

Clinically Relevant Concentrations of Olprinone Reverse Attenuating Effect of Propofol on Isoproterenol-induced Cyclic Adenosine Monophosphate Accumulation in Cardiomyocytes

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ABSTRACT

Propofol has been shown to attenuate β -adrenoreceptor-mediated signal transduction in cardiomyocytes. Cyclic adenosine monophosphate (cAMP) is an essential second messenger of β -signal transduction, while olprinone, a phosphodiesterase-III inhibitor, improves poor cardiac performance by increasing cAMP levels. In the present study, we investigated the effects of olprinone toward the reducing effect of propofol on β -adrenoreceptor-mediated increases in cAMP production. First, suspensions of rat ventricular myocytes were incubated with isoproterenol or olprinone and the effects on cAMP concentrations were assessed. Next, propofol was added prior to the addition of isoproterenol or olprinone. Finally, following preincubation with propofol, isoproterenol with or without olprinone was added. Both isoproterenol and olprinone increased cAMP production in a dose-dependent manner. However, clinically relevant concentrations of olprinone (up to 10^{-7} M) did not cause a significant increase. Propofol (10^{-7} ~ 10^{-4} M) attenuated isoproterenol-stimulated increases in cAMP production (decrease of $2 \pm 4\%$ ~ $43 \pm 1\%$, as compared to the isoproterenol-stimulated state). However, the agent did not alter olprinone (10^{-7} M)-stimulated cAMP production. Olprinone (10^{-8} ~ 10^{-6} M) reversed the attenuating effect of propofol (10^{-5} M) toward isoproterenol (10^{-7} M)-stimulated cAMP production dose-dependently (increase of $10 \pm 5\%$ ~ $79 \pm 4\%$ as compared to the propofol-attenuated state). Our results suggest that an improvement in cardiac function is provided by olprinone when the β -adrenoreceptor-mediated signaling pathway is inhibited by propofol.

Key words: *Propofol, β -adrenoreceptor, Phosphodiesterase-III inhibitor, Olprinone*

Due to recent progress in medical technology, the number of surgical operations for patients with deteriorated cardiac function has been increasing. As a result, many patients with a failing heart require anesthesia after already being administered inotropic agents. It is necessary to perform all anesthesia safely, therefore, it is important to elucidate the potential interactions between anesthetic agents and inotropic agents such as catecholamines.

Propofol is an intravenous anesthetic agent that

has become one of the most popular among available drugs used for anesthesia. We previously reported that propofol attenuated β -adrenoreceptor-mediated signal transduction in cardiomyocytes⁷⁾, which suggested that it is important to use the agent carefully for critically ill patients receiving inotropic agents for hemodynamic support. Olprinone hydrochloride, 1, 2-dihydro-6-methyl-2-oxo-5-[imidazo (1, 2-a) pyridin-6-yl]-3-pyridine carbonitrile hydrochloride monohydrate, is a newly developed phosphodiesterase III inhibitor

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with positive inotropic and vasodilatory actions that improves myocardial mechanical efficiency without increasing O_2 consumption⁹. Several clinical reports have shown its beneficial effects, such as improving hemodynamics and peripheral circulation after a cardiopulmonary bypass (CPB) procedure^{1,17}, and providing easy weaning from CPB¹³. Further, basic studies have found that pretreatment with olprinone elicits cardioprotective effects in a failing heart after myocardial infarction¹⁰, possibly via a cyclic adenosine monophosphate (cAMP)-dependent mechanism¹⁵. cAMP is an important second messenger of intracellular β -adrenoreceptor-mediated signal transduction and olprinone improves cardiac performance, which is attributable to an increase in intracellular cAMP. Thus, we hypothesized that olprinone was able to reverse the reducing effect of propofol toward isoproterenol-stimulated increases in cAMP production.

