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Lysophosphatidylcholine-induced myocardial damage is inhibited by pretreatment with poloxamer 188 in isolated rat heart

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Abstract

Lysophosphatidylcholine (LPC) accumulates in myocardial tissues and coronary sinus during ischemia, and plays important role in the development of ischemia-reperfusion injury and ischemic ventricular arrhythmia. The aim of this study was to examine whether pretreatment of poloxamer 188 (P-188), a nonionic and non-toxic surfactant, can prevent the cardiac dysfunction induced by exogenous LPC perfusion in Langendorff perfused rat heart model. LPC (6 μM) significantly ($p < 0.05$) decreased heart rate (HR) and left ventricular developed pressure (LVDP) from 274.3 ± 23.2 to 175.0 ± 42.9/min and from 115.9 ± 11.3 to 26.7 ± 7.1 mmHg, respectively. The LPC-induced reduction of HR and LVDP did not recover by washout of LPC. Pretreatment with P-188 (1 mM for 30 min) inhibited completely the LPC-induced decreases of HR and LVDP. The pretreatment with P-188 also prevented the LPC-induced increases of left ventricular end-diastolic pressure (LVEDP) and GOT release, significantly ($p < 0.05$). The coronary perfusion pressure (CPP) rose ($p < 0.01$) by the LPC perfusion from 71.9 ± 5.3 to 121.9 ± 13.0 mmHg, significantly, but pretreatment of P-188 did not affect the LPC-induced vasoconstriction. Our results suggest that exogenous LPC causes irreversible cardiac injury by the sarcolemmal membrane disruption followed by Ca overload, and this LPC-induced cardiac injury, probably, can be prevented by the pretreatment with poloxamer 188. (Mol Cell Biochem **248**: 209–215, 2003)

Key words: Langendorff perfusion, ischemia-reperfusion, cardioprotection, left ventricular developed pressure, left ventricular end-diastolic pressure

Introduction

In myocardial ischemia-reperfusion injury, various factors, such as generation of oxygen free radicals [1, 2], increased or decreased autacoids release [3], activation of proteases [4, 5] and activation of the cascade for apoptotic cell death [6–8], play important rolls. Among them, effects of the generation of lysophosphatidylcholine (LPC) during the ischemic period has been widely investigated. Although LPC is continuously generated from phosphatidylcholine (PC) by the catalytic action of phospholipase A_2, LPC is rapidly converted to PC by lysophospholipid acyltransferase under normoxic condition. Under ischemic condition, however, since activity of phospholipase A_2 is increased and acyl CoA, a cofactor for phospholipid acyltransferase, is decreased, LPC is accumulated in intra- and extra-cellular space. Exogenous application of LPC induces damages on the heart very similar to those caused by ischemia-reperfusion, i.e. development of various types of arrhythmias and Ca overload [27, 28]. The Na^+/Ca^{2+} exchange mechanism can contribute to the LPC-induced Ca overload, but we have previously reported that LPC causes cell membrane poration in cultured endothelial cells and rabbit ventricular myocytes [9, 10]. This membrane poration forms large pores which can uptake trypan blue into the cells [9], indicating that Ca^{2+} passes through these pores, and Ca overload is elicited.

Poloxamer 188 (pluronic F-68), a nontoxic, nonionic surface active agent, is clinically used as emulsifier for artificial blood and as an anticoagulator in the microcirculation [11]. Sharma *et al*. suggested that in the artificial lipid mem-

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branes, poloxamer 188 is incorporated into the lipid bilayers, thereby decreasing their susceptibility to electroporation [12]. Another experiment showed that injection of poloxamer 188 facilitates the recovery from electroporation induced by electrical shock [13]. It has been also reported that administration of poloxamer 188 reduces ischemia-reperfusion injury in the rabbit hearts [14] and rat testicles [15]. These results suggest that poloxamer 188 increase the resistance to membranes damages induced by electroporation and ischemia-reperfusion injury by sealing the membranes. However, it is not yet clear that poloxamer 188 protect heart from which factor(s) contributing to the development of ischemia-reperfusion injury. The aim of this study was to examine whether pretreatment of poloxamer 188 can prevent LPC-induced cardiac dysfunction, one of the major contributors for ischemia-reperfusion injury, in Langendorff perfused rat heart, and investigate dose- and time-dependency for protective effects of poloxamer 188-pretreatment.

