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Polydatin, a natural polyphenol, protects arterial smooth muscle cells against mitochondrial dysfunction and lysosomal destabilization following hemorrhagic shock

Wang X, Song R, Bian HN, Brunk UT, Zhao M, Zhao K. Polydatin, a natural polyphenol, protects arterial smooth muscle cells against mitochondrial dysfunction and lysosomal destabilization following hemorrhagic shock. Am J Physiol Regul Integr Comp Physiol 302: R805–R814, 2012. First published January 25, 2011; doi:10.1152/ajpregu.00350.2011.—The main objective of this study was to investigate the activity of polydatin on mitochondrial dysfunction and lysosomal stability of arteriolar smooth muscle cells (ASMCs) in severe shock. The experimental animals (rats) were divided into five groups: control, hemorrhagic shock, shock + CsA, shock + Res, and shock + PD (exposed to cyclosporin A, resveratrol, or polydatin following induction of hemorrhagic shock, respectively). The calcine-CeO$_2$ technique revealed opening of ASMC mitochondrial permeability transition pores (mPTP) after shock with resulting mitochondrial swelling, decreased mitochondrial membrane potential ($\Delta$Vm), and reduced intracellular ATP levels. These alterations were all inhibited by exposure to PD, which was significantly more effective than CsA and Res. PD also preserved lysosomal stability, suppressed activation of KATP channels, ASMC hyperpolarization, and reduced vasoresponsiveness to norepinephrine that normally follows severe shock. The results demonstrate that exposure to PD after initiation of severe shock effectively preserves ASMC mitochondrial integrity and has a significant therapeutic effect in severe shock. The effects may partially result from lysosomal stabilization against shock-induced oxidative stress and depressed relocation of hydrolytic enzymes and redox-active lysosomal iron that, in turn, may induce mPTP opening.

hemorrhagic shock; hypotension; polydatin; lysosomes

PERSISTENT OR REFRACTORY HYPOTENSION is one of the major causes of mortality in severe shock (5). It has been shown by us and others, that activation of ATP-sensitive potassium channels ($K_{\text{ATP}}$) on arteriolar smooth muscle cells (ASMCs) results in hypopolarization with inhibition of L-type calcium channels in norepinephrine-stimulated ASMCs. The consequently reduced influx of Ca$^{2+}$ leads to depressed contractile vasoresponsiveness and persistent hypotension (21, 23, 35, 37, 38, 40). Recently, it was found that depressed ASMC ATP levels, with ensuing activation of $K_{\text{ATP}}$ channels, might not only result from insufficient microcirculatory delivery of nutrients and oxygen but also from mitochondrial injury and dysfunction (12, 29). Therefore, it is important to find new ways to prevent mitochondrial injury to improve the outcome of severe shock.

Polydatin (PD, 3, 4', 5-trihydroxystibene-3-monoglucoside) is a monocrystalline drug isolated from a plant (Polygonum cuspidatum) used in traditional Chinese herbal medicine (14, 22, 34). Polydatin medication is a new modality, which has obtained permission for phase II clinical trials from the Chinese Food and Drug Administration. The substance is related to the nonglucoside resveratrol (Res, 3,4',5-trihydroxystibene). It has been shown that Res, like cyclosporine A, improves mitochondrial function in many pathological conditions (4, 17, 24, 33). The aim of the present study was to investigate whether PD may have superior protective effects on ASMCs in shock compared with those offered by Res and CsA.

MATERIALS AND METHODS

Chemicals. Polydatin (PD) and resveratrol (Res) were supplied by Neptunus (Shenzhen, China), while cyclosporin A (CsA) was purchased from Novartis Pharmaceuticals (Basel, Switzerland). The cell-Titer-Glo kit was from Promega (Madison, WI). JC-1, calcine-AM, and mitoTracker were obtained from Molecular Probes (Invitrogen, Carlsbad, CA). Lipid hydroperoxide (LPO) assay kit was from Cayman (Cayman Chemical, Ann Arbor, MI). Acridine orange base (AO) and all other chemicals were from Sigma (St. Louis, MO).