METHODS

All experimental procedures and protocols were approved by the Hiroshima University Institutional Animal Care and Use Committee (Hiroshima, Japan). Propofol was obtained from Research Biochemicals International (Natick, MA) and solubilized in dimethylsulfoxide to appropriate stock concentrations. Pure propofol was used to avoid any possible effect of the intralipid emulsion diluent on the cell signaling pathways. Isoproterenol was obtained from Sigma Chemical Co. (St. Louis, MO) and olprinone from Eizai Co. Ltd. (Tokyo, Japan).

Ventricular myocytes were freshly isolated from adult male Sprague-Dawley rat hearts by enzyme digestion using a Langendorff apparatus, as previously described⁷. Immediately after euthanasia, the hearts were rapidly removed and perfused in a retrograde manner at a constant flow rate (8 ml/min) with oxygenated (95% O_2 /5% CO_2) Krebs-Henseleit buffer (KHB; 35°C) containing the following: 118 mM NaCl, 4.8 mM KCl, 1.2 mM $MgCl_2$, 1.2 mM KH_2PO_4 , 1.2 mM $CaCl_2$, 37.5 mM $NaHCO_3$, and 16.5 mM dextrose, at pH 7.35. After a 5-min equilibration period, the perfusion buffer was changed to Ca^{2+} -free KHB containing collagenase type II (347 U/ml; Worthington Biochemical Corp., Freehold, NJ). After digestion with collagenase for 20 min, the ventricles were minced and shaken in KHB, and the resulting cellular digest was washed, filtered, and resuspended in phosphate-free HEPES-buffered saline (HBS; 25°C) containing the following: 118 mM NaCl, 4.8 mM KCl, 1.2 mM $MgCl_2$, 1.25 mM $CaCl_2$, 11.0 mM dextrose, 25.0 mM HEPES, and 5.0 mM pyruvate, at pH 7.35.

cAMP production was assessed in suspensions of freshly isolated myocytes using an enzyme

immunoassay kit. The experimental buffer (HBS) was the same as that used for the resuspension of myocytes during the isolation process. At the end of the protocol, the cells were quickly pelleted using a microfuge (500 $\times g$, 5 sec), then the buffer was aspirated, and the pellets resuspended in ice-cold HBS and centrifuged again (500 $\times g$, 5 sec). The supernatant was aspirated and frozen using liquid nitrogen, after which the samples were thawed on ice, with freezing and thawing repeated 3 times. The preparations were homogenized using a plastic homogenizer in ice-cold ethanol (0.5 ml) to extract cAMP. The homogenates were centrifuged (1500 $\times g$, 10 min) and the supernatants were collected. Each pellet was then washed with 0.5 ml of 75% ethanol and centrifuged again at 1500 $\times g$ for 10 min, after which the supernatants were combined and dried under clear oxygen. Samples were stored at $-20^\circ C$. The production of cAMP was assessed using an enzyme-linked immunoassay kit (Cayman Chemical, Ann Arbor, MI).

First, to determine the dose-dependent effects of isoproterenol and olprinone on cAMP production by myocytes, suspensions of rat ventricular myocytes were incubated with isoproterenol (10^{-9} – 10^{-6} M) or olprinone (10^{-9} – 10^{-4} M) for 10 min at 37°C. Second, to identify the extent to which propofol alters β -adrenoreceptor-mediated increases in cAMP production or increases in cAMP production mediated by the inhibition of PDE-III, we activated β -adrenoreceptors with isoproterenol or inhibited PDE-III with olprinone after exposure to propofol. Propofol (10^{-7} – 10^{-4} M) was added 5 min prior to the addition of isoproterenol (10^{-7} M) or olprinone (10^{-7} M), and the effects on cAMP concentrations were assessed. Finally, to determine whether olprinone alters the effect of propofol toward isoproterenol-induced cAMP production, following preincubation with propofol (10^{-4} M) for 5 min, isoproterenol (10^{-7} M) with or without olprinone (10^{-8} – 10^{-6} M) was added for 10 min.

The experiments were repeated at least 3 times, with measurements performed in triplicate. Data are shown as the mean \pm standard error of the mean. Statistical comparisons were made using a one-way analysis of variance with a post hoc test (Fisher's PLSD). p values < 0.05 were considered to be statistically significant.