Materials and methods

Preparation of hearts and perfusion protocols

This investigation conformed with the Guide for the Care and Use of Laboratory Animals published US National Institute of Health (NIH publication no. 85-23, revised 1985) and was approved by our institution's animal experimentation committee.

Male Sprague-Dawley rats, weighing 285–340 g, were used. Rats were sacrificed and their hearts were excised quickly to establish Langendorff perfusion. Each heart was perfused with modified Krebs-Henseleit solution (containing in mM: NaCl 116.0, $NaHCO_3$ 25.0, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KCl 4.7, KH_2PO_4 1.2 and glucose 5.5; pH 7.4) in a retrograde direction at a constant flow rate of 13 ml/min without recirculation. The perfusate was warmed to 38°C and oxygenated with a 95% O_2-5% CO_2 gas mixture to elevate the P_{O2} to over 400 mmHg. A latex balloon was inserted through the mitral annulus into the left ventricular cavity, and distilled water (0.2–0.4 ml) was injected into the balloon until it was inflated to just above the level required to produce visible elevation of the left ventricular end-diastolic pressure (LVEDP). The left ventricular developed pressure (LVDP), LVEDP, heart rate (HR), and coronary perfusion pressure (CPP) were monitored throughout the experiment. The extent of irreversible myocardial damage was assessed by determining the activity of released GOT into coronary effluent. The GOT activity in 10 µl aliquots was estimated by dry chemical method with commercially available kit (Fuji Film Ltd. Co., Tokyo, Japan). The GOT activity was normalized to IU/1 g dry tissue/min.

Experimental protocols

After a 15-min stabilization, hearts were challenged with the following treatments (Fig. 1). Control: The hearts were perfused with normal Krebs solution for 35 min, and they were applied vehicle (ethanol; 0.2% solvent of LPC) for 15 min. The hearts were then washed out for 20 min (Fig. 1, top). LPC treatment: The hearts were perfused for 35 min with normal Krebs solution, and then L-α-lysophosphatidylcholine palmitoyl (LPC: 6 µM) was added for 15 min. After the LPC perfusion, hearts were washed out for 20 min (Fig. 1, middle). P-188 treatment: The hearts were perfused with Krebs solution containing Poloxamer 188 (P-188: 1 mM) for 30 min and P-188 was washed out for 5 min. After the P-188 pretreatment, LPC (6 µM) was applied without P-188 for 15 min followed by a 20 min of wash out period (Fig. 1, bottom). In additional experiments, the concentration of P-188 was decreased to 0.24 mM or the duration of pretreatment decreased to 10 min.

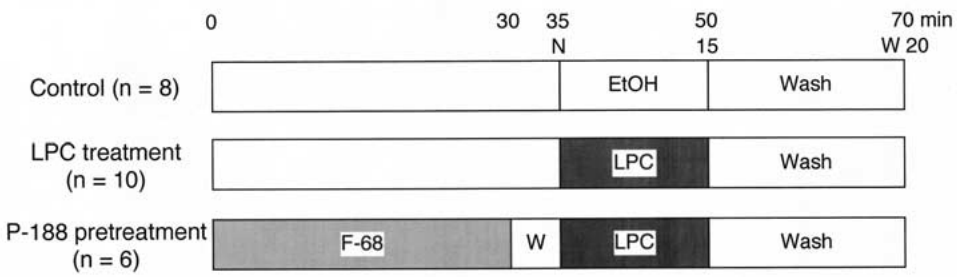

Fig. 1. Experimental protocols. After a 15 min stabilization period, the hearts were divided into 3 groups. Time-matched control hearts (top: n = 8); perfusion with normal Krebs solution for 35 min and followed by the vehicle (0.2% ethanol) and the vehicle was washed out for 20 min. LPC treatment group (middle, n = 10): the hearts were perfused with 6 µM LPC containing Krebs solution instead of vehicle. P-188 pretreatment group (bottom, n = 6): the hearts were perfused with P-188 containing Krebs solution for 30 min (indicated as F-68 in this figure), followed by washout of P-188 with normal Krebs solution for 5 min. The following perfusion protocol was the same as those for the LPC treatment group. At several time points (N, 5, 10, 15, W5 and W10 min), coronary effluent was collected and the GOT activity level was determined.