Setting up of a rat hemorrhagic shock model and assaying vascular reactivity. All experimental procedures were carried out in accordance with the U.S. National Institutes of Health’s “Guide for the Care and Use of Laboratory Animals,” with the approval of Ethics Committee from the Southern Medical University, Guangzhou, China. Wistar rats (180–220 g) were anesthetized with a mixture of 13.3% urethane and 0.5% chloralose-$\alpha$ (0.65 ml/100 g body wt). The animals were instrumented to measure mean arterial blood pressure (MAP), and the spino-trapezius muscle was prepared for assaying vascular reactivity, as previously described (9, 36). The arteriolar reactivity to norepinephrine (NE) was measured by topical application of increasing concentration of NE until a threshold concentration was reached,
resulting in complete cessation of blood flow in the transverse arteriole for 10–20 s.

Upon completion of the surgical procedure, all animals were allowed a 30-min equilibration period. To produce shock, blood was withdrawn from a catheter placed in the femoral artery into a syringe containing diluted heparin solution (125 units/ml) until the mean arterial pressure (MAP) stabilized at 30 mmHg (usually within 10 min). When MAP had been maintained at 30 mmHg for 120 min, a bolus dose of NE (10 μg/kg) was applied intravenously, and 5 min later, the shed blood was reinfused.

Animals were randomly assigned into five groups, and the applied doses of CsA, Res, and PD were based on literature (4, 14, 33). Preliminary dose-response experiments (CsA, 3, 6, and 9 mg/100 g body wt; Res, 7.5, 15, and 22.5 mg/100 g body wt; PD, 15, 30, and 45 mg/100 g body wt) were carried out to find out survival times. It was found that the high and middle dose of Res and PD resulted in the similar survival time, while the survival time using the high CsA dose was shorter than that of the middle one, which might be a consequence of the serious immunosuppression side effect of the highest dose of CsA (see Supplemental Table S1). Therefore, the intermediate doses of all three drugs were chosen in the main experiments. Experiments were performed on the following groups: 1) the control (sham) group, in which the rats were only anesthetized and operated on; 2) the Shock group, in which rats were subjected to hemorrhage to maintain MAP at 30 mmHg for 120 min, followed by administration of normal saline and NE (10 μg/kg) and reinfusion of the shed blood with an observation period of 120 min posttreatment; 3) the Shock + CsA group, in which rats were subjected to shock for 120 min followed by administration of cyclosporine A (6 mg/kg) and NE (10 μg/kg), and then, the shed blood was reinfused using the above observation period; 4) the Shock + Res group, in which rats were exposed to hemorrhagic shock for 120 min followed by administration of Res (15 mg/kg) and NE (10 μg/kg), infusion of the shed blood, and observation as above; and 5) the Shock + PD group, in which rats were subjected to shock for 120 min followed by administration of PD (30 mg/kg) and NE (10 μg/kg) before reinfusion and observation as above.

At the end of observation period, catheters were removed and skin wounds were sutured. Survival time and 24-h survival rate were recorded.

Cell isolation procedures. Other animals, similarly treated, were used for cellular tests. Following completion of the above procedures, ASMCs were isolated from mesenteric arterioles (the second A2 branch) (29). Briefly, after dissociation with 0.3 mg/ml papain and 1 mg/ml collagenase, cells were maintained at 4°C in HPSS buffer by 10.220.32.246 on June 9, 2017 http://ajpregu.physiology.org/ Downloaded from

![Ultrastructural alterations of arteriolar smooth muscle cells (ASMC) mitochondria following shock.](image-url)

**Fig. 1. Ultrastructural alterations of arteriolar smooth muscle cells (ASMC) mitochondria following shock.** A: mitochondria are normal (some arrowed) in the control (sham) group. B: mitochondria (some arrowed) are swollen with poorly defined cristae in the shock group. These alterations are partially prevented in the shock + CsA (C), shock + Res (D), and shock + PD groups (E), respectively (some mitochondria arrowed). Scale bars: 1.0 μm. The column graphs (F) show results of morphologic assessments of ASMC total mitochondria areas from the five groups.*P < 0.05, **P < 0.01 vs. sham group; ###P < 0.01 vs. shock group.
consisting of 130 mM NaCl, 5 mM KCl, 1.2 mM MgCl₂, 0.1 mM CaCl₂, 10 mM HEPES, and 10 mM glucose.