RESULTS

The levels of cAMP production following stimulation by isoproterenol and olprinone are shown in Fig. 1 and 2, respectively. Isoproterenol (10^{-9} – 10^{-6} M) increased cAMP in a dose-dependent manner ($96 \pm 4\%$, $128 \pm 7\%$, $146 \pm 7\%$, and $165 \pm 26\%$, respectively, of the control). Further, olprinone in a range of 10^{-9} – 10^{-4} M increased cAMP accumulation in a dose-dependent manner ($96 \pm 6\%$, $104 \pm$

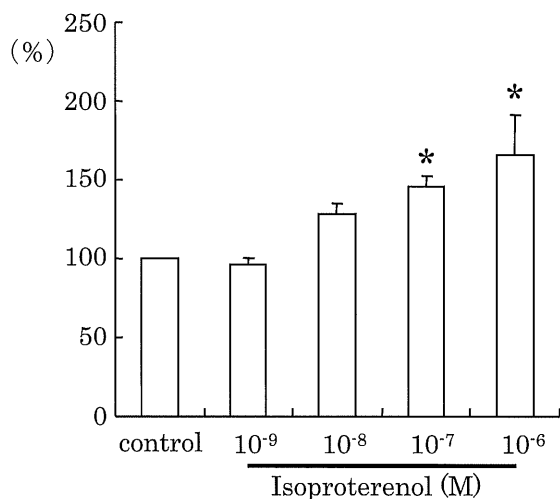


Fig. 1. Summarized data for the effect of isoproterenol on cyclic adenosine monophosphate production. Data are expressed as % of control. * $p < 0.05$ compared with control. $n = 3$ hearts.

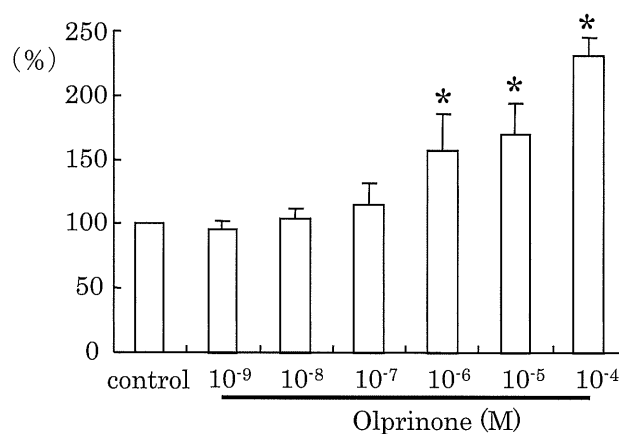


Fig. 2. Summarized data for the effect of olprinone on cyclic adenosine monophosphate production. Data are expressed as % of control. * $p < 0.05$ compared with control. $n = 3$ hearts.

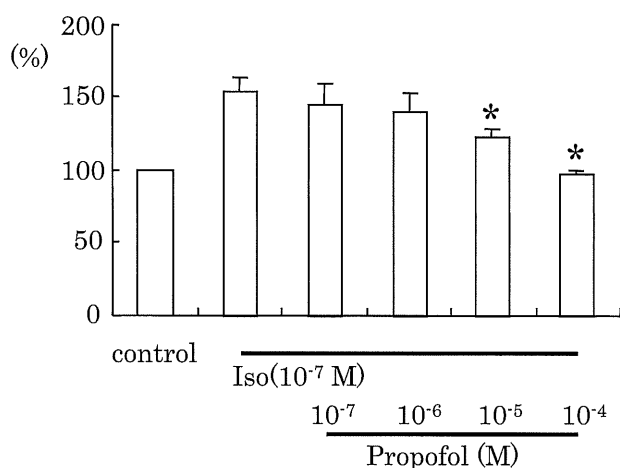


Fig. 3. Summarized data for the effect of propofol on isoproterenol (Iso) (10^{-7} M) - stimulated cyclic adenosine monophosphate production. Data are expressed as % of control. * $p < 0.05$ compared with isoproterenol - stimulated state. $n = 3$ hearts.

