Targeted Inactivation of Cystic Fibrosis Transmembrane Conductance Regulator Chloride Channel Gene Prevents Ischemic Preconditioning in Isolated Mouse Heart

Hong Chen, MD, PhD*; Luis L. Liu, MD, MSc*; Linda L. Ye, MD; Conor McGuckin, BS; Susan Tamowski, BS; Paul Scowen, BS; Honglin Tian, PhD; Keith Murray, BS; William J. Hatton, PhD; Dayue Duan, MD, PhD

Background—Recent evidence suggests that chloride channels may be involved in ischemic preconditioning (IPC). In this study, we tested whether the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channels, which are expressed in the heart and activated by protein kinase A and protein kinase C, are important for IPC in isolated heart preparations from wild-type (WT) and CFTR knockout (CFTR−/−) mice.

Methods and Results—Hearts were isolated from age-matched WT or CFTR−/− (B6.129P2-Cftt1Unc and STOCKCftt1Unc-TgN 1Jaw) mice and perfused in the Langendorff or working-heart mode. All hearts were allowed to stabilize for 10 minutes before they were subjected to 30 or 45 minutes of global ischemia followed by 40 minutes of reperfusion (control group) or 3 cycles of 5 minutes of ischemia and reperfusion (IPC group) before 30 or 45 minutes of global ischemia and 40 minutes of reperfusion. Hemodynamic indices were recorded to evaluate cardiac functions. Release of creatine phosphate kinase (CPK) in the samples of coronary effluent and infarct size of the ventricles were used to estimate myocardial tissue injury. In WT adult hearts, IPC protected cardiac function during reperfusion and significantly decreased ischemia-induced CPK release and infarct size. A selective CFTR channel blocker, gemfibrozil, abrogated the protective effect of IPC. Furthermore, targeted inactivation of the CFTR gene in 2 different strains of CFTR−/− mice also prevented IPC’s protection of cardiac function and myocardial injury against sustained ischemia.

Conclusions—CFTR Cl− channels may serve as novel and crucial mediators in mouse heart IPC. (Circulation. 2004;110:700-704.)

Key Words: ischemia ■ myocardial infarction ■ hemodynamics ■ ion channels ■ genes

Numerous studies have examined the signaling pathways involved in ischemic preconditioning (IPC), a phenomenon in which brief ischemic episodes elicit protection of the heart against sustained ischemia. It has been suggested that both sarcolemmal and mitochondrial ATP-sensitive potassium channels (sarc-KATP and mito-KATP, respectively) may serve as triggers or end-effectors.1 Protein kinase C (PKC) may link cellular signal events during ischemia to the activation of end-effectors.2,3 The precise mechanism of IPC, however, remains to be elucidated.

Recent studies have pointed to a potential role of anion channels in IPC. A cell swelling–induced Cl− current (I\(_{\text{Cl,swell}}\)) has been suggested to contribute to IPC in rabbit heart,4 although the causal role for I\(_{\text{Cl,swell}}\) in IPC has yet to be confirmed.5 Previous studies have demonstrated that a different class of anion channels, cystic fibrosis transmembrane conductance regulator (CFTR), are expressed in mammalian heart.6,7 Several lines of evidence suggest that CFTR channels may be involved in IPC, including the possibilities that (1) sarc-K\(_{\text{ATP}}\) blockers, such as glibenclamide, which suppress IPC protection, also block CFTR channels in noncardiac8 and cardiac cells;6,9 (2) PKC and PKA, 2 essential second messengers in IPC,2,3 can activate CFTR channels;6,7 and (3) triggers of IPC (nitric oxide, opioids, adenosine, etc) can all regulate CFTR channel function.8,9 Because much of the evidence favoring a role of CFTR in IPC is circumstantial, we tested this hypothesis directly by studying hemodynamics and tissue injury of hearts isolated from wild-type (WT) and 2 strains of CFTR-knockout (CFTR−/−) mice subjected to ischemia and reperfusion. The results from these experiments are consistent with the conclusion that CFTR Cl− channels may serve as novel and important mediators of IPC.

Methods

This investigation conforms to the Guide for the Care and Use of Laboratory Animals (US National Institute of Health publication No.

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From the Department of Pharmacology and Center of Biomedical Research Excellence, University of Nevada School of Medicine, Reno. Dr. Hong Chen is now at the Department of Pharmacology and Shanghai Institute of Hypertension, Shanghai Second Medical University, Shanghai, China.

*The first 2 authors contributed equally to this work.