Drugs

LPC and P-188 were purchased from Sigma (St. Louis, MO, USA). LPC was dissolved in ethanol at concentration of 10 mM and 600 µl of the stock solution was added to 1L of Krebs solution. P-188 (10% solution or flakeable solid) was directly added to the perfusate to obtain the final concentration of 1 mM.

Statistical analysis

Data are expressed as mean ± S.E.M. Differences between means were analyzed by unpaired Student's *t*-test, as deemed appropriate, and those at $p < 0.05$ were considered significant.

Results

Time-dependent changes in heart function

During the first 35 min of experiment (pretreatment period), contractile activities of time-matched control and LPC group showed no significant changes, that is, at the beginning of experiment and at after 35 min perfusion, LVDP was 113.9 ± 8.2 and 102.3 ± 11.7 mmHg, HR was 291.5 ± 9.5 and 285.2 ± 11.0/min, and CPP was 64.2 ± 2.7 and 64.7 ± 1.8 mmHg (data of both groups were summed). Similarly, 30 min of P-188 pretreatment did not affect cardiac activity except for CPP. At the same time points, LVDP was 101.8 ± 7.2 and 98.2 ± 9.7 mmHg, and HR was 299.8 ± 16.4 and 277.3 ± 17.4/min. P-188 perfusion, however significantly elevated CPP; CPP elevated from 52.7 ± 9.3 mmHg at the beginning of P-188 perfusion to 109.5 ± 15.6 mmHg at the end (30 min) of P-188 perfusion. During the later half of experiment (LPC perfusion and washout period), time-matched controls showed no significant contractile changes with time, i.e. from the beginning of vehicle perfusion to the end of the experiment (70 min), HR increased by 0.8% (N.S.), LVDP decreased by 14.9% (N.S.), LVEDP increased by 2.3 mmHg (N.S.), the amount of GOT released decreased by 6.8% (N.S.), and CPP increased by 13.5% (N.S.). There was no significant difference in these parameters between at the beginning and at the end of the vehicle perfusion, too. The time-dependent changes in these parameters, were shown in the corresponding figures.

Effect of LPC on cardiac function and protective effects of P-188 pretreatment against LPC-induced injury

In 10 hearts which were treated with LPC without P-188 pretreatment, 3 hearts developed ventricular fibrillation (VF). Among the 3 hearts, one heart developed VF during LPC application, and the other 2 hearts developed VF during washout period and it continued until the end of washout period. Data from these three hearts were excluded from the following analysis. In P-188 pretreated hearts, none of the hearts developed VF throughout the experiment.

The cardiac contractile activity was evaluated with pressure-rate product (PRP = LVDP × HR). Each data was normalized with PRP at 35 min (before LPC application: N in Fig. 1). In control hearts, PRP at 15 min vehicle application and at 20 min washout were 98.8 ± 11.9 and 94.9 ± 13.0%, respectively (Fig. 2). LPC (6 µM) significantly ($p < 0.01$) decreased PRP, to 21.6 ± 5.9% at 15 min LPC perfusion. The reduction of PRP did not recover by 20 min washout of LPC and it remained at 10.0 ± 5.4% ($p < 0.01$) of control, suggesting that the LPC-induced reduction of PRP is irreversible (Fig. 2, closed square). In P-188 pretreated group (1 mM, 30 min), LPC did not decrease PRP; PRP at 15 min LPC perfusion and 20 min washout were 95.3 ± 9.9 and 83.2 ± 10.5%, respectively (Fig. 2, open square).

Effects of LPC on HR and LVDP were examined separately (Fig. 3). In LPC group, HR decreased ($p < 0.05$) gradually during LPC perfusion (from 274.3 ± 23.2 to 175.0 ± 42.9/min at the end of LPC perfusion; Fig. 3A). Upon washout of LPC, however, HR abruptly and substantially ($p < 0.01$) decreased; HR decreased to 59.3 ± 22.5/min at the end of washout period. In P-188 pretreatment group, neither LPC