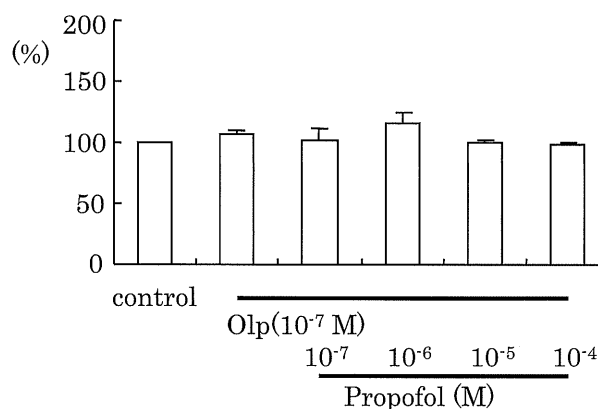


Fig. 4. Summarized data for the effect of propofol on olprinone (Olp) (10^{-7} M) - stimulated cyclic adenosine monophosphate production. Data are expressed as % of control. $n = 3$ hearts.

8%, $124 \pm 16\%$, $158 \pm 28\%$, $170 \pm 24\%$, and $230 \pm 16\%$, respectively, of the control), whereas clinically relevant concentrations¹²⁾ (up to 10^{-7} M) did not cause a significant increase in cAMP levels, in contrast to isoproterenol.

Propofol (10^{-7} ~ 10^{-4} M) attenuated the isoproterenol (10^{-7} M)-stimulated increase in cAMP production (decrease of $2 \pm 4\%$, $5 \pm 5\%$, $17 \pm 7\%$, and $43 \pm 1\%$, respectively, of isoproterenol-stimulated state) (Fig. 3). Propofol did not significantly alter olprinone (10^{-7} M)-stimulated cAMP production (Fig. 4).

The effect of olprinone toward the propofol-induced reduction of cAMP production stimulat-

ed by isoproterenol is shown in Fig. 5. Olprinone (10^{-8} ~ 10^{-6} M) reversed the attenuating effect of propofol (10^{-5} M) toward isoproterenol (10^{-7} M)-stimulated cAMP production dose-dependently (increase of $10 \pm 5\%$, $28 \pm 6\%$, and $79 \pm 4\%$, respectively, of propofol-attenuated state). Notably, clinically relevant concentrations of olprinone (10^{-7} M) increased cAMP levels significantly ($p < 0.05$).

DISCUSSION

The present results demonstrated that olprinone was able to reverse the propofol-attenuated production of cAMP stimulated by

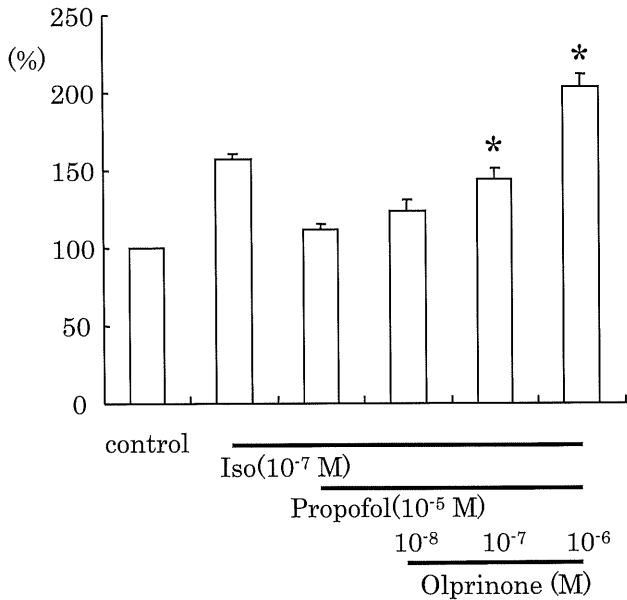


Fig. 5. Summarized data for the effect of olprinone on propofol (10^{-5} M)-induced attenuation of isoproterenol (Iso) (10^{-7} M)-stimulated cyclic adenosine monophosphate production.