Transmission electron microscopy. Mitochondrial ASMC morphology was evaluated as previously described (4, 29). Isolated ASMCs were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, postfixed in 1% cacodylate-buffered osmium tetroxide (OsO₄) and flat-embedded in Epon-Araldite. Ultra-thin sections were stained with 1% uranyl acetate and lead citrate and examined using a transmission electron microscope (Philips CM10; Philips, Eindhoven, The Netherlands).

Determination of mitochondrial permeability transition pore opening. The opening of ASMC mitochondrial permeability transition pores (mPTP) was monitored by the calcein-Co²⁺ technique using both laser scanning confocal microscopy and fluorescence-activated cell sorter (FACS) analysis (15, 25). Calcein is a fluorescent molecule. Upon its esterification to generate calcein-AM, it turns nonfluorescent, but it acquires the ability to cross membranes. Once inside the cell, the probe is deesterified and trapped in its free, fluorophoric form. Co²⁺ causes quenching of cytosolic and nuclear calcein fluorescence, whereas mitochondria fluorescence is mainly unaffected because Co²⁺ does not easily permeate the normal inner mitochondrial membrane. However, if pores open in the inner membrane, calcein in the mitochondrial matrix is quenched by inpassing Co²⁺, leading to quenched mitochondrial fluorescence. MitoTracker is a mitochondri-on-selective probe. For confocal analysis of mPTP, cells were incubated with 1 μM calcein-AM and 2 mM CoCl₂ for 15 min and then exposed to 25 μM mitoTracker for another 30 min at room temperature. Cells were plated in 35-mm Petri dishes and excited at 488 nm (calcein) or 543 nm (mitoTracker) using a Leica TCS SP confocal system (Leica Microsystems, Wetzlar, Germany). For FACS analysis, cells were incubated with 1 μM calcein-AM and 2 mM CoCl₂ for 15 min and then examined in a Gallios Flow Cytometer (Beckman Coulter, Brea, CA) using 488-nm excitation. FACS data were analyzed (10,000 cells/sample) with Gallios software (Beckman Coulter).

Fig. 2. Alterations of ASMC mitochondrial permeability transition pores following shock. The left column of the image shows ASMCs mitochondria exposed to calcein-AM and CoCl₂, the middle column shows exposure to MitoTracker Red, and the right column presents overlay images of the left and middle pictures. The images show normal mitochondrial fluorescence in the sham group (A, left), apparently decreased fluorescence in the shock group (B, left), and partial preservation of the fluorescence in the shock + C₅₇ (C, left) and the shock + Res (D, left) groups. The best preservation is found in the shock + PD group (E, left). Scale bar: 20 μm. F–K: flow cytometric recordings of mitochondrial calcein fluorescence, showing the mean intensity of fluorescence (MIF) to be clearly reduced in the shock group (G), but partially preserved in the three treated groups (H–J and K), especially in the shock + PD group (J and K). *P < 0.01 vs. sham group; #P < 0.01 vs. shock group; ΔP < 0.05, ΔΔP < 0.01 vs. shock + PD group.
**RESULTS**

**PD attenuates mitochondrial damage following severe shock.** ASMC mitochondria from control (sham operated) animals appeared sausage-shaped with electron-dense matrixes and normal cristae (Fig. 1A). Cyclosporin A (CsA) and resveratrol (Res), which are known protectors of mitochondria, partly protected against shock-induced mitochondrial swelling (Fig. 1, C and D). In comparison, a significant protection was also observed in the shock + PD group (Fig. 1, E and F). The findings indicated that mitochondrial injury following initiation of severe shock could be significantly attenuated by administration of PD during the reperfusion phase, which is known to be accompanied by substantial oxidative stress (13).