Correspondence to Dayue Duan, MD, PhD, FAHA, Associate Professor, Center of Biomedical Research Excellence, Department of Pharmacology, University of Nevada School of Medicine, Manville Medical Building Room 9/MS 318, Reno, NV 89557-0270. E-mail dduan@med.unr.edu

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Results

To test whether CFTR Cl⁻ channels are involved in IPC, we first applied gemfibrozil (Gem), a selective blocker of CFTR channels in guinea pig and mouse heart (D. Duan, unpublished data, 2000), to isolated WT mouse hearts perfused in the working-heart mode. Hearts from age- and size-matched C57BL/6J mice were divided randomly into control, IPC, and IPC+Gem (200 μmol/L) groups and subjected to the experimental protocols shown in Figure 1A. Under control conditions, 30 minutes of ischemia caused a significant decrease in cardiac function as estimated by LVP, LVDP, +dP/dt, −dP/dt, and cardiac output measured at the end of 40 minutes of reperfusion. Postischemic heart function recovered to the same level as that before sustained ischemia in IPC hearts. Administration of Gem during the preconditioning reperfusion abolished IPC-induced heart function recovery with a significant decrease in LVDP, +dP/dt, and −dP/dt (Figure 1, B through D). Table 1 summarizes the hemodynamic changes in the control, IPC, and IPC+Gem groups. Consistent with these functional changes, CPK release after sustained ischemia was significantly reduced in the IPC hearts. CPK release in the IPC+Gem group, however, remained as high as in the control group (Figure 1E). These results suggest that blockade of CFTR channels may prevent IPC in mouse heart.

To confirm that the effects of Gem on IPC are a result of the blockade of CFTR channels and are not of other effects, such activation of peroxisome proliferator–activated nuclear receptors, we first used B6-CFTR⁻/⁻ mice to examine the effect of IPC on ischemia-induced myocardial CPK release and infarct size. B6-CFTR⁻/⁻ mice on normal diet are significantly smaller in both body and heart than WT and CFTR⁺/⁺ mice (Table 2) and can live for only up to 5 weeks because of severe intestinal disease. Therefore, experiments were performed using 3- to 4-week-old WT (C57BL/6J), CFTR⁺/⁺, and CFTR⁻/⁻ littermates. These hearts, especially the hearts of CFTR⁻/⁻ mice, are too small to be perfused in working-heart mode. Therefore, these hearts were perfused only in the Langendorff mode, and no hemodynamic data were collected. For the same reason, it is impossible to accurately measure the infarct size of these small hearts, so CPK release (units per heart tissue weight per minute) was used as the index for myocardial injury. As shown in Table 2, IPC effectively reduced myocardial CPK release in WT and CFTR⁺/⁺ mice but not in the age-matched CFTR⁻/⁻ mice. To test whether the failure to precondition the CFTR⁻/⁻ mouse heart is because of severe intestinal disease and lower body and heart weights, a special liquid diet food, peptamin, was used to feed the B6-CFTR⁻/⁻ mice so that they were able to survive up to 8 weeks with body and heart weights (20.3±1.0 g and 104.3±2.9 mg, respectively, n=6) similar to those of WT mice (21.9±0.5 g and 104.3±2.9 mg, respectively, n=6, P>0.05). Hearts from these mice were also perfused only in the Langendorff mode, and infarct sizes were measured (Figure 1F). Consistent with the results in the 3- to 4-week-old mice, IPC failed to reduce the infarct size induced by 30 minutes of sustained global ischemia in the 8-week-old CFTR⁻/⁻ mice, whereas it significantly limited the infarct size in both WT and CFTR⁺/⁺ mouse hearts, suggesting that
targeted inactivation of the CFTR gene prevented IPC in isolated mouse heart.

To confirm these observations and ensure that the results in the peptamin-fed CFTR<sup>−/−</sup> mice are not because of the special diet, we used another strain of CFTR<sup>−/−</sup> mice. Homozygous FABPCFTR<sup>−/−</sup> mice are produced from FVB/NJ background and can live on normal mouse chow to a normal age and body weight, because the lethal intestinal defect seen in the B6-CFTR<sup>−/−</sup> mice is corrected.12 Hearts from these mice therefore offered an alternative approach to assess the effects of CFTR gene disruption on IPC-induced reduction in infarct size and protection of cardiac function. Hearts from age- and size-matched male WT (FVB/NJ), FABPCFTR<sup>−/−</sup>, and FABPCFTR<sup>−/−</sup> mice were perfused in the working-heart mode,