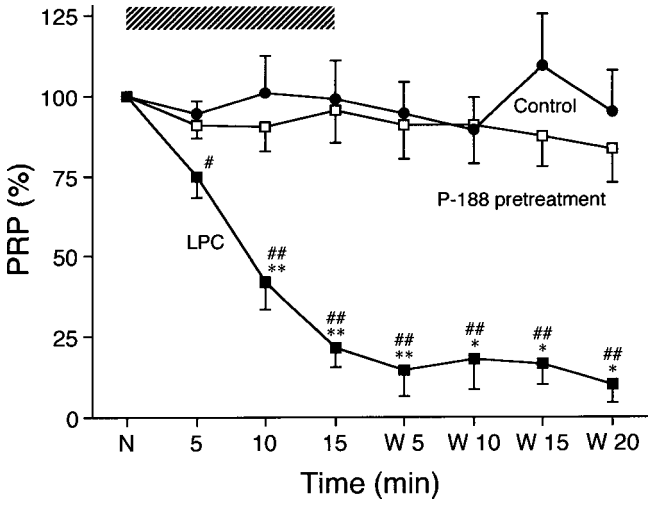

Fig. 2. Effects of pretreatment with P-188 on LPC-induced decrease of the pressure-rate product (PRP = HR × LVDP). LPC (6 µM) caused acute decrease of PRP (closed square). The pretreatment with P-188 (1 mM for 30 min) decreased this LPC-induced PRP decrease (open circles). The striped bar in upper left of graph indicates LPC perfusion time (15 min). The closed circles represent time-matched control. $^{**}p < 0.01$ and $^{*}p < 0.05$: vs. control group at the same time points. $^{\#\#}p < 0.01$ and $^{\#}p < 0.05$: vs. prior to LPC perfusion (N).

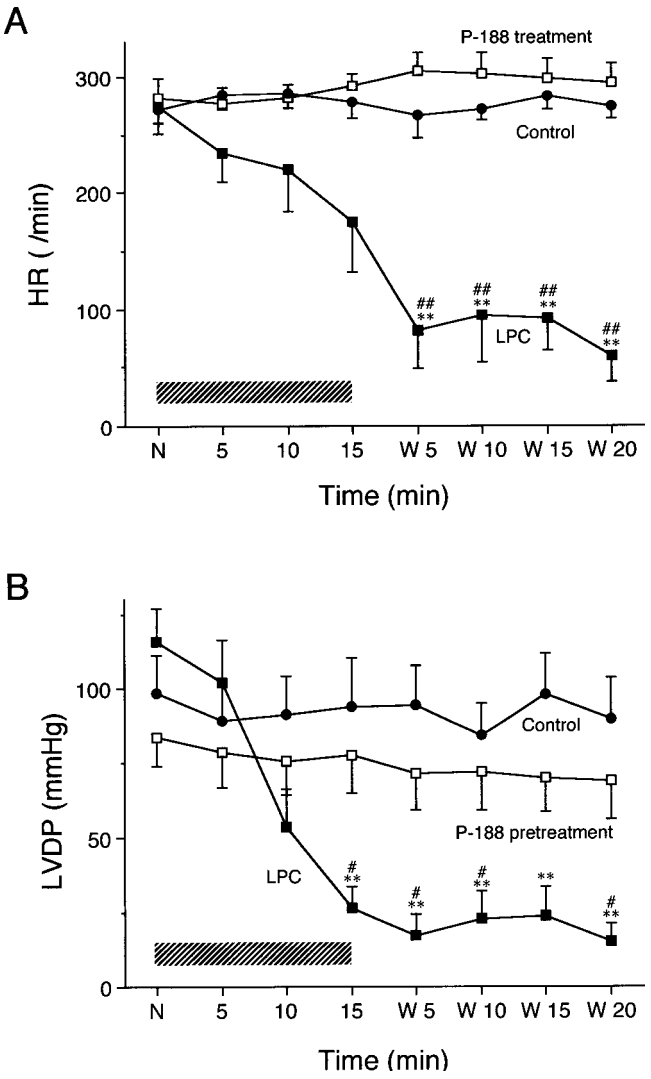

Fig. 3. Effects of LPC on HR (panel A) and LVDP (panel B). LPC decreased both HR and LVDP, and washout of LPC did not result in the recovery of LPC-induced decreases (closed square). These LPC-induced decreases of HR and LVDP were prevented by pretreatment with P-188 (open square).