Data are expressed as % of control. * $p < 0.05$ compared with propofol-induced attenuated state. $n = 3$ hearts.

isoproterenol. cAMP is an important second messenger of intracellular β -adrenoreceptor-mediated signal transduction, and all currently available inotropic agents increase myocardial contractility by increasing it as a final common pathway. Therefore, they suggest that an improvement of cardiac function is potentially provided by olprinone when the β -adrenoreceptor-mediated signaling pathway is inhibited by propofol.

Olprinone is a newly developed phosphodiesterase III inhibitor characterized by several properties. It has positive inotropic and vasodilatory actions, and has been shown to improve myocardial mechanical efficiency without increasing O_2 consumption⁹, as also seen with other PDE III inhibitors such as amrinone and milrinone. There have also been several clinical reports that showed its beneficial effects, especially for patients undergoing cardiac surgery. Orime et al reported that olprinone increased the cardiac index and decreased systemic vascular resistance to almost the same extent as milrinone, and also allowed for easy weaning from CPB¹³. Arai also reported the effectiveness of olprinone for improvement of hemodynamics and peripheral circulation following CPB in a randomized controlled trial¹. Further, Sha et al found that administration of olprinone as a continuous infusion reduced the number of cases that required combined catecholamine administration during coronary artery bypass grafting¹⁷. Compared with dopamine, olprinone improved sevoflurane-

induced acute myocardial depression with lower ventricular energy expenditure than dopamine³. This beneficial effect will become more important in rational selection of inotropic agents when increased myocardial metabolic demand should be avoided. Basic research studies have shown that repetitive pre-ischemic infusions of olprinone elicited cardioprotective effects in failing hearts after myocardial infarction¹⁰. The mechanism of this direct cardioprotective effect of olprinone is thought to be via a cAMP- and PKA-dependent pathway¹⁵. Compared with other PDE III inhibitors, such as amrinone and milrinone, olprinone showed the most potent inotropic effect, with a peripheral vasodilating effect similar to that of milrinone but less than that of amrinone, and a lower chronotropic effect than that of milrinone². Taken together, these results suggest that application of olprinone for cardiac support is useful for patients with poor cardiac performance.

Propofol has been extensively used in diverse populations of patients, even those with deteriorated cardiac function, even though it decreases systemic vascular resistance, sympathetic activity, and cardiac output, and its cardiac effects have several characteristics. Propofol has been reported to inhibit the L-type Ca^{2+} channel¹⁹, as well as intracellular Ca^{2+} transients and contraction⁵, and increase myofilament Ca^{2+} sensitivity⁶. Further, the agent has either no direct effect on contractile function¹⁴ or only a modest negative inotropic effect at supra-clinical concentrations¹⁸. However, a very recent study demonstrated that clinically relevant concentrations of propofol can depress cardiac function in isolated perfused hearts when the experiments were performed at physiologic temperatures and physiologic stimulation frequencies⁴. In clinical study, prophylactic administration of ephedrine, up to 0.2 mg/kg IV, attenuated, but did not abolish the hypotensive effect of propofol in elderly female patients⁸. Even though the extent to which the direct negative inotropic effect may contribute to the decrease in blood pressure in their study is unclear, we have to use propofol with the utmost care. As for the β -signaling pathway, we previously reported that propofol at clinically relevant concentrations attenuated β -adrenoreceptor-mediated signal transduction in cardiomyocytes⁷, which implies the need for care when using propofol for critically ill patients who are receiving inotropic agents like catecholamines for hemodynamic support.