**PD inhibits opening of mitochondrial permeability transition pores following severe shock.** To explore the molecular mechanisms for the protection of mitochondria by PD, evaluation of the opening of mitochondrial permeability transition pores (mPTPs) was considered critical (13, 27). Fluorescence microscopy of ASMCs from the shock group (Fig. 2B) showed a population of cells with decreased intensity of calcein-dependent mitochondrial fluorescence compared with control cells (Fig. 2A), indicating opening of ASMC mPTPs during severe shock. ASMCs of the shock + CsA group showed partially preserved fluorescence intensity (Fig. 2C) and about the same was found for the shock + Res group (Fig. 2D). The best preservation of mitochondrial fluorescence was found in the shock + PD group (Fig. 2E).

To further quantify the effect of PD on mitochondrial permeability transition, calcein fluorescence was assessed by flow cytometry (n = 5 for each group). This analysis showed a mean fluorescence intensity (MIF) of 156.6 ± 11.8 in the control group and 48.9 ± 7.1 in the shock group, which means that the calcein MIF value was decreased by 68.8% in the shock-group ASMCs (P < 0.01), indicating substantial mPTP opening. The MIF values were mildly preserved to 59.7 ± 13.4 and 62.3 ± 24.8 in the CsA- and Res-treated groups, respectively. Meanwhile, the MIF value was 79.6 ± 8.6 in the shock + PD group, which is significantly different not only from the shock group (P < 0.01; Fig. 2K) but also from the CsA and Res groups (P < 0.05; Fig. 2K), showing that PD is a better protector than both CsA and Res.

**PD suppresses the drop in ASMCs mitochondrial membrane potential following shock.** Decreased potential over the inner mitochondrial membrane (ΔΨm) has been reported in relation to opening of mPTPs (27). To find further support for our notion that PD protects mitochondria by somehow guarding the mPTPs, we investigated the influence on ΔΨm by PD exposure. Mitochondrial depolarization results in increased percent-

Table 1. Changes of ASMC mitochondrial membrane potential in shock (ΔΨm) (χ ± s)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Percentage of Cells with JC-1 Monomer (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>5</td>
<td>13.4 ± 7.73</td>
</tr>
<tr>
<td>Shock</td>
<td>5</td>
<td>80.34 ± 9.01**</td>
</tr>
<tr>
<td>Shock + CsA</td>
<td>5</td>
<td>75.38 ± 18.33**Δ</td>
</tr>
<tr>
<td>Shock + Res</td>
<td>5</td>
<td>53.69 ± 17.10*Δ</td>
</tr>
<tr>
<td>Shock + PD</td>
<td>5</td>
<td>31.57 ± 6.12##</td>
</tr>
</tbody>
</table>

Quantification of mitochondrial depolarization expressed as JC-1 monomer (green fluorescence) in different treatments. ASMC, arteriolar smooth muscle cells; CsA, cyclosporin A; Res, resveratrol; PD, polydatin. *P < 0.05, **P < 0.01 vs. sham group; #P < 0.01 vs. shock group; ΔP < 0.05, ΔΔP < 0.01 vs. shock + PD group.
age of organelles showing JC-1 monomer (green) cells. The shock group contained 80.34 ± 9.01% green cells, which was substantially higher than the value of 13.44 ± 7.73% in the control group (P < 0.01; Table 1). The percentage of cells with low \( \Delta \Psi_m \) was 75.38 ± 18.33% in the shock + CsA group and 53.69 ± 17.10% in the shock + Res group. Interestingly, the percentage of cells with low \( \Delta \Psi_m \) in the shock + PD group was only 31.57 ± 6.12%, which was significantly (\( P < 0.05 \)) different from that of the groups exposed to CsA or Res, indicating a better protective effect of PD than of CsA or Res on ASMCs mitochondrial depolarization in shock.