perfusion nor washout of LPC changed HR: at 15 min of LPC and 20 min of washout were 291.8 ± 11.2 and 294.8 ± 16.5/min, respectively. In LPC group, LVDP decreased ($p < 0.01$) from 115.9 ± 11.3 to 26.7 ± 7.1 mmHg during the LPC perfusion (Fig. 3B) and the level did not recover by the following washout period (15.6 ± 6.4 mmHg at 20 min of washout). Pretreatment with P-188 inhibited the LVDP decrease by LPC, completely ($p < 0.05$): at 15 min of LPC and 20 min of washout were 77.8 ± 12.8 and 68.7 ± 12.5 g, respectively.

The changes in LVEDP were shown in Fig. 4. In the presence of 6 μM LPC, LVEDP rose remarkably ($p < 0.01$); it was elevated by 53.5 ± 10.6 mmHg at the end of LPC period. During the following washout period, LVEDP did not decrease and remained at high level: 50.9 ± 9.9 mmHg at 20 min of washout. P-188 pretreatment almost completely inhibited the LPC-induced LVEDP increase; elevation (N.S.) was 3.7 ± 2.3 mmHg at the end of washout.

LPC-induced myocardial damage

The activity of released GOT increased significantly during the perfusion of 6 μM LPC (Fig. 5); at 20 min after LPC application it reached 3.998 ± 0.891 IU/min/g; it was significantly ($p < 0.01$) higher than that of time-matched control (0.344 ± 0.051 IU/min/g). The increase in GOT release during the washout period was remained at high level (2.696 ± 0.445 IU/min/g at 20 min of washout). In P-188 pretreatment group, the activity of released of GOT slightly increased to 0.612 ± 0.087 IU/min/g by the pretreatment of P-188 (at 35 min: N in Fig. 5). During the LPC perfusion and washout, GOT activity was significantly ($p < 0.05$) higher than that of time-matched control, but LPC perfusion and washout did not affect significantly GOT activity; GOT activity at 15 min after LPC perfusion and 10 min after washout were 0.868 ± 0.153 and 0.781 ± 0.145 IU/min/g, respectively ($p < 0.05$ vs. LPC perfused group).

Concentration- and time-dependency of P-188 pretreatment

In order to determine the concentration- and time-dependency of protective effects of P-188, we changed the incubation time

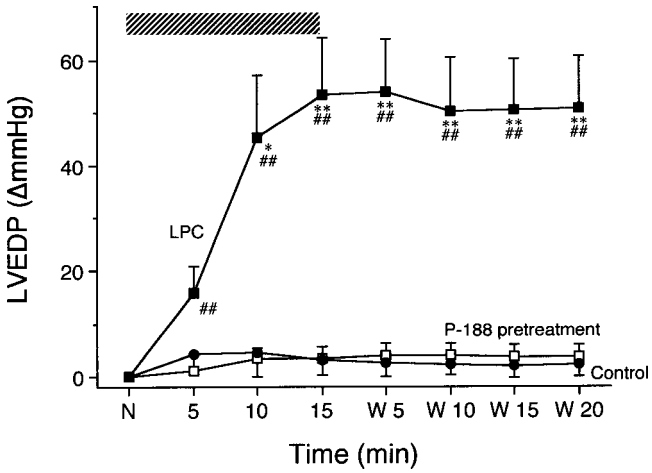

Fig. 4. Effect of P-188 pretreatment on LPC-induced LVEDP increase. The LVEDP was normalized at point N and then comparatively evaluated at various time points. LVEDP increased by more than 50 mmHg with LPC perfusion without P-188 pretreatment (closed square). Pretreatment with 1 mM P-188 for 30 min completely inhibited the LPC-induced LVEDP significantly increased (open square). $^{**}p < 0.01$ and $^{*}p < 0.05$: vs. control group at the same time points. $^{\#\#}p < 0.01$: vs. before LPC perfusion (N).