cAMP is an important second messenger of intracellular β -adrenoreceptor-mediated signal transduction, and measurement of its concentration may indicate interactions between various drugs and the β -signaling pathway. In our previous study, we found that propofol interacts with the β -signaling pathway that exists between the β -receptor and adenylyl cyclase⁷. The current

findings also showed that propofol attenuated isoproterenol-induced cAMP production, which we considered to be from a countering effect of propofol toward the isoproterenol-induced positive inotropic effect⁷). Olprinone improves cardiac performance by increasing cAMP levels⁹). Thus, we hypothesized that olprinone can reverse the reducing effect of propofol toward the isoproterenol-stimulated increase in cAMP production. When olprinone was administered alone in an *in vitro* study¹⁶), 10^{-5} M increased myocardial cAMP levels along with the force of contraction, which is similar to our present results. Further, in the present study we showed that olprinone at a lower concentration (10^{-7} M) increased the ability of propofol to attenuate cAMP production induced by isoproterenol. Previous studies have reported an inotropic response by papillary muscles to isoproterenol potentiated by pretreatment with olprinone at a minimally-effective inotropic concentration (3×10^{-7} M) in guinea pig cardiac muscle¹¹) and canine ventricular muscle¹⁶) tissues, implying that a small dose of olprinone in combination with isoproterenol could potentiate inotropic action in cardiac muscle. Our data are consistent with these reports. Further, in clinical settings, the maximum therapeutic plasma concentrations of olprinone are generally accepted to be 10^{-7} M¹²). Therefore, the present results suggest that a clinical dose of olprinone overcomes the disadvantage caused by propofol toward cardiac function, when the β -adrenoreceptor-mediated signaling pathway is inhibited by propofol.

Due to limitations in our laboratory equipment, we only assessed the interactions among propofol, isoproterenol, and olprinone intracellularly measuring cAMP. Additional study is needed to determine if these findings are consistent with those in a clinical setting. Nevertheless, since the increase in contractile force occurred concomitantly with the increase in cAMP level by the all currently available inotropic agents, we believe that the present findings are applicable, at least in part, to supracellular situations.

In summary, we demonstrated that clinically relevant concentrations of olprinone, a phosphodiesterase-III inhibitor, reversed the attenuating effect of propofol toward β -adrenoreceptor-mediated cAMP production in rat cardiomyocytes. Our results suggest that improvement in cardiac function is potentially provided by olprinone when the β -adrenoreceptor-mediated signaling pathway is inhibited by propofol.

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REFERENCES

1. **Arai, T.** 2004. Effects of olprinone hydrochloride after coronary artery bypass grafting. *Masui* **53**: 122-130.
2. **Hirabayashi, Y., Igarashi, T., Saitoh, K., Fukuda, H., Suzuki, H. and Shimizu, R.** 2000. Comparison of the effects of amrinone, milrinone and olprinone in reversing bupivacaine induced cardiovascular depression. *Acta Anaesthesiol. Scand.* **44**: 1128-1133.
3. **Iwade, M., Nomura, M., Uezono, S., Ashikari, E. and Ozaki, M.** 2006. Effects of dopamine and olprinone on ventricular energetics in sevoflurane-induced acute left ventricular depression in dogs. *J. Cardiothorac. Vasc. Anesth.* **20**: 358-363.
4. **Kanaya, N., Gable, B., Wickley, P.J., Murray, P.A. and Damron, D.S.** 2005. Experimental conditions are important determinants of cardiac inotropic effects of propofol. *Anesthesiology* **103**: 1026-1034.
5. **Kanaya, N., Murray, P.A. and Damron, D.S.** 1998. Propofol and ketamine only inhibit intracellular Ca^{2+} transients and contraction in rat ventricular myocytes at supraclinical concentrations. *Anesthesiology* **88**:781-791.
6. **Kanaya, N., Murray, P.A. and Damron, D.S.** 2001. Propofol increases myofilament Ca^{2+} sensitivity and intracellular pH via activation of Na^+-H^+ exchange in rat ventricular myocytes. *Anesthesiology* **94**: 1096-1104.
7. **Kurokawa, H., Murray, P.A. and Damron, D.S.** 2002. Propofol attenuates β -adrenoreceptor-mediated signal transduction via a protein kinase C-dependent pathway in cardiomyocytes. *Anesthesiology* **96**: 688-698.
8. **Michelsen, I., Helbo-Hansen, H.S., Kohler, F., Lorenzen, A.G., Rydlund, E. and Bentzon, M.W.** 1998. Prophylactic ephedrine attenuates the hemodynamic response to propofol in elderly female patients. *Anesth. Analg.* **86**: 477-481.
9. **Mizushige, K., Ueda, T., Yukiiri, K. and Suzuki, H.** 2002. Olprinone: a phosphodiesterase III inhibitor with positive inotropic and vasodilator effects. *Cardiovasc. Drug Rev.* **20**:163-174.
10. **Nomura, Y., Horimoto, H., Mieno, S., Nakahara, K., Okawa, H., Yoshida, M. and Shinjiro, S.** 2003. Repetitive preischemic infusion of phosphodiesterase III inhibitor olprinone elicits cardioprotective effects in the failing heart after myocardial infarction. *Mol. Cell. Biochem.* **248**: 179-184.
11. **Ogawa, T., Ohhara, H., Tsunoda, H., Kuroki, J. and Shoji, T.** 1989. Cardiovascular effects of the new cardiotonic agent 1, 2-dihydro-6-methyl-2-oxo-5-(imidazo [1, 2-a]pyridin-6-yl) -3-pyridine carbonitrile hydrochloride monohydrate. 1st communication: studies on isolated guinea pig cardiac