![Figure 1. Effects of CFTR channel blocker and CFTR gene knockout on IPC in isolated C57BL mouse heart. A, Experimental protocols. Hearts were perfused in working-heart mode (W) for 10 minutes before 3 cycles of IPC protocol (5 minutes of ischemia and 5 minutes of reperfusion). Global ischemia (I) was induced by clamping both atrial inflow and aortic outflow. At end of 30 minutes of sustained ischemia, hearts were reperfused (R) for 15 minutes in Langendorff mode to restore spontaneous heart beating and 25 minutes in working heart mode. B through D, Recovery of LV contractile (B, LVDP; C, +dP/dt) and relaxation (D, −dP/dt) function after 30 minutes of ischemia and 40 minutes of reperfusion. E, Myocardial injury after ischemia (lsch) and IPC in WT male mice. Cumulative CPK release during reperfusion after ischemia was measured with a Sigma CPK kit and spectrophotometer according to manufacturer’s instructions. Compared with ischemia control group (lsch, n=8), IPC significantly decreased myocardial CPK release (n=9, P<0.01), whereas CFTR blocker gemfibrozil (Gem, 200 μmol/L) abolished effect of IPC on CPK release (n=6, P<0.01). F, Mean infarct sizes measured from age-matched (8-week) WT (C57BL/6J), CFTR<sup>−/−</sup>, or CFTR<sup>−/−</sup> (B6129P2-CFTR<sup>tm1Unc</sup>) mouse heart after ischemia or IPC (n=6 for each group). Hearts were reperfused in Langendorff mode. *P<0.05, **P<0.01, ***P<0.001.](http://circ.ahajournals.org/doi/10.1161/01.CIR.0000126420.40954.9F)

**TABLE 1. Chloride Channel Blocker on IPC-Induced Changes in Hemodynamics of Isolated Perfused Wild-Type Working Heart**

<table>
<thead>
<tr>
<th></th>
<th>Control (n=7)</th>
<th>IPC (n=9)</th>
<th>IPC+Gem (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>40 min R</td>
<td>Baseline</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>336±22</td>
<td>319±27</td>
<td>320±17</td>
</tr>
<tr>
<td>LVP, mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>106±1.3</td>
<td>71±8.1†</td>
<td>107±2.9</td>
</tr>
<tr>
<td>LVDP, mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>102±1.7</td>
<td>64±7.6†</td>
<td>102±2.7</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.7±0.8</td>
<td>6.4±0.6†</td>
<td>4.0±0.4</td>
</tr>
<tr>
<td>+dP/dt, mm Hg/s</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6404±366</td>
<td>3782±428‡</td>
<td>6309±456</td>
</tr>
<tr>
<td>−dP/dt, mm Hg/s</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5084±344</td>
<td>2832±407†</td>
<td>5273±312</td>
</tr>
<tr>
<td>Coronary flow, mL/min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.0±0.4</td>
<td>2.4±0.4</td>
<td>2.6±0.2</td>
</tr>
<tr>
<td>CO, mL/min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.9±0.6</td>
<td>3.5±0.5‡</td>
<td>6.2±0.7</td>
</tr>
</tbody>
</table>

Experimental protocols are shown in Figure 1A. Hemodynamic parameters at baseline were measured at the end of the 10 minutes of working heart perfusion before ischemia/reperfusion protocol or exposure to drugs. In control group, results measured at the end of 40 minutes of reperfusion (40 min R) after 30 minutes of global ischemia were compared with those measured at baseline (†P<0.05, ‡P<0.01, ††P<0.001). In IPC and IPC+Gem group, results measured at the end of 40 minutes of reperfusion (40 min R) after 30 minutes of global ischemia were compared with those measured at the end of the last reperfusion of the 3-cycle brief ischemia/reperfusion (I/R) (§P<0.05, ||P<0.01).
and the infarct sizes of the ventricles were measured. In the FVB/NJ WT mouse hearts, 30-minute sustained ischemia caused a significantly smaller infarct size (19.1 ± 2.3%, n = 5) than that in the C57BL/6J WT mouse (31.4 ± 2.5%, n = 6, P = 0.006). Furthermore, IPC in FVB/NJ WT mice caused only a mild reduction in the infarction (17.5 ± 2.3%, P = 0.5) and no significant recovery in cardiac function compared with the ischemia group (83.1 ± 1.4% versus 84.4 ± 1.6% in LVDP, 64.4 ± 0.3% versus 62.6 ± 3.7% in +dP/dt, and 80.6 ± 2.4% versus 70.3 ± 4.6% in −dP/dt, P = NS). Apparently, the response to ischemia and IPC varies in different genetic backgrounds of mice. Similar observations of different response to ischemia/reperfusion injury and preconditioning in different mouse strains have been reported by Bao et al.15 In fact, variations in cardiovascular traits occur naturally among inbred mouse strains.16 Therefore, we used a 45-minute sustained global ischemia protocol (Figure 2A). Postischemic cardiac function (LVDP and ±dP/dt) recovered significantly to a level close to that before 45 minutes of sustained ischemia in ischemic preconditioned hearts of WT FVB/NJ and CFTR+/−/− mice but not of CFTR−/− mice (Figure 2, B through D). Consistent with these functional changes, IPC significantly decreased myocardial infarct size in both WT and CFTR+/−/− mice but not in the CFTR−/− mice (Figure 2, E and F).

**Discussion**

In this report, we present compelling evidence for an important functional role of CFTR Cl− channels in IPC of isolated mouse heart.

The CFTR Cl− channel protein is a member of the ATP-binding cassette (ABC) superfamily of transporters. **TABLE 2. Characterization of 3- to 4-Week-Old C57BL/6J WT and CFTR-Knockout B6.129P2-Cftrtm1Unc Mouse Hearts and CPK Release After Ischemia/Reperfusion and Preconditioning**

<table>
<thead>
<tr>
<th></th>
<th>WT Control</th>
<th>WT IPC</th>
<th>CFTR+/− IPC</th>
<th>CFTR−/− IPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart weight, mg</td>
<td>56.0 ± 5.5</td>
<td>61.4 ± 4.9</td>
<td>60.3 ± 4.5</td>
<td>28.9 ± 7.8†</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>10.0 ± 0.8</td>
<td>10.2 ± 1.2</td>
<td>9.9 ± 1.1</td>
<td>5.6 ± 1.1†</td>
</tr>
<tr>
<td>Heart/body wt, mg/g</td>
<td>5.5 ± 0.3</td>
<td>6.2 ± 0.4</td>
<td>6.3 ± 0.5</td>
<td>5.0 ± 0.5</td>
</tr>
<tr>
<td>CPK, U/100 mg·40 min−1</td>
<td>54.1 ± 9.9</td>
<td>27.2 ± 4.5*</td>
<td>25.5 ± 6.3*</td>
<td>90.8 ± 14.9†</td>
</tr>
</tbody>
</table>

Experimental protocols are similar to those described in Figure 1A (Groups 1 and 2) except that the reperfusion was only in Langendorff mode.

*P < 0.01, WT IPC or CFTR+/− IPC vs WT Control group.
†P < 0.01, ‡P < 0.001, CFTR−/− IPC vs WT IPC or CFTR+/− IPC group.

**Figure 2.** Effects of CFTR gene knockout (FABPCFTR) on IPC in isolated working mouse heart. A, Experimental protocol. B through D, Recovery of LV contractile (B and C) and relaxation (D) function of WT (FVB/NJ), CFTR+/− and CFTR−/− (FABPCFTR) mice after 45 minutes of ischemia and 40 minutes of reperfusion. E and F, IPC on infarct size of ventricles. E, Representative ventricle transverse slices after ischemia (Isch) or IPC. F, Mean infarct size measured from age-matched WT, CFTR+/−, or CFTR−/− mouse heart after ischemia (Isch) or IPC (n = 6 for each group). *P < 0.05, **P < 0.01, ***P < 0.001.
Members in the ABC superfamily, including the sulfonylurea receptor, which combines with inward rectifier K⁺ (Kir6.1, Kir6.2) channel subunits to form functional sarc-KₐT₃P channels, share similar structure of transmembrane domains and nucleotide binding domains. However, CFTR seems to be unique in forming a PKA- and PKC-regulated anion channel.

It is highly unlikely that the ex vivo IPC response of CFTR⁺⁺ hearts are the result of chronic hemodynamic effects caused by hypoxia or pulmonary hypertension, because these CFTR⁺⁺ mice do not develop any lung disease or pulmonary hypertension, although lung disease is the leading cause of mortality and morbidity in humans with cystic fibrosis. Several mechanisms may be responsible for a functional role of CFTR channels in mouse heart IPC. (1) It has been demonstrated that cardiac CFTR plays a role in early action potential shortening during hypoxia and ischemia. Activation of CFTR will also decrease resting membrane potential and action potential duration, thereby limiting intracellular Ca²⁺ overload and cell damage. (2) The CFTR channel is an important transporter of sphingosine 1-phosphate (S-1-P), which has recently emerged as an important lipid messenger involved in IPC. (3) CFTR is impermeable not only to Cl⁻ but also to larger organic ions, as well as reduced and oxidized forms of glutathione. Therefore, CFTR may contribute to the control of oxygen stress–induced apoptosis and the regulation of inflammation and the immune responses. (4) CFTR may decrease intracellular pH and modulate apoptosis. (5) CFTR functions as a regulator of volume-dependent homeostatic cell mechanisms in cell proliferation and apoptosis. We are currently in the process of investigating these potential mechanisms and the relative role of CFTR in early and late preconditioning.

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