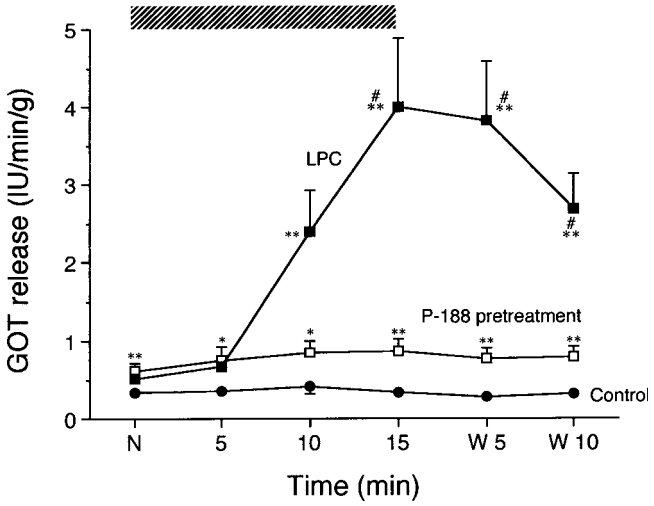

Fig. 5. LPC-induced irreversible cardiac damage and protective effect of P-188 pretreatment. In the hearts perfused with LPC, the activity of released GOT increased significantly, and did not decrease with washout of LPC (closed square). In P-188 pretreated hearts, although basal GOT activity was slightly higher than those of time-matched controls and LPC treated hearts, LPC-induced cardiac damage was significantly inhibited (open square). **p < 0.01 for experimental vs. control groups at the same time points. ##p < 0.01 and #p < 0.05 vs. P-188 pretreatment group at the same time points.

and concentration of P-188 (Fig. 6). In 7 hearts, duration of P-188 (1 mM)-pretreatment was decreased to 10 min (short-pretreatment group). In short-pretreatment group, during the LPC perfusion, the reduction of PRP, the elevation of LVEDP and the activity of released GOT were not different from those of LPC group (32.0 ± 17.3%, 57.4 ± 16.1 mmHg and 4.089 ± 0.913 IU/min/g at 15 min, respectively). In 7 hearts, the concentration of P-188 was decreased to 0.24 mM (pretreatment duration was 30 min: low concentration group). Its protective effect on PRP was comparable to that of 1 mM pretreated group at each time point during experiments; PRP at 15 min LPC perfusion and at 20 min washout of LPC were 90.5 ± 17.3 and 83.0 ± 14.0%, respectively. However, protection of the 0.24 mM P-188 was incomplete on the elevation of LVEDP and increase in GOT release, i.e. peak value of LVEDP, and peak amount of GOT release were 24.7 ± 9.0 mmHg and 1.500 ± 0.431 IU/min/g, respectively ($p < 0.05$).

One heart in short-time group and 2 hearts in low concentration group developed VF during LPC perfusion, and VF continued until the end of washout period.

LPC-induced changes in coronary vasotone

In the present preparations, since hearts were perfused at a constant flow rate, elevation and depression of CPP directly indicate coronary vasoconstriction and vasodilation, respectively. Perfusion with 6 μM LPC induced significant ($p <$