**PD preserves lysosomal stability following severe shock.** Lysosomal rupture with release of chelatable ferrous iron and a number of powerful proteases (cathepsins) has been demonstrated to induce mitochondrial damage, including mPTP opening (19). Ischemia-reperfusion is well known to induce severe oxidative stress that induces iron-mediated intralysosomal production of hydroxyl radicals (Fenton-type reactions) with ensuing lysosomal membrane permeabilization (LMP). To find out whether LMP was involved in mPTP opening following severe shock, and whether PD had a protective influence, we estimated the influence on lysosomal stability by PD-treatment using the AO-uptake method (39). Fluorescence microscopy of ASMCs from the shock group (Fig. 3B) showed a population of cells with decreased intensity of AO-dependent red, granular fluorescence compared with control cells (Fig. 3A), indicating

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**Fig. 3.** Changes in ASMC lysosomal stability following shock. A–E: confocal microscopy. The top row in the image presents ASMCs exposed to acridine orange (AO), showing red, granular, lysosomal fluorescence as a result of AO accumulation in the form of dimers and/or polymers, indicating preserved pH and membrane stability. The images show normal lysosomal fluorescence in the sham group (A), clearly decreased red fluorescence in the shock group (B), partial preservation of the fluorescence in the shock + CsA (C) and the shock + Res (D) groups, while the best preservation is found in the shock + PD group (E). Scale bar: 30 μm. F–K: flow cytometric recordings of the percentage of “pale” cells (i.e., cells with fewer than normal intact, red lysosomes) was performed as described in MATERIALS AND METHODS. Quantization of the results (K) shows that the “pale” cells of the shock group (G) increased to 51.6 ± 4.8% from 28.3 ± 5.2% of the sham group (F). The ASMC lysosomal stability was significantly better preserved in the shock + PD (J) group than in the shock + CsA (H) and shock + Res (I) groups (n = 6 for each group). *P < 0.01 vs. sham group; #P < 0.01, vs. shock group; 130P < 0.05, \( \Delta \Delta P < 0.01 \) vs. shock + PD group.
LMP to be induced by shock. The cells from shocked animals and then exposed to CsA and Res showed partial preservation of red granular fluorescence (Fig. 3, C and D). A higher protective effect on lysosomal red fluorescence was found in the shock + PD group (Fig. 3E).

To further quantify the effect of PD on lysosomal stability, AO fluorescence was analyzed by flow cytometry (n = 6 for each group). This analysis showed 51.6 ± 4.8% pale cells in the shock group, which was much higher than the observed value of 28.3 ± 5.2% in the control group (P < 0.01; Fig. 3, F and K). The percentage of pale cells was 42.0 ± 2.8% in the shock + CsA group (Fig. 3H) and 47.5 ± 3.1% in the shock + Res group (Fig. 3I). Simultaneously, the percentage of pale cells in the shock + PD group was only 36.8 ± 3.8%, which was significantly lower than that of the shock group (P < 0.01; Fig. 3, G and K), indicating a pronounced inhibitory effect of PD on ASMC lysosomal destabilization during shock. The preservation of lysosomal stability in the shock + PD group was significantly different from that of the CsA and Res groups (P < 0.05; Table 1), showing that PD is a better protector than both CsA and Res.

Because oxidative stress has been reported to induce LPM and play a key role in the lysosome-mitochondria pathway of cell death (20). Obviously, both PD and Res acted as redox protectants, and it is possible they do so by being iron chelators (14, 17). To explore the mechanism of PD-induced lysosomal stability, the next step was to investigate the effect of PD on LPO in shock (n = 6 for each group). It was found that the LPO level in ASMCs was increased from 10.01 ± 1.56 nmol in the sham group to 15.00 ± 2.23 nmol in the shock group (P < 0.01), while the LPO content was reduced to 10.42 ± 0.99 nmol in the shock + PD group, which was significantly lower than that in shock group (P < 0.01). The PD antioxidative effect was significantly better than that of the CsA and Res groups (P < 0.01; Table 2).