- muscles. *Arzneimittelforschung* **39**:33-37.
12. **Onomoto, M., Tsuneyoshi, I., Yonetani, A., Suehiro, S., Matsumoto, K., Sakata, R. and Kanmura, Y.** 2005. Differential pharmacologic sensitivities of phosphodiesterase-3 inhibitors among human isolated gastroepiploic, internal mammary, and radial arteries. *Anesth. Analg.* **101**:950-956.
 13. **Orime, Y., Shiono, M., Hata, H., Yagi, S., Tsukamoto, S., Kimura, S., Ohmiya, S., Sezai, A., Yamada, H., Obana, M. and Sezai, Y.** 1998. Post-operative effects of olprinone after coronary artery bypass grafting. *Ann. Thorac. Cardiovasc. Surg.* **4**:340-346.
 14. **Riou, B., Besse, S., Lecarpentier, Y. and Viars, P.** 1992. In vitro effects of propofol on rat myocardium. *Anesthesiology* **76**:609-616.
 15. **Sanada, S., Kitakaze, M., Papst, P.J., Asanuma, H., Node, K., Takashima, S., Asakura, M., Ogita, H., Liao, Y., Sakata, Y., Ogai, A., Fukushima, T., Yamada, J., Shinozaki, Y., Kuzuya, T., Mori, H., Terada, N. and Hori, M.** 2001. Cardioprotective effect afforded by transient exposure to phosphodiesterase III inhibitors: the role of protein kinase A and p38 mitogen-activated protein kinase. *Circulation* **104**:705-710.
 16. **Satoh, H. and Endoh, M.** 1990. Effects of a new cardiotonic agent 1, 2-dihydro -6-methyl-2-oxo-5-[imidazo (1, 2-a) pyridin-6-yl]-3-pyridine carbonitrile hydrochloride monohydrate (E-1020) on contractile force and cyclic AMP metabolism in canine ventricular muscle. *Jpn. J. Pharmacol.* **52**: 215-224.
 17. **Sha, K., Iwata, M., Mori, M., Motozu, Y., Yonemoto, N., Kitaguchi, K., Hirai, K. and Furuya, H.** 2003. Evaluation of the different doses of olprinone for continuous infusion after cardiopulmonary bypass for coronary artery bypass surgery. *Masui* **52**:621-625.
 18. **Stowe, D.F., Bosnjak, Z.J. and Kampine, J.P.** 1992. Comparison of etomidate, ketamine, midazolam, propofol, and thiopental on function and metabolism of isolated hearts. *Anesth. Analg.* **74**:547-558.
 19. **Yang, C-Y., Wong, C.S., Yu, C.C., Luk, H.N. and Lin, C.I.** 1996. Propofol inhibits cardiac L-type calcium current in guinea pig ventricular myocytes. *Anesthesiology* **84**:626-635.