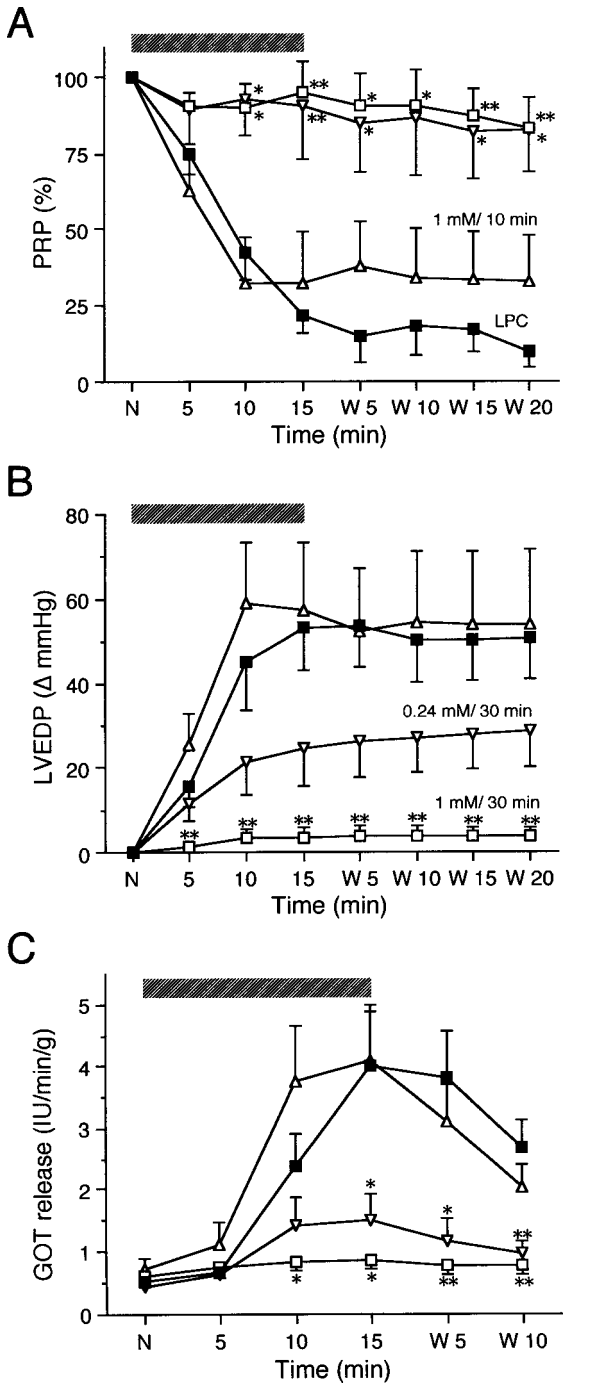

Fig. 6. Concentration- and time-dependency of the protective effects of P-188 (panel A): Change in PRP (%), (panel B): changes in LVEDP (mmHg) and (panel C): changes in released GOT activity. Symbol description for each panel; closed square: LPC perfusion group, open square: normal pretreatment group, open triangle with apex pointing down (∇): low concentration group, open triangle with apex pointing up (Δ): short pretreatment group. Low concentration group inhibited LPC-induced PRP decrease, but the inhibitory effects on LVEDP increase was incomplete. Short pretreatment group failed to protect the heart from LPC-induced functional and irreversible damages. **p < 0.01 and *p < 0.05: vs. LPC perfused group at the same time points.

0.01) vasoconstriction (Fig. 6); CPP increased from 71.9 ± 5.3 to 121.9 ± 13.0 mmHg after 15 min of LPC perfusion. This level did not fall after washout (116.6 ± 11.4 mmHg at washout 20 min). In P-188 pretreated hearts, CPP was elevated to 109.5 ± 15.6 mmHg by the P-188 perfusion and then decreased to 97.3 ± 16.8 mmHg by the 5 min of P-188 washout; the level was still significantly higher than those of control and LPC group. The level of CPP further rose to 124.2 ± 15.9 mmHg within 5 min LPC perfusion and remained at that level during the LPC perfusion. The following 20 min washout failed to recover the elevated level of CPP (110.3 ± 16.0 mmHg). Neither short-pretreatment group nor low concentration group inhibited the elevation of CPP by LPC perfusion.

Discussion

In this study, we have demonstrated that exogenous LPC reduces HR, LVDP, PRP and increases LVEDP, CPP and GOT release resulting in irreversible cardiac injury in isolated Langendorff perfused rat heart. Our study also showed that this LPC-induced cardiac injury is blocked almost completely by the pretreatment with nonionic surfactant, poloxamer 188 (pluronic F-68).

The LPC-induced decrease of PRP (Fig. 2) was caused by the reduction of both of HR and LVDP (Fig. 3), and the significant elevation of LVEDP (Fig. 4) was also observed. Our and published [16] results suggest that the sarcolemmal membrane was damaged irreversibly, resulting continuous Ca^{2+} influx and myocardial Ca overload. There are various possibilities for the Ca overload by LPC; LPC activates non-selective cation channel, resulting direct increase of Ca^{2+} influx [17] and/or increased Na^+ influx through the non-selective cation channel elevates intracellular Na^+ concentration and then increase the Ca^{2+} influx via Na^+/Ca^{2+} exchange mechanism [17, 18]; LPC causes membrane poration directly in cultured endothelial cells and ventricular myocytes [9, 10, 19]. It has been reported that poloxamer 188 prevents electroporation in the artificial lipid membranes, and this effect is provided by the incorporation of poloxamer 188 into the lipid bilayers [12]. Therefore, we hypothesized that the pretreatment of heart with poloxamer 188 protect heart from LPC-induced injury. Pretreatment of heart with poloxamer 188 (1 mM for 30 min) could inhibit completely LPC-induced depression of PRP (Fig. 2), elevation of LVEDP (Fig. 4) and GOT release (Fig. 5). These protective effects can be explained by sealing effect of poloxamer 188 [12, 13], i.e. LPC could not be included into the sarcolemmal membrane sealed with poloxamer 188, and failed to form pores on it. Hashizume *et al.* suggested that the some Ca^{2+} channel blockers and β receptor antagonists with high lipophilicity, have protective effects on cell injury induced by LPC independent of their Ca^{2+} channel blocking effects [20]. Their observation may be the similar lines of the present results.