PD restored ASMC ATP levels following severe shock. To further study PD-mediated mitochondrial protection, the effect of PD on ATP levels during shock was examined using the CellTiter-Glo luciferase bioluminescence method. Because oxidative stress has been reported to induce LPM (14, 17), it was again found that severe shock caused significant decrease in the ATP levels of ASMCs to 17.6 ± 7.9% of the control value (P < 0.01; Fig. 4). Treatment with CsA or Res increased ATP levels to 32.7 ± 5.4% or 62.1 ± 11.5% of normal values, respectively (P < 0.05; Fig. 4), while exposure to PD was almost fully protective and resulted in 90.7 ± 7.5% of the control level, which was significantly higher than the values found in CsA and Res-treated animals (P < 0.01). There was even no difference from the control group (P > 0.05).

The role of PD in the activation of KATP and changes of ASMC membrane potential during shock. Previous studies have demonstrated that depletion of ATP plays an important role in activation of the KATP-channel during ASMC membrane hyperpolarization (23, 29, 37). Therefore, the effect of PD on KATP current and membrane potential of ASMCs was investigated. The results showed that the KATP current densities increased remarkably during shock (Fig. 5B) at voltages ranging from −80 to +80 mV compared with control values (Fig. 5A) (P < 0.05; n = 10 cells for each group). Along the voltage range of −80 to +80 mV, CsA (n = 10) and Res (n = 16) decreased the KATP current density compared with the shock group (Fig. 5, C, D, F). Similarly, PD (n = 11) also inhibited the KATP change in ASMCs, although even further (Fig. 5E). At +80 mV, the KATP current density was decreased by 40%, i.e., 15.7 ± 7.3 pA/pF in the shock group and 9.4 ± 4.2 pA/pF in the shock + PD group (P < 0.05; Fig. 5F).

Consistent with the alteration of KATP current density, the ASMCs membrane potential significantly increased from −31.7 ± 5.3 mV in the control group (n = 25) to −49.7 ± 5.3 mV in the shock group (n = 29; P < 0.01), indicating that ASMCs hyperpolarization is a feature of shock. Exposure to PD before reperfusion led to reduction of the membrane potential to −36.9 ± 7.2 mV (n = 27; P < 0.01), indicating a significant PD-mediated suppression of membrane hyperpolarization (Table 3).

Effect of mitochondrial PD protection on vasoreactivity and MAP. ASMCs membrane hyperpolarization has been suggested to result in depressed vasoresponsiveness and persistent hypotension (3, 14, 37, 38, 40). The next step of the study was, therefore, to determine whether mitochondrial PD protection would normalize vasoreactivity. The results showed that the NE threshold concentration increased to 29.3 times of that of the prehemorrhage level at the end of a period, including 2 h of hemorrhage and 2 h of treatment and MAP had then decreased to 47.23 ± 11.28 mmHg in the shock group (Fig. 6A). In the shock + CsA and shock + Res groups, the NE threshold concentration increased to 10.4 and 11.8 times of the prehemorrhage level, respectively, while MAP increased to 55.23 ± 9.92 and 57.10 ± 15.74 mmHg, respectively, at the same time points. Meanwhile, in the shock + PD group, the NE threshold

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Table 2. Changes of ASMC intracellular LPO content in shock (X ± s)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>LPO Content, nmol</th>
</tr>
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<tbody>
<tr>
<td>Sham</td>
<td>5</td>
<td>10.01 ± 1.56</td>
</tr>
<tr>
<td>Shock</td>
<td>5</td>
<td>15.00 ± 2.23**</td>
</tr>
<tr>
<td>Shock + CsA</td>
<td>5</td>
<td>14.10 ± 1.42**ΔΔ</td>
</tr>
<tr>
<td>Shock + Res</td>
<td>5</td>
<td>14.18 ± 1.59**ΔΔ</td>
</tr>
<tr>
<td>Shock + PD</td>
<td>5</td>
<td>10.41 ± 0.99##</td>
</tr>
</tbody>
</table>

LPO, lipid hydroperoxide. **P < 0.01 vs. sham group; #P < 0.01 vs. shock group; ΔΔP < 0.01 vs. shock + PD group.
concentration increased to 4.8 times of the prehemorrhage level during the same period, while MAP increased to 89.38 ± 16.31 mmHg, which was significantly higher than that of the shock group (P < 0.01; Fig. 6).

