Anesthesiology 2004; 100:581–8

Desflurane-induced Preconditioning Alters Calcium-induced Mitochondrial Permeability Transition

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Background: **Recent investigations have focused on the pivotal role of the mitochondria in the underlying mechanisms volatile anesthetic-induced myocardial preconditioning. This study aimed at examining the effect of anesthetic preconditioning on mitochondrial permeability transition (MPT) pore opening.**

Methods: **Anesthetized open chest rabbits were randomized to one of four groups and underwent 10 min of ischemia, except for the sham 1 group (n = 12). Before this, they underwent a treatment period consisting of (1) no intervention (ischemic group; n = 12), (2) 30 min of desflurane inhalation (8.9% end-tidal concentration) followed by a 15-min washout period (desflurane group; n = 12), or (3) ischemic preconditioning (IPC group; n = 12). A second set of experiments was performed to evaluate the effect of a putative mitochondrial adenosine triphosphate–sensitive potassium channel antagonist, 5-hydroxydecanoate (5-HD). The animals underwent the same protocol as previously, plus pretreatment with 5 mg/kg 5-HD. They were randomized to one of five groups: the sham 2 group, receiving no 5-HD (n = 12); the sham 5-HD group (n = 12); the ischemic 5-HD group (n = 12), the desflurane 5-HD group (n = 12), and the IPC 5-HD group (n = 12). At the end of the protocol, the hearts were excised, and mitochondria were isolated. MPT pore opening was assessed by measuring the amount of calcium required to trigger a massive calcium release indicative of MPT pore opening.**

Results: **Desflurane and IPC group mitochondria needed a higher calcium load than ischemic group mitochondria (362 ± 84, 372 ± 74, and 268 ± 110 μM calcium, respectively; $P < 0.05$) to induce MPT pore opening. The sham 1 and sham 2 groups needed a similar amount of calcium to trigger mitochondrial calcium release (472 ± 70 and 458 ± 90 μM calcium, respectively). 5-HD preadministration had no effect on sham animals (458 ± 90 and 440 ± 128 μM calcium without and with 5-HD, respectively) and ischemic group animals (268 ± 110 and 292 ± 102 μM calcium without and with 5-HD, respectively) but abolished the effects of desflurane on calcium-induced MPT pore opening (362 ± 84 μM calcium without 5-HD *vs.* 238 ± 96 μM calcium with 5-HD; $P < 0.05$) and IPC (372 ± 74 μM calcium without 5-HD *vs.* 270 ± 104 μM calcium with 5-HD; $P < 0.05$).**

Conclusion: **Like ischemic preconditioning, desflurane improved the resistance of the transition pore to calcium-induced opening. This effect was inhibited by 5-HD, suggesting a link between mitochondrial adenosine triphosphate–sensitive potassium and MPT.**

ISCHEMIC preconditioning (IPC) represents an endogenous defense against ischemia. Several exogenous substances are known to trigger preconditioning, such as adenosine, bradykinin, noradrenaline, δ-opioid agonists, and inhalational volatile anesthetics.[1,2]

Recent attention has been focused on mitochondria that may play a pivotal role in volatile anesthetic–induced preconditioning. However, the mitochondrial molecular target responsible for the protection afforded by volatile anesthetic anesthetics remains unclear. Tanaka *et al.*[3] showed that mitochondrial adenosine triphosphate–sensitive potassium (mito K_{ATP}) channels are clearly involved in anesthetic preconditioning. Volatile anesthetics can induce flavoprotein oxidation[4] and decrease mitochondrial calcium overload during reperfusion.[5] Hanley *et al.*[6] demonstrated that volatile anesthetics can also block mitochondrial respiratory chain complex I. Several recent studies have shown the importance of mitochondrial permeability transition (MPT) pore opening in ischemia and mito K^+_{ATP} agonist–induced protection.[7] However, the link between mito K_{ATP} channel and MPT opening remains unclear. The current study was designed to investigate the effects of anesthetic preconditioning by volatile anesthetic agents on MPT. Specifically, we sought to determine whether desflurane-induced preconditioning is mediated by MPT and related to mito K_{ATP} activation.