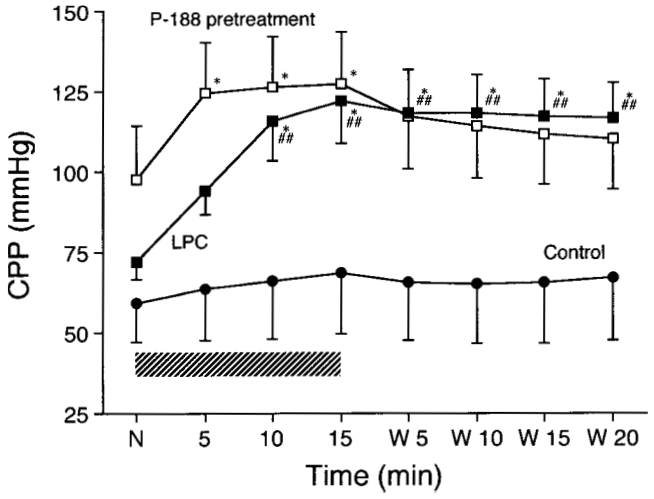

Fig. 7. Effect of P-188 on LPC-induced vasoconstriction. LPC caused vasoconstriction (closed square). In the P-188 pretreatment group, the basal coronary perfusion pressure was higher than that of control, and it further increased with LPC-perfusion (open square). *$p < 0.05$ vs. before LPC perfusion (N). #$p < 0.05$ for experimental group vs. control group.

When the concentration of poloxamer 188 was decreased from 1 mM to 0.24 mM during pretreatment period (30 min), although the LPC-induced depression of PRP and increase of GOT release were completely inhibited, elevation of LVEDP was suppressed only by 50% (Fig. 6). In hearts pretreated with poloxamer 188 for 10 min (short-time pretreatment group), protective effect against LPC-induced contractile depression became slight. These results indicate that pretreatment of poloxamer 188 protect heart from LPC-induced cardiac damage, and its protective effect is time- and concentration-dependent.

Pretreatment of poloxamer 188 did not affect LPC-induced coronary constriction. It is reported that LPC not only causes Ca overload but also modulate signal transduction in vascular smooth muscle and endothelial cell; LPC inhibits delayed rectifier K^+ current in rabbit coronary smooth muscle cells [21], selectively inhibits a G_i protein-dependent pathway in porcine coronary endothelial cells [22], and inhibits endothelium-derived nitric oxide release in porcine coronary artery [23]. All of the above effects of LPC induce vasoconstriction and the activation of intracellular signal transduction mechanisms would be the cause of LPC-induced coronary constriction observed in this study. Pretreatment with poloxamer 188 did not affect LPC-induced elevation of CPP indicating that it has no effect on LPC-induced intracellular signal transduction in coronary smooth muscle cells.

LPC accumulates in myocardial tissues and coronary sinus during ischemia [24–26], and is supposed to be, at least

in part, responsible for the development of ischemia-reperfusion injury and ischemic ventricular arrhythmia [27, 28]. Our results suggest a possibility that the pretreatment of hearts with poloxamer 188 is effective for the protection of the heart from myocardial ischemia-reperfusion injury. We conclude that LPC-induced cardiac damage is caused by the sarcolemmal membrane disruption followed by Ca overload, and this damage can be prevented by the pretreatment with sufficient dose and duration of poloxamer 188 perfusion. The protection induced by poloxamer 188 can be attributed to the sealing effect of the membranes and increased strength of membranes to pathophysiological damages.

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