To describe a reproducible model for irreversible hemorrhagic shock, we previously reported that MAP of rat was reduced by bleeding to 40 mmHg and being kept at that level for 60 min, which caused 65–70% animal death within 24 h following blood replacement (36). To investigate whether PD could truly protect against mitochondrial dysfunction, MAP was decreased to 30 mmHg and lasted for 120 min in the study, which led to a mean survival time of only 5.4 ± 2.6 h and an 100% animal death within 24 h after reinfusion of shed blood in the shock group. It was shown that the survival time in the shock + CsA and shock + Res groups was prolonged 2.05 and 1.95 times that of the shock group, respectively, which was significantly longer than that in shock group (P < 0.01; Table 4). In the shock + PD group, the survival time was significantly prolonged to 4.35 times that of the shock group, and the 24-h survival rate was 5/8, which was significantly higher than the values of the shock group (P < 0.01; Table 4) and also much longer than the values of shock + CsA and shock + Res group (P < 0.01; Table 4).

**DISCUSSION**

It is well recognized that mitochondrial dysfunction plays a crucial role in the pathogenesis of several diseases, involving depressed circulatory capacity and that abnormality of the mPTP is a critical determinant in the genesis of mitochondrial dysfunction and apoptosis (1, 2). The mPTPs of the inner mitochondrial membranes normally remain closed but can open under a pathological condition, such as following ischemia-reperfusion injury (11, 18). Such opening results in mitochondrial swelling with consequent general mitochondrial dysfunction and relocation of apoptogenic substances. Previous work from our laboratory showed that ASMC mitochondrial dysfunction results in persistent low vasoreactivity and hypotension after establishment of severe shock.

The present study revealed that mPTP opening, indeed, occurred in rat ASMCs during severe shock, as assayed by the calcein-AM and CoCl2 fluorescent technique. As a result of mPTP opening, molecules of moderate size (<1.5 kDa) move freely across the membrane, while most proteins do not. As a result, they exert a colloidal osmotic pressure that drives influx of water into the mitochondrial matrix with resultant mitochondrial swelling and injury (20, 24), which was observed here in electron microscopic images. The mPTP opening also causes equilibration of H⁺ across the inner membrane, which, as shown, dissipates ΔΨm and inhibits ATP production (10, 18). The low ATP levels that result from mitochondrial dysfunction activate the KATP channels with resulting ASMC hyperpolarization and low vasoreactivity. Needless to say, inhibition of mPTP opening would represent a new therapeutic approach in the treatment of severe shock.

**Table 3. Changes of ASMC membrane potential in shock (X ± s)**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Membrane Potential, mV</th>
</tr>
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<tbody>
<tr>
<td>Sham</td>
<td>25</td>
<td>−31.7 ± 5.3</td>
</tr>
<tr>
<td>Shock</td>
<td>29</td>
<td>−49.7 ± 9.3**</td>
</tr>
<tr>
<td>Shock + CsA</td>
<td>27</td>
<td>−42.0 ± 9.5*#</td>
</tr>
<tr>
<td>Shock + Res</td>
<td>24</td>
<td>−41.5 ± 10.3**##</td>
</tr>
<tr>
<td>Shock + PD</td>
<td>27</td>
<td>−36.9 ± 7.2*##</td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.01 vs. sham group; #P < 0.05, ###P < 0.01 vs. shock group.
PD is a glucoside of Res, a natural polyphenolic compound. Both PD and Res belong to the stilbene-type of compounds and share some pharmacological effects (14, 22, 34). It has been suggested that Res protects mitochondria and prevents reperfusion injury by targeting the mPTP through translocation of GSK-3, which allegedly interacts with cyclophilin D to modulate the mPTP permeability (33). In addition, PD and Res protect the heart and brain against hypoxia and ischemic-reperfusion injury through still not well-understood antioxidative mechanisms (17, 31, 34). Because polyphenols often are potent iron chelators, it is possible that they prevent Fenton-type reactions by binding lysosomal redox-active low mass iron and, thereby, the formation of aggressive hydroxyl radicals that may induce LMP and consequent cellular damage (13). CsA is well known to be an mPTP inhibitor, since it binds one of the mPTP constituent proteins, cyclophilin D in the matrix (4).

