cardiac protection afforded by Cav-3 over-expression is under investigation. A limitation of the current study is the lack of use of selective opioid receptor antagonists.

In conclusion, the expression of Cav-3 appears essential for δ -opioid receptor-induced cardiac protection from myocardial I/R injury. Our results suggest the caveolae and caveolins within the heart are critical for opioid-induced preconditioning and that caveolins may be novel therapeutic targets for preconditioning the heart to myocardial I/R injury.

Acknowledgments

Financial Support:

Supported by Grant-in-Aid for Young Scientists (A) 20591833 (to Dr. Tsutsumi) from Japan Society for the Promotion of Science, Tokyo; Takeda Science Foundation, Tokyo (to Dr. Tsutsumi); Scientist Development Grant

060039N (to Dr. Patel) from American Heart Association, Burlingame, California; a VA Merit Grant (to Dr. Roth) from the Department of Veterans Affairs, Washington, D.C.; and National Institutes of Health grants HL081400 (to Dr. Roth), HL066941 (to Dr. Roth), and HL091071 (to Dr. Patel) from the United States Public Health Service, Bethesda, Maryland.

References

1. Peart JN, Headrick JP. Clinical cardioprotection and the value of conditioning responses. *Am J Physiol Heart Circ Physiol*. 2009; 296:H1705–20. [PubMed: 19363132]
2. Frassdorf J, De Hert S, Schlack W. Anaesthesia and myocardial ischaemia/reperfusion injury. *Br J Anaesth*. 2009; 103:89–98. [PubMed: 19502287]
3. Patel HH, Murray F, Insel PA. Caveolae as organizers of pharmacologically relevant signal transduction molecules. *Annu Rev Pharmacol Toxicol*. 2008; 48:359–91. [PubMed: 17914930]
4. Tsutsumi YM, Horikawa YT, Jennings MM, Kidd MW, Niesman IR, Yokoyama U, Head BP, Hagiwara Y, Ishikawa Y, Miyanochara A, Patel PM, Insel PA, Patel HH, Roth DM. Cardiac-specific overexpression of caveolin-3 induces endogenous cardiac protection by mimicking ischemic preconditioning. *Circulation*. 2008; 118:1979–88. [PubMed: 18936328]
5. Tsutsumi YM, Kawaraguchi Y, Horikawa YT, Niesman IR, Kidd MW, Chin-Lee B, Head BP, Patel PM, Roth DM, Patel HH. Role of caveolin-3 and glucose transporter-4 in isoflurane-induced delayed cardiac protection. *Anesthesiology*. 2010; 112:1136–45. [PubMed: 20418694]
6. Patel HH, Head BP, Petersen HN, Niesman IR, Huang D, Gross GJ, Insel PA, Roth DM. Protection of adult rat cardiac myocytes from ischemic cell death: role of caveolar microdomains and delta-opioid receptors. *Am J Physiol Heart Circ Physiol*. 2006; 291:H344–50. [PubMed: 16501018]
7. Horikawa YT, Patel HH, Tsutsumi YM, Jennings MM, Kidd MW, Hagiwara Y, Ishikawa Y, Insel PA, Roth DM. Caveolin-3 expression and caveolae are required for isoflurane-induced cardiac protection from hypoxia and ischemia/reperfusion injury. *J Mol Cell Cardiol*. 2008; 44:123–30. [PubMed: 18054955]
8. Tsutsumi YM, Yokoyama T, Horikawa Y, Roth DM, Patel HH. Reactive oxygen species trigger ischemic and pharmacological postconditioning: in vivo and in vitro characterization. *Life Sci*. 2007; 81:1223–7. [PubMed: 17915258]
9. Patel HH, Tsutsumi YM, Head BP, Niesman IR, Jennings M, Horikawa Y, Huang D, Moreno AL, Patel PM, Insel PA, Roth DM. Mechanisms of cardiac protection from ischemia/reperfusion injury: a role for caveolae and caveolin-1. *FASEB J*. 2007; 21:1565–74. [PubMed: 17272740]
10. Safran N, Shneyvays V, Balas N, Jacobson KA, Nawrath H, Shainberg A. Cardioprotective effects of adenosine A1 and A3 receptor activation during hypoxia in isolated rat cardiac myocytes. *Mol Cell Biochem*. 2001; 217:143–52. [PubMed: 11269659]
11. Shainberg A, Safran N, Balas N, Zinman T, Isaac A, Schwab K, Akabayov B, Shneyvays V. The role of A1/A3 adenosine receptor activation in reduction of cardiomyocyte injury caused by hypoxic stress and in induction of apoptosis in rat cardiomyocyte cultures. *Adv Exp Med Biol*. 2000; 486:201–5. [PubMed: 11783485]
12. De Jonge R, Out M, Maas WJ, De Jong JW. Preconditioning of rat hearts by adenosine A1 or A3 receptor activation. *Eur J Pharmacol*. 2002; 441:165–72. [PubMed: 12063088]
13. Gao F, Chen J, Lopez BL, Christopher TA, Gu J, Lysko P, Ruffolo RR Jr, Ohlstein EH, Ma XL, Yue TL. Comparison of bisoprolol and carvedilol cardioprotection in a rabbit ischemia and reperfusion model. *Eur J Pharmacol*. 2000; 406:109–16. [PubMed: 11011041]
14. Yao Z, Gross GJ. Acetylcholine mimics ischemic preconditioning via a glibenclamide-sensitive mechanism in dogs. *Am J Physiol Heart Circ Physiol*. 1993; 264:H2221–5.
15. Yao Z, Gross GJ. Role of nitric oxide, muscarinic receptors, and the ATP-sensitive K⁺ channel in mediating the effects of acetylcholine to mimic preconditioning in dogs. *Circ Res*. 1993; 73:1193–201. [PubMed: 8222090]
16. Li Y, Sato T. Dual signaling via protein kinase C and phosphatidylinositol 3'-kinase/Akt contributes to bradykinin B2 receptor-induced cardioprotection in guinea pig hearts. *J Mol Cell Cardiol*. 2001; 33:2047–53. [PubMed: 11708848]

