Role of Tyrosine Kinase in Desflurane-induced Preconditioning


Background: Short administration of volatile anesthetics precondition myocardium and protects the heart against the consequences of subsequent ischemia. Activation of tyrosine kinase is implicated in ischemic preconditioning. The authors investigated whether desflurane-induced preconditioning depends on activation of tyrosine kinase.

Methods: Sixty-four rabbits were instrumented for measurement of left ventricular pressure, cardiac output, and myocardial infarct size (IS). All rabbits were subjected to 30 min of occlusion of a major coronary artery and 2 h of subsequent reperfusion. Rabbits underwent a treatment period consisting of either no intervention for 35 min (control group, n = 12) or 15 min of 1 minimum alveolar concentration desflurane inhalation followed by a 10-min washout period (desflurane group, n = 12). Four additional groups received the tyrosine kinase inhibitors genistein (5 mg/kg) or lavendustin A (1.3 mg/kg) at the beginning of the treatment period with (desflurane–genistein group, n = 11; desflurane–lavendustin A group, n = 12) or without desflurane inhalation (genistein group, n = 9; lavendustin A group, n = 8).

Results: Hemodynamic values were similar in all groups during baseline (left ventricular pressure, 87 ± 14 mmHg [mean ± SD]; cardiac output, 198 ± 47 ml/min), during coronary artery occlusion (left ventricular pressure, 78 ± 12 mmHg; cardiac output, 173 ± 39 ml/min), and after 2 h of reperfusion (left ventricular pressure, 59 ± 17; cardiac output, 154 ± 43 ml/min). IS in the control group was 55 ± 10% of the area at risk. The tyrosine inhibitors had no effect on IS (genistein group, 56 ± 13%; lavendustin A group, 49 ± 13%; each P = 1.0 vs. control group). Desflurane preconditioning reduced IS to 40 ± 15% (P = 0.04 vs. control group). Tyrosine kinase inhibitor administration had no effect on IS reduction (desflurane–genistein group, 44 ± 13%; desflurane–lavendustin A group, 44 ± 16%; each P = 1.0 vs. desflurane group).

Conclusion: Desflurane-induced preconditioning does not depend on tyrosine kinase activation.

SHORT periods of myocardial ischemia protect the heart against a subsequent longer ischemia. This phenomenon, first described by Murry et al.,1 is known as ischemic preconditioning and provides strong protection against the consequences of myocardial ischemia, such as arrhythmias,2 postischemic dysfunction,3 metabolic changes, and cell death.1 The protection induced by ischemic preconditioning can be mimicked by several agents, such as adenosine,4 opioid,5 and inhalational anesthetics,6–8 including desflurane.9 This preconditioning effect of volatile anesthetics has also been shown in clinical settings in humans.10,11

The precise signaling pathway of volatile anesthetic-induced preconditioning is only partly understood. Activation of mitochondrial adenosine triphosphate–sensitive potassium (K\textsubscript{ATP}) channels12 and consecutive intracellular release of free oxygen radicals is important for both ischemic13 and anesthetic-induced preconditioning.14 Regarding ischemic preconditioning, downstream activation cascade of protein kinases, including tyrosine kinase (TK), seems likely, modulating a yet unidentified end effector that actually protects the heart.13 Blockade of TK also blocks protection provided by ischemic preconditioning.15 Whether TK activation is also involved in volatile anesthetic-induced preconditioning is not known.

Therefore, the objective of the current study was to determine whether activation of TK is involved in desflurane-induced cardioprotection. Specifically, we investigated whether the two structurally different TK blocking agents genistein and lavendustin A can block desflurane-induced preconditioning in the rabbit heart in vivo.

Materials and Methods

The current study conforms with the Guiding Principles in the Care and Use of Animals, endorsed by the Council of the American Physiologic Society, and was approved by the Animal Care Committee of the district of Düsseldorf (Düsseldorf, Germany).

General Preparation

The animal preparation has been described in detail previously.16 Briefly, 64 α-chloralose-anesthetized New Zealand white rabbits (mean weight, 2.5 ± 0.5 kg) were instrumented for measurement of aortic pressure (Statham transducer; Gould Instruments, Cleveland, OH), cardiac output (ultrasonic flow probe), and left ventricular (LV) pressure (Millar tip manometer; Millar Instruments, Houston, TX). A ligature snare was passed around a major coronary artery for later occlusion. The effectiveness of coronary artery occlusion was verified by the appearance of epicardial cyanosis and changes in surface electrocardiogram. Ventricular fibrillation during coronary occlusion was treated with electrical defibrillation (5 J). After coro-
nary occlusion, the snare occluder was released, and reperfusion was verified by the disappearance of epicardial cyanosis. Temperature was measured inside the pericardial cradle and maintained between 38.3° and 38.7°C by adjusting a heating pad and an infrared lamp.

### Experimental Protocol

The experimental protocol is shown in figure 1. Twenty minutes after completion of the surgical preparation, baseline measurements were performed and the animals received a TK blocker (genistein or lavendustin A) dissolved in 0.5 ml dimethyl sulfoxide (DMSO; 99.7%) or DMSO alone. All rabbits underwent 30 min of coronary artery occlusion followed by 2 h of reperfusion.

Twelve rabbits underwent the ischemia-reperfusion procedure without further treatment (control group). Rabbits in the desflurane group (n/H11005/12) received the anesthetic in an end-tidal concentration of 8.9% (corresponding to 1 minimum alveolar concentration in rabbits) for 15 min followed by a 10-min washout period. End-tidal desflurane concentrations were measured (Capnomac Ultima; Datex, Helsinki, Finland) and never exceeded 0.2 vol% at the beginning of coronary artery occlusion. In the desflurane-genistein (n = 11) and the desflurane-lavendustin A (n = 12) groups, 10 min before desflurane inhalation, the animals received the TK blocker genistein (5 mg/kg) or lavendustin A (1.3 mg/kg) intravenously. Two further groups were treated with genistein (n = 9) or lavendustin A (n = 8) without desflurane.

### Infarct Size Assessment

After 2 h of reperfusion, the heart was arrested by injection of potassium chloride solution into the left atrium and quickly excised. The area at risk size was then determined by Evans blue staining of the nonischemic area, and infarct size (IS) within the area at risk was determined by triphenyltetrazolium chloride staining as described in detail previously.16

### Data Analysis

Left ventricular pressure, its first derivative, the rate of pressure increase (dP/dt), aortic pressure, and cardiac output were recorded continuously on an ink recorder (Recorder 2800; Gould Inc., Cleveland, OH). The data were digitized using an analog-to-digital converter (Data Translation, Marlboro, MA) at a sampling rate of 500 Hz and were processed later on a personal computer.

### Hemodynamic Variables

Global systolic function was measured in terms of LV systolic pressure and maximum dP/dt. Global LV end-systole was defined as the point of minimum dP/dt, and LV end-diastole was defined as the beginning of the sharp upslope of the LV dP/dt tracing. The time constant of decrease in LV isovolumic pressure was used as an index of LV relaxation. Stroke volume was calculated from heart rate and cardiac output, rate pressure product from heart rate and LV peak systolic pressure, and systemic vascular resistance from mean aortic pressure and cardiac output, assuming a right atrial pressure of 0 mmHg in the open chest preparation.

### Statistical Analysis

Data are presented as mean ± SD. Group differences were analyzed with use of the Student t test followed by the Bonferroni correction for multiple comparisons. Changes were considered statistically significant when the P value was less than 0.05.

### Results

#### Hemodynamic Variables

Hemodynamic variables are summarized in figure 2 and table 1. During baseline recordings, no hemody-
This reduction was not influenced by pretreatment with the TK blockers genistein (desflurane–genistein, 44 ± 13%; P = 1.0 vs. desflurane) and lavendustin A (desflurane–lavendustin A, 44 ± 16%; P = 1.0 vs. desflurane). Treatment with genistein and lavendustin A alone had no effect on IS (genistein, 56 ± 13%; P = 1.0 vs. control; lavendustin A, 49 ± 13; P = 1.0 vs. control).

Discussion

The main finding of our study is that two structurally different inhibitors of TK do not block the cardioprotective effect of desflurane-induced preconditioning in the rabbit heart in vivo. Therefore, desflurane-induced preconditioning is independent of TK activation.

The mechanism of protection by ischemic or anesthetic induced preconditioning is not fully understood. However, some steps in the signal transduction have been elucidated. Ischemic preconditioning depends on the opening of mitochondrial K\textsubscript{ATP} channels.\textsuperscript{12,18} Volatile anesthetics have also been shown to activate K\textsubscript{ATP} channels, thereby inducing myocardial protection.\textsuperscript{19} The mitochondrial K\textsubscript{ATP} channel was considered to be the end-effector of ischemic preconditioning until Pain et al.\textsuperscript{13} demonstrated that opening of mitochondrial K\textsubscript{ATP} channels is a trigger rather than a mediator of preconditioning, which leads to release of free oxygen radicals, inducing further steps in the signal transduction cascade. Müllenhuis et al.\textsuperscript{14} recently showed that anesthetic preconditioning with isoflurane also depends on the release of free oxygen radicals.

Another step in the signal transduction cascade that is shared by ischemia- and anesthetic-induced preconditioning is the activation of protein kinase C (PKC).\textsuperscript{18,20} Direct stimulation of PKC mimics\textsuperscript{21} and specific blockade of PKC abolishes\textsuperscript{22} the preconditioning-induced protection. There is evidence identifying PKC as a mediator rather than a trigger of protection, and, therefore, the activation of PKC is most likely downstream of mitochondrial K\textsubscript{ATP} channel opening.\textsuperscript{18,22} Activation of PKC is closely related to protein TK phosphorylation, and evidence has been reported that TKs can be upstream of,\textsuperscript{23,24} parallel to,\textsuperscript{15,25,26} or downstream of PKC.\textsuperscript{21,27} TKs also play an important role in the signal transduction cascade of ischemic preconditioning, and TK inhibition has been shown to block cardioprotection.\textsuperscript{21,28} However, the role of TK activation and its relation to PKC seems to depend on the species and the severity of the preconditioning stimulus. Valhaus et al.\textsuperscript{26} demonstrated in pigs that a combined inhibition of PKC and TK is needed to sufficiently block cardioprotection of ischemic preconditioning. Fryer et al.\textsuperscript{15} successfully abolished cardioprotection induced by one 5-min cycle of preconditioning ischemia using genistein but failed to abrogate preconditioning by three 5-min cycles of ischemia in rats.
Table 1. Hemodynamic Variables

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Inhibitor</th>
<th>Desflurane</th>
<th>Washout</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEDP, mmHg</td>
<td>Control</td>
<td>2.6 ± 1.1</td>
<td>3.1 ± 1.4</td>
<td>3.4 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>Desflurane</td>
<td>3.0 ± 1.4</td>
<td>2.5 ± 1.3</td>
<td>1.5 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>Desflurane–genistein</td>
<td>2.5 ± 1.2</td>
<td>2.3 ± 0.9</td>
<td>2.0 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>Desflurane–lavendustin A</td>
<td>3.0 ± 1.1</td>
<td>3.1 ± 1.1</td>
<td>2.7 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>Genistein</td>
<td>2.4 ± 0.9</td>
<td>2.2 ± 1.2</td>
<td>2.4 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>Lavendustin A</td>
<td>2.3 ± 0.9</td>
<td>2.3 ± 0.6</td>
<td>2.0 ± 0.9</td>
</tr>
<tr>
<td>dP/dt max, mmHg/s</td>
<td>Control</td>
<td>3.555 ± 1.064</td>
<td>4.453 ± 880</td>
<td>4.357 ± 944</td>
</tr>
<tr>
<td></td>
<td>Desflurane</td>
<td>3.695 ± 987</td>
<td>4.594 ± 983</td>
<td>2.818 ± 1.251</td>
</tr>
<tr>
<td></td>
<td>Desflurane–genistein</td>
<td>3.696 ± 825</td>
<td>4.574 ± 1.076</td>
<td>3.263 ± 1.107</td>
</tr>
<tr>
<td></td>
<td>Desflurane–lavendustin A</td>
<td>4.081 ± 1.000</td>
<td>4.299 ± 818</td>
<td>2.743 ± 1.065</td>
</tr>
<tr>
<td></td>
<td>Lavendustin A</td>
<td>3.789 ± 858</td>
<td>4.188 ± 744</td>
<td>4.434 ± 785</td>
</tr>
<tr>
<td>SVR, mmHg · min · ml⁻¹</td>
<td>Control</td>
<td>0.44 ± 0.09</td>
<td>0.49 ± 0.12</td>
<td>0.51 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>Desflurane</td>
<td>0.43 ± 0.13</td>
<td>0.50 ± 0.13</td>
<td>0.34 ± 0.07*</td>
</tr>
<tr>
<td></td>
<td>Desflurane–genistein</td>
<td>0.42 ± 0.15</td>
<td>0.46 ± 0.10</td>
<td>0.33 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>Desflurane–lavendustin A</td>
<td>0.41 ± 0.11</td>
<td>0.42 ± 0.08</td>
<td>0.34 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>Genistein</td>
<td>0.41 ± 0.15</td>
<td>0.44 ± 0.09</td>
<td>0.49 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>Lavendustin A</td>
<td>0.39 ± 0.08</td>
<td>0.42 ± 0.09</td>
<td>0.46 ± 0.10</td>
</tr>
<tr>
<td>t, ms</td>
<td>Control</td>
<td>18 ± 3</td>
<td>16 ± 2</td>
<td>16 ± 2</td>
</tr>
<tr>
<td></td>
<td>Desflurane</td>
<td>17 ± 3</td>
<td>17 ± 4</td>
<td>18 ± 9</td>
</tr>
<tr>
<td></td>
<td>Desflurane–genistein</td>
<td>16 ± 3</td>
<td>15 ± 3</td>
<td>16 ± 8</td>
</tr>
<tr>
<td></td>
<td>Desflurane–lavendustin A</td>
<td>16 ± 3</td>
<td>17 ± 4</td>
<td>17 ± 5</td>
</tr>
<tr>
<td></td>
<td>Genistein</td>
<td>20 ± 5</td>
<td>17 ± 3</td>
<td>16 ± 3</td>
</tr>
<tr>
<td></td>
<td>Lavendustin A</td>
<td>16 ± 4</td>
<td>16 ± 2</td>
<td>17 ± 3</td>
</tr>
</tbody>
</table>

In the current investigation, pretreatment with 8.9% end-tidal desflurane for 15 min reduced IS by 28% in comparison with controls, thereby confirming previous studies of desflurane-induced preconditioning. However, Piriou et al.3 found a much more profound protection by desflurane preconditioning (IS reduction from 54% to 16% of the area at risk) in a similar in vitro rabbit heart model. The only relevant difference in experimental protocol was the duration of 30 min of desflurane preconditioning versus 15 min in our study. This may explain the differences in the resulting protection. There are also differences in anesthesia (ketamine-xylazine vs. α-chloralose), body temperature (39.0°C–40.5°C vs. 38.3°C–38.7°C), and fluid replacement (hetastarch vs. normal saline) between the two studies. These differences may also have affected the resulting protection by anesthetic preconditioning. Interestingly, the same study by Piriou et al. could not detect an effect of sevoflurane preconditioning as it was shown by Müllenhaim et al.30 from our laboratory and by other investigators.31,32 Desflurane washout time was also different between the two studies (15 min vs. 10 min in our study). Because end-tidal desflurane concentration did not exceed 0.2 vol% at the beginning of coronary artery occlusion, a remaining effect of desflurane during ischemia can be excluded. In the current study, the administration of two structurally different TK inhibitors, i.e., genistein and lavendustin A, did not affect the protection of desflurane-induced preconditioning. This could be an indication for a different downstream mechanism of anesthetic preconditioning compared with ischemic preconditioning. There are no studies available that directly compare signal transduction of ischemic and anesthetic preconditioning. Although it has been shown that both mechanisms involve opening of mitochondrial KATP channels,12,18,19 probably followed by intracellular release of free oxygen radicals13,14 and PKC activation,18,20 it remains unclear whether downstream mechanisms are also similar. A limitation of our study is the lack of a positive control showing that, also under our experimental conditions, TK activation leads to a preconditioning effect and/or blockade of TK abolishes preconditioning. However, the
absence of a TK-dependent pathway of preconditioning in the rabbit heart seems unlikely because ischemic as well as pharmacologically induced preconditioning have been blocked by TK inhibitors in the rabbit heart. In these studies, an isolated rabbit heart model was used. It cannot be ruled out that the role of TKs in preconditioning is different in the isolated rabbit heart compared with hearts in vivo. The role of TK seems to be dependent at least on species and preconditioning stimulus. Several other factors may also have an influence (temperature, baseline anesthesia, in vivo vs. in vitro, and others). The choice and dosage of TK inhibitors were adequate in our study. Genistein, 5 mg/kg, and 1–1.3 mg/kg lavendustin A is the common dosage used to block TK effectively in the rabbit heart in vivo, and others have been successfully used to block preconditioning in the

Table 2. Weight and Area at Risk

<table>
<thead>
<tr>
<th>Time</th>
<th>Control</th>
<th>Desflurane</th>
<th>Desflurane-Genistein</th>
<th>Desflurane-Lavendustin A</th>
<th>Genistein</th>
<th>Lavendustin A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>2,555 ± 497</td>
<td>2,527 ± 553</td>
<td>2,583 ± 491</td>
<td>2,346 ± 482</td>
<td>2,543 ± 589</td>
<td>2,557 ± 436</td>
</tr>
<tr>
<td>LV weight, g</td>
<td>0.72 ± 0.18</td>
<td>0.67 ± 0.16</td>
<td>0.71 ± 0.13</td>
<td>0.64 ± 0.14</td>
<td>0.75 ± 0.17</td>
<td>0.71 ± 0.09</td>
</tr>
<tr>
<td>Area at risk, g</td>
<td>0.22 ± 0.08</td>
<td>0.16 ± 0.08</td>
<td>0.21 ± 0.09</td>
<td>0.17 ± 0.07</td>
<td>0.21 ± 0.07</td>
<td>0.19 ± 0.05</td>
</tr>
<tr>
<td>Area at risk/LV, %</td>
<td>30 ± 7</td>
<td>23 ± 6</td>
<td>29 ± 9</td>
<td>27 ± 8</td>
<td>26 ± 5</td>
<td>26 ± 6</td>
</tr>
<tr>
<td>Infarct size, g</td>
<td>0.12 ± 0.05</td>
<td>0.07 ± 0.04*</td>
<td>0.10 ± 0.06</td>
<td>0.08 ± 0.05</td>
<td>0.12 ± 0.06</td>
<td>0.09 ± 0.04</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD. The given heart weights are dry weights.

* P = 0.03 vs. control.
LV = left ventricular.
Therefore, we cannot exclude that genistein had already in vitro increased tolerance to sustained low-flow ischemia by a brief episode of no-flow ischemia without intermittent reperfusion. Circ Res 1995; 76:942-50

Further investigations are needed to completely elucidate the mechanism of anesthetic preconditioning.

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