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Role of Tyrosine Kinase in Desflurane-induced Preconditioning

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Background: **Short administration of volatile anesthetics preconditions 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 inhibitor 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_{ATP}) channels[12] and consecutive intracellular release of free oxygen radicals is important for both ischemic[13] 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-

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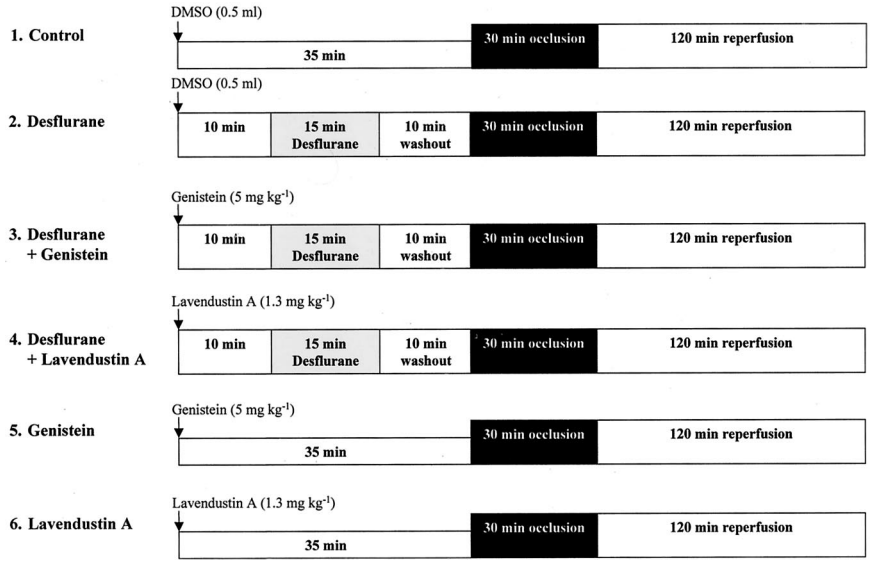

Fig. 1. Experimental protocol. DMSO = dimethyl sulfoxide (99.7%, the solvent of genistein and lavendustin A).

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 = 12) received the anesthetic in an end-tidal concentration of 8.9% (corresponding to 1 minimum alveolar concentration in rabbits)[17] 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-

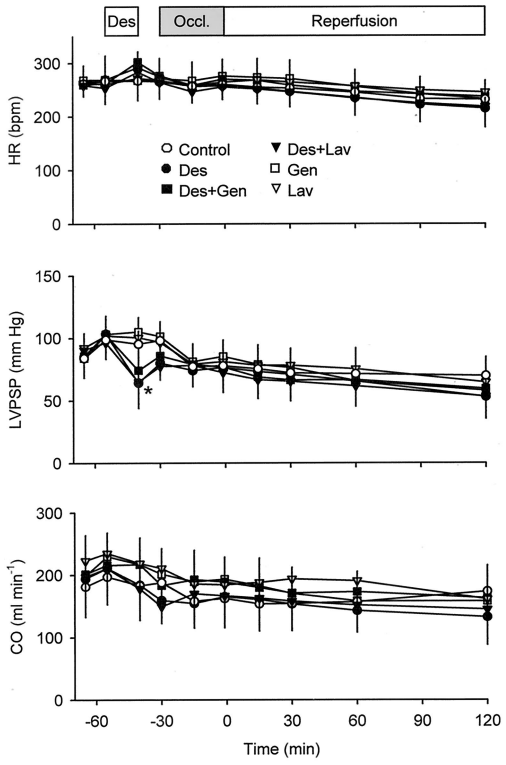

Fig. 2. Line plots showing the time course of heart rate (HR), left ventricular peak systolic pressure (LVPSP), and cardiac output (CO) during experiments in the control, desflurane (Des), desflurane + genistein (Des + Gen), desflurane + lavendustin A (Des + Lav), genistein (Gen), and lavendustin A (Lav) groups. Data are presented as mean ± SD. * $P = 0.01$, desflurane *versus* control. bpm = beats/min; occl. = occlusion.

namic differences between the groups were detected. Administration of genistein and lavendustin A did not lead to hemodynamic differences between the study groups. During desflurane administration, LV peak systolic pressure (desflurane, 64 ± 20 mmHg; control, 95 ± 10 mmHg; $P = 0.01$) and systemic vascular resistance (desflurane, 0.34 ± 0.07 mmHg · min · ml^{-1}; control, 0.51 ± 0.11 mmHg · min · ml^{-1}; $P = 0.01$) were reduced, whereas no effect on cardiac output was detected (desflurane, 184 ± 56 ml/min; control, 183 ± 48 ml/min). There was a tendency toward increased heart rate during desflurane administration (desflurane, 292 ± 30 beats/min; control, 267 ± 27 beats/min), but this did not reach statistical significance.

Area at Risk and Infarct Size

The mean LV dry weight of all hearts studied was 0.70 ± 0.15 g, with no differences between the groups. The mean ischemic–reperfused area (area at risk) was 0.19 ± 0.07 g and constituted 27 ± 7% of the left ventricle (data from the individual groups are given in table 2). The results of the IS measurements are shown in figure 3. Anesthetic preconditioning with desflurane reduced IS from 55 ± 10% of the area at risk (control group) to 40 ± 15% (desflurane, $P = 0.04$ *vs.* control). 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_{ATP} channels.[12,18] Volatile anesthetics have also been shown to activate K_{ATP} channels, thereby inducing myocardial protection.[19] The mitochondrial K_{ATP} channel was considered to be the end-effector of ischemic preconditioning until Pain *et al.*[13] demonstrated that opening of mitochondrial K_{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. Müllenheim *et al.*[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).[18,20] Direct stimulation of PKC mimics[21] and specific blockade of PKC abolishes[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_{ATP} channel opening.[18,22] Activation of PKC is closely related to protein TK phosphorylation, and evidence has been reported that TKs can be upstream of,[23,24] parallel to,[15,25,26] or downstream of PKC.[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.[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.*[26] demonstrated in pigs that a combined inhibition of PKC and TK is needed to sufficiently block cardioprotection of ischemic preconditioning. Fryer *et al.*[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

	Baseline	Inhibitor	Desflurane	Washout
LVEDP, mmHg				
Control	2.6 ± 1.1	3.1 ± 1.4	3.4 ± 1.5	3.5 ± 1.5
Desflurane	3.0 ± 1.4	2.5 ± 1.3	1.5 ± 0.9	2.3 ± 1.2
Desflurane–genistein	2.5 ± 1.2	2.3 ± 0.9	2.0 ± 1.4	2.5 ± 0.9
Desflurane–lavendustin A	3.0 ± 1.1	3.1 ± 1.1	2.7 ± 1.4	3.0 ± 1.4
Genistein	2.4 ± 0.9	2.2 ± 1.2	2.4 ± 0.9	2.0 ± 1.0
Lavendustin A	2.3 ± 0.9	2.3 ± 0.6	2.0 ± 0.9	2.7 ± 1.4
$dPdt_{max}$, mmHg/s				
Control	3,555 ± 1,064	4,453 ± 880	4,357 ± 944	4,460 ± 725
Desflurane	3,695 ± 987	4,594 ± 983	2,818 ± 1,251	3,515 ± 1,113
Desflurane–genistein	3,696 ± 825	4,574 ± 1,076	3,263 ± 1,107	3,907 ± 1,130
Desflurane–lavendustin A	4,081 ± 1,000	4,299 ± 818	2,743 ± 1,065	3,075 ± 647
Genistein	3,681 ± 1,094	4,556 ± 940	4,859 ± 964	4,700 ± 1,129
Lavendustin A	3,789 ± 858	4,188 ± 744	4,434 ± 785	4,269 ± 852
$dPdt_{min}$, mmHg/s				
Control	−3,084 ± 1,017	−3,994 ± 787	−3,761 ± 829	−3,984 ± 722
Desflurane	−3,288 ± 1,043	−4,239 ± 1,006	−2,342 ± 1,256	−3,111 ± 1,018
Desflurane–genistein	−3,159 ± 778	−4,245 ± 1,055	−2,865 ± 1,031	−3,206 ± 658
Desflurane–lavendustin A	−3,418 ± 935	−3,868 ± 734	−2,208 ± 963	−2,681 ± 761
Genistein	−2,986 ± 969	−4,225 ± 916	−4,396 ± 1,086	−4,284 ± 1,167
Lavendustin A	−3,590 ± 1,208	−4,352 ± 1,146	−4,316 ± 1,162	−4,084 ± 1,193
SVR, mmHg · min · ml^{-1}				
Control	0.44 ± 0.09	0.49 ± 0.12	0.51 ± 0.11	0.52 ± 0.13
Desflurane	0.43 ± 0.13	0.50 ± 0.13	0.34 ± 0.07*	0.51 ± 0.14
Desflurane–genistein	0.42 ± 0.15	0.46 ± 0.10	0.33 ± 0.06	0.46 ± 0.12
Desflurane–lavendustin A	0.41 ± 0.11	0.42 ± 0.08	0.34 ± 0.09	0.48 ± 0.07
Genistein	0.41 ± 0.15	0.44 ± 0.09	0.49 ± 0.13	0.50 ± 0.11
Lavendustin A	0.39 ± 0.08	0.42 ± 0.09	0.46 ± 0.10	0.45 ± 0.11
τ, ms				
Control	18 ± 3	16 ± 2	16 ± 2	16 ± 2
Desflurane	17 ± 3	17 ± 4	18 ± 9	17 ± 3
Desflurane–genistein	16 ± 3	15 ± 3	16 ± 8	17 ± 4
Desflurane–lavendustin A	16 ± 3	17 ± 4	17 ± 5	20 ± 4
Genistein	20 ± 5	17 ± 3	16 ± 3	16 ± 3
Lavendustin A	16 ± 4	16 ± 2	17 ± 3	16 ± 4

(continues)

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.[9,29] However, Piriou *et al.*[9] found a much more profound protection by desflurane preconditioning (IS reduction from 54% to 16% of the area at risk) in a similar *in vivo* 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°–40.5° *vs.* 38.3°–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 Müllenheim *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 K_{ATP} channels,[12,18,19] probably followed by intracellular release of free oxygen radicals[13,14] 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

Table 1. (Continued)

Occlusion		Reperfusion			
15 min	29 min	15 min	30 min	60 min	120 min
4.0 ± 1.6	3.6 ± 1.7	3.6 ± 1.2	3.1 ± 1.1	3.4 ± 1.1	3.6 ± 1.0
3.2 ± 1.7	3.0 ± 1.6	2.6 ± 1.4	2.6 ± 1.2	2.6 ± 1.1	2.4 ± 1.0
3.9 ± 2.4	3.0 ± 1.2	2.4 ± 1.1	2.3 ± 1.0	2.4 ± 1.1	2.4 ± 1.1
4.2 ± 2.1	3.4 ± 2.1	2.8 ± 1.1	2.6 ± 1.0	2.5 ± 1.2	2.8 ± 1.3
3.6 ± 2.9	3.1 ± 1.4	2.9 ± 2.0	3.2 ± 1.8	2.7 ± 1.4	2.8 ± 1.7
3.2 ± 1.4	2.8 ± 1.3	2.4 ± 1.2	2.6 ± 0.8	2.6 ± 0.6	2.5 ± 0.6
3,140 ± 565	3,397 ± 706	2,916 ± 797	2,828 ± 785	2,765 ± 778	2,718 ± 818
3,013 ± 726	3,175 ± 652	2,802 ± 733	2,730 ± 823	2,427 ± 823	1,952 ± 806
3,669 ± 1,123	3,735 ± 907	2,824 ± 898	2,712 ± 800	2,643 ± 846	2,191 ± 748
3,093 ± 1,136	3,113 ± 906	2,696 ± 661	2,541 ± 630	2,326 ± 608	1,966 ± 843
3,537 ± 1,099	4,020 ± 831	3,476 ± 769	3,229 ± 849	2,640 ± 931	2,138 ± 820
3,222 ± 741	3,619 ± 737	3,215 ± 383	3,269 ± 314	3,098 ± 672	2,493 ± 720
−2,836 ± 466	−2,901 ± 621	−2,779 ± 1,221	−2,544 ± 938	−2,517 ± 1,134	−2,436 ± 880
−2,630 ± 618	−2,708 ± 737	−2,516 ± 765	−2,410 ± 922	+2,150 ± 945	−1,540 ± 906
−2,838 ± 725	−2,845 ± 372	−2,266 ± 679	−2,244 ± 705	−2,225 ± 779	−2,804 ± 772
−2,533 ± 782	−2,439 ± 616	−2,187 ± 675	−2,039 ± 710	−1,804 ± 750	−1,365 ± 700
−3,161 ± 757	−3,354 ± 858	−2,985 ± 931	−2,903 ± 1,008	−2,294 ± 1,076	−1,800 ± 996
−2,960 ± 570	−3,052 ± 746	−2,874 ± 443	−2,898 ± 434	−2,757 ± 722	−2,159 ± 750
0.48 ± 0.08	0.48 ± 0.10	0.45 ± 0.10	0.42 ± 0.10	0.40 ± 0.08	0.34 ± 0.06
0.49 ± 0.11	0.47 ± 0.09	0.45 ± 0.09	0.45 ± 0.11	0.44 ± 0.14	0.36 ± 0.12
0.41 ± 0.09	0.40 ± 0.09	0.35 ± 0.05	0.35 ± 0.05	0.34 ± 0.07	0.31 ± 0.06
0.44 ± 0.10	0.41 ± 0.09	0.38 ± 0.09	0.38 ± 0.09	0.35 ± 0.06	0.31 ± 0.05
0.43 ± 0.10	0.44 ± 0.09	0.42 ± 0.08	0.44 ± 0.12	0.39 ± 0.09	0.33 ± 0.07
0.42 ± 0.08	0.44 ± 0.09	0.41 ± 0.09	0.40 ± 0.09	0.38 ± 0.09	0.37 ± 0.10
20 ± 3	19 ± 3	18 ± 4	18 ± 3	19 ± 3	20 ± 4
20 ± 4	20 ± 5	20 ± 5	21 ± 5	21 ± 7	25 ± 8
19 ± 5	18 ± 5	18 ± 4	18 ± 5	19 ± 6	20 ± 7
22 ± 3	20 ± 3	21 ± 4	22 ± 5	24 ± 7	23 ± 6
19 ± 3	18 ± 3	17 ± 3	17 ± 2	19 ± 4	19 ± 3
18 ± 6	18 ± 6	18 ± 5	17 ± 5	17 ± 6	19 ± 7

dP/dt_{max} = maximum rate of increase in left ventricular pressure; dP/dt_{min} = minimum rate of decay in left ventricular pressure; LVEDP = left ventricular end-diastolic pressure; SVR = systemic vascular resistance; τ = time constant of decrease in isovolumic left ventricular pressure.

* $P = 0.01$ compared with the control group.

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.[21,33] 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*,[27,34,35] and both agents have been successfully used to block preconditioning in the

Table 2. Weight and Area at Risk

	Control	Desflurane	Desflurane–Genistein	Desflurane–Lavendustin A	Genistein	Lavendustin A
Body weight, g	2,555 ± 497	2,527 ± 553	2,583 ± 491	2,346 ± 482	2,543 ± 589	2,557 ± 436
LV weight, g	0.72 ± 0.18	0.67 ± 0.16	0.71 ± 0.13	0.64 ± 0.14	0.75 ± 0.17	0.71 ± 0.09
Area at risk, g	0.22 ± 0.08	0.16 ± 0.06	0.21 ± 0.09	0.17 ± 0.07	0.21 ± 0.07	0.19 ± 0.05
Area at risk/LV, %	30 ± 7	23 ± 8	29 ± 9	27 ± 8	28 ± 5	26 ± 6
Infarct size, g	0.12 ± 0.05	0.07 ± 0.04*	0.10 ± 0.06	0.08 ± 0.05	0.12 ± 0.06	0.09 ± 0.04

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

* $P = 0.03$ *vs.* control.

LV = left ventricular.

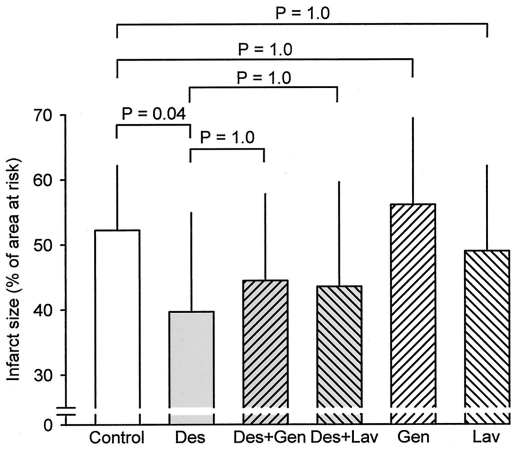

Fig. 3. Infarct size as a percentage of the area at risk in the control, desflurane (Des), desflurane + genistein (Des + Gen), desflurane + lavendustin A (Des + Lav), genistein (Gen), and lavendustin A (Lav) groups. Data are presented as mean ± SD.

rabbit heart *in vitro*.[36] A parallel pathway of TK and PKC, dependent on the force of the stimulus as suggested by Fryer *et al.*,[15] seems a possible explanation for our findings. One could speculate that 15 min of inhalation of 8.9% desflurane activates more than one pathway leading to protection and that a shorter administration time could then induce a TK-dependent pathway, but this issue has not been investigated. However, especially for rabbit hearts, there is evidence of a downstream localization of TK in signal transduction.[21,27]

With genistein and lavendustin A, we used two structurally different inhibitors of TK. The isoflavone genistein blocks TK by competitive inhibition at the adenosine triphosphate–binding site of the enzyme.[37] We did not measure genistein plasma concentration. Therefore, we cannot exclude that genistein had already left the binding site when TK was activated by desflurane inhalation. However, lavendustin A is a extremely potent and selective inhibitor of TK,[38] with a different mode of action from that of genistein, and acts as a noncompetitive inhibitor at the adenosine triphosphate–binding site as well as an uncompetitive inhibitor at the substrate-binding site.[39] The block of TK is irreversible.[40] Therefore, a relevant decrease of action of lavendustin A during our experimental protocol can be excluded, and the blockade of at least this agent was effective during desflurane inhalation and the subsequent index ischemia. Because of the high specificity of both blockers, undesired side effects that affected our results seem to be unlikely. The solvent DMSO was applied at the beginning of each experiment (with or without blocker). An effect of DMSO on IS cannot be ruled out but seems unlikely because control groups in other studies with the same *in vivo* rabbit model from our laboratory not receiving DMSO had similar ISs.[14,41]

In summary, we conclude that desflurane-induced preconditioning is independent of activation of TK. A parallel pathway of TK and PKC could be an explanation. Further investigations are needed to completely elucidate the mechanism of anesthetic preconditioning.

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