17. Lagneux C, Adam A, Lamontagne D. A study of the mediators involved in the protection induced by exogenous kinins in the isolated rat heart. *Int Immunopharmacol*. 2003; 3:1511–8. [PubMed: 12946448]
18. Schultz JE, Rose E, Yao Z, Gross GJ. Evidence for involvement of opioid receptors in ischemic preconditioning in rat hearts. *Am J Physiol Heart Circ Physiol*. 1995; 268:H2157–61.
19. Lamontagne D, Lepicier P, Lagneux C, Bouchard JF. The endogenous cardiac cannabinoid system: a new protective mechanism against myocardial ischemia. *Arch Mal Coeur Vaiss*. 2006; 99:242–6. [PubMed: 16618028]
20. Schultz JEJ, Hsu AK, Gross GJ. Morphine mimics the cardioprotective effect of ischemic preconditioning via a glibenclamide-sensitive mechanism in the rat heart. *Circ Res*. 1996; 78:1100–4. [PubMed: 8635241]
21. Tomai F, Crea F, Gaspardone A, Versaci F, Ghini AS, Ferri C, Desideri G, Chiariello L, Gioffré PA. Effects of naloxone on myocardial ischemic preconditioning in humans. *J Am Coll Cardiol*. 1999; 33:1863–9. [PubMed: 10362186]
22. Weil J, Eschenhagen T, Fleige G, Mittmann C, Orthey E, Scholz H. Localization of preproenkephalin mRNA in rat heart: selective gene expression in left ventricular myocardium. *Am J Physiol Heart Circ Physiol*. 1998; 275:H378–H84.
23. Schultz JE, Hsu AK, Gross GJ. Ischemic preconditioning in the intact rat heart is mediated by delta1- but not mu- or kappa-opioid receptors. *Circulation*. 1998; 97:1282–9. [PubMed: 9570199]
24. Head BP, Patel HH, Roth DM, Lai NC, Niesman IR, Farquhar MG, Insel PA. G-protein coupled receptor signaling components localize in both sarcolemmal and intracellular caveolin-3-associated microdomains in adult cardiac myocytes. *J Biol Chem*. 2005; 280:31036–44. [PubMed: 15961389]

Figure 1.

Schematic illustration of the experimental protocol. Control (n=10) and Cav-3 KO (n=8) mice were treated with the δ -opioid receptor agonist, SNC-121 (SNC; n=8 and Cav-3 KO +SNC; n=6, respectively), 15 minutes before myocardial ischemia. Additionally, cardiac-specific Cav-3 over-expressing (Cav-3 OE, n=7) mice were pretreated with the opioid antagonist, naloxone (Cav-3 OE+Nal; n=7).

Figure 2.

(A) Western blot analysis confirmed the absence of Cav-3 protein in the hearts of the Cav-3 KO mice. Cav-3 over-expressing (OE) mice have increased expression of Cav-3 protein when compared to control mice. (B-D) Electron micrographs of cardiac myocytes from control (B), Cav-3KO (C), and Cav-3 OE mice (D). Arrows denote caveolae. CCP=clathrin coated pit. Bar length=100nm.

Figure 3.

(A) Area at risk for myocardial ischemia as a percent of the left ventricle was no different among groups. (B) Myocardial infarct size was reduced by SNC in control mice, however, Cav-3 KO mice were not able to be protected with SNC. Treatment with naloxone abolished the innate cardiac protection in Cav-3 overexpressing (OE) mice. (C) Serum cardiac troponin-I, a marker of cardiac myocyte damage, confirmed the findings of the infarct size measurements. Sample size is depicted within parentheses (n) in panel A.

Table 1**Hemodynamics**

	Pre-occlusion	Ischemia	Reperfusion
Heart rate, beats · min ⁻¹			
Control	417 ± 22	417 ± 52	315 ± 17 *
SNC	421 ± 48	403 ± 27	403 ± 46 #
Cav-3 KO	426 ± 31	415 ± 26	382 ± 24 *, #
Cav-3KO+SNC	413 ± 37	408 ± 36	386 ± 29
Cav-3 OE	418 ± 44	396 ± 38	405 ± 40 #
Cav-3 OE+Naloxone	414 ± 41	411 ± 56	374 ± 81
Mean arterial pressure, mmHg			
Control	68 ± 4	69 ± 4	61 ± 7 *
SNC	69 ± 7	68 ± 6	67 ± 10
Cav-3 KO	68 ± 5	66 ± 6	62 ± 3
Cav-3 KO+SNC	71 ± 4	73 ± 4	66 ± 9
Cav-3 OE	69 ± 5	68 ± 5	70 ± 6
Cav-3 OE+Naloxone	70 ± 3	71 ± 6	66 ± 5
Rate-Pressure Product, beats · min ⁻¹ · mmHg · 10 ³			
Control	28.6 ± 2.2	28.6 ± 2.7	19.1 ± 2.5 *
SNC	29.0 ± 4.8	27.5 ± 2.5	27.3 ± 5.4 #
Cav-3 KO	28.9 ± 3.5	27.3 ± 3.5	23.8 ± 1.7 *
Cav-3 KO+SNC	29.4 ± 2.4	29.8 ± 3.1	25.3 ± 3.5
Cav-3 OE	29.0 ± 3.7	27.1 ± 3.6	28.5 ± 3.9 #
Cav-3 OE+Naloxone	29.1 ± 3.6	29.3 ± 4.6	24.8 ± 5.6

Data are mean ± SD.

SNC, SNC-121; KO, knockout; OE, overexpression.

* Significantly ($P < 0.05$) different from pre-occlusion (intragroup comparison).

Significantly ($P < 0.05$) different from Control (intergroup comparison).