In this study, the effects of PD, CsA, and Res on mitochondrial dysfunction following severe shock were determined on ASMCs. It was shown that PD, CsA, and Res partially protected ASMC mitochondria. Of note, CsA also inhibits calcineurin, which is involved in antiapoptotic effects of cardiomyocytes in vitro and in vivo (6). Obviously, there is a CsA-mediated fine balance between prosurvival and prodeath signals, which may be why CsA is not an optimal protector. The PD-protective effect was especially evident on the cellular ATP content that was preserved to 90.7% of that of the control group, being much higher than those of 17.6%, 32.3%, and 62.1% in the shock only, CsA-treated, and Res-treated groups, respectively.

The finding that ASM C ATP levels were preserved post PD-treatment initiated a study of what happened to the $K_{\text{ATP}}$ channels and the vasoresponsiveness to NE. In accordance with the changes of ASMCs, ATP levels among the three treated groups, the best preservation of $K_{\text{ATP}}$ current density, ASMC membrane potential, and the vasoreactivity was observed in the shock + PD group. For example, the NE threshold concentration 2 h postreinfusion was 29.3, 10.4, and 11.8 times of the prebleeding level in the shock, shock + CsA, and shock + Res group, respectively. Meanwhile, it was 4.8 times in the shock + PD group, which led to restoration of blood pressure and prolonged survival time after PD treatment.

Recent reports indicate that LMP caused by oxidative stress can give rise to relocalization of redox-active ferrous iron and lysosomal proteases, which may trigger mPTP opening (19). Therefore, an alternative hypothesis is proposed, considering how PD and other herbal polyphenols with iron-chelating capacity act. They may chelate lysosomal redox-active iron and prevent intralysosomal Fenton-type reactions with ASMC LPO levels reduced to near normal in the shock + PD group. Because it restores capillary microcirculation during severe shock, Polydatin was first used by us to treat shock (14). Here, it was found

**Table 4. Rat survival time following hemorrhagic shock ($\bar{x} \pm s$)**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Body Weight, g</th>
<th>Blood Loss, ml/100 g body wt</th>
<th>Survival Time, h</th>
<th>24-h Survival Rate</th>
</tr>
</thead>
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<td>Sham</td>
<td>8</td>
<td>207.1 ± 6.2</td>
<td>0</td>
<td>&gt;72.0</td>
<td>8/8</td>
</tr>
<tr>
<td>Shock</td>
<td>8</td>
<td>208.9 ± 5.4</td>
<td>2.9 ± 0.1</td>
<td>5.4 ± 2.6</td>
<td>0/8</td>
</tr>
<tr>
<td>Shock + CsA</td>
<td>8</td>
<td>210.4 ± 10.0</td>
<td>3.0 ± 0.1</td>
<td>9.0 ± 4.8</td>
<td>0/8</td>
</tr>
<tr>
<td>Shock + Res</td>
<td>8</td>
<td>215.4 ± 5.2</td>
<td>2.9 ± 0.1</td>
<td>10.6 ± 6.7</td>
<td>0/8</td>
</tr>
<tr>
<td>Shock + PD</td>
<td>8</td>
<td>211.6 ± 5.6</td>
<td>2.9 ± 0.1</td>
<td>23.7 ± 3.7##</td>
<td>5/8##</td>
</tr>
</tbody>
</table>

##P < 0.01 vs. sham group.
that PD has another therapeutic effect—protection of ASMC mitochondrial dysfunction, which might result from upstream protection against destabilization of the lysosomal-mitochondrial axis and, thereby, reduction of ROS formation. The study indicates that protection of ASMCs against mitochondrial dysfunction may be a new way of treating refractory hypotension in severe shock. Polydatin, which serves as a protector of mitochondrial dysfunction, may also be a new remedy for the treatment of disease connected with cytopathic hypoxia (8).

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DISCLOSURES
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