Materials and Methods

This study, including care of the animals involved, was conducted according to the official edict of the French Ministry of Agriculture (Paris, France) and the recommendations of the Helsinki Declaration. Therefore, these experiments were conducted in an authorized laboratory and under the supervision of an authorized researcher (M. O.).

Surgical Preparation

Male New Zealand white rabbits weighing 2.2–2.5 kg were anesthetized by means of intramuscular injection of xylazine (5 mg/kg) and ketamine (50 mg/kg). An intravenous infusion of a mixture of xylazine (20–50 $\mu g \cdot kg^{-1} \cdot min^{-1}$) and ketamine (40–100 $\mu g \cdot kg^{-1} \cdot min^{-1}$) was then maintained throughout the experiment. Before the surgical procedure, adequate depth of anesthesia

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Received from the Université Claude Bernard, Lyon, France. Submitted for publication August 29, 2003. Accepted for publication November 24, 2003. Support was provided solely from institutional and/or departmental sources.

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was ensured after determination of the absence of pedal and palpebral reflexes. After a midline cervical incision, a tracheotomy was performed, and animals underwent mechanical ventilation (Servo Ventilator 900 B; Siemens-Elema, Solna, Sweden) with use of 50% oxygen. The tidal volume was set at 15 ml/kg, and the respiratory rate was set at 35 strokes/min. Ventilation was adjusted to keep the end-tidal carbon dioxide concentration within the physiologic range. End-tidal gas concentration was measured continuously (gas analyzer Capnomac Ultima; Datex, Helsinki, Finland). Body temperature, recorded by use of a thermistor inserted into the esophagus, was maintained between 39° and 40.5°C. Limb lead II of the electrocardiograph was continually monitored by means of subcutaneous needle electrodes. Systemic blood pressure was monitored continuously throughout the experiment with use of a Gould® pressure transducer (Gould Inc., Cleveland, OH) connected to a 1-mm fluid-filled catheter inserted in the central ear artery. A cannula was inserted into an ear vein for administration of drugs and fluids. The heart was exposed *via* a left thoracotomy in the fourth left intercostal space and suspended in a pericardial cradle. The first large marginal branch of the circumflex artery was identified, and a 3.0 silk suture attached to a small curved needle was passed around this artery. Both ends of the thread were passed through a small vinyl tube to form a snare that could be tightened to occlude and loosened to reperfuse the artery. After the surgical procedure, a 20-min stabilization period was allowed.

In all groups except the sham groups, the coronary artery was occluded for 10 min after 55 min. Myocardial ischemia was confirmed by the appearance of a regional cyanosis on the epicardium distal to the snare, akinesia or bulging in the area, and a progressive marked ST segment elevation in the electrocardiogram. After 10 min, the snare was released, and reperfusion was allowed for a period of 5 min. Reperfusion was visually confirmed by the appearance of hyperemia. The thread passed around the marginal artery was left in place, and the heart was excised. A piece of myocardium (0.6–1.0 g) in the ischemic area (except in the sham group) was cut and immediately placed in a buffer solution.

Preparation of Isolated Mitochondria

Preparation of mitochondria was adapted from a previously described procedure.[8] All operations were performed in the cold. Heart pieces (0.6–1.0 g) were placed in isolation buffer A, containing 70 mM sucrose, 210 mM mannitol, and 1 mM EDTA in 50 mM Tris–HCl (pH 7.4). The tissue was finely minced with scissors and then homogenized in the same buffer (1 ml buffer/g tissue) using successively a Kontes tissue grinder and a Potter-Elvehjem (Glass Company, Vineland, NJ). The homogenate was centrifuged at 1,300*g* for 3 min. The supernatant was poured through cheesecloth and centrifuged at 10,000*g* for 10 min. The mitochondrial pellet was resuspended in isolation buffer B, containing 70 mM sucrose and 210 mM mannitol in 50 mM Tris–HCl (pH 7.4). After aliquots were removed for protein measurements, the mitochondria (by aliquots of 5 mg protein) were washed in isolation buffer B, centrifuged at 6,500*g* for 10 min, and stored as pellets on ice before MPT pore opening experiments. Protein content was routinely assayed according to the procedure of Gornall *et al.*[9] using bovine serum albumin as a standard. To check the validity of the fractionation procedure, the purity and integrity of isolated mitochondria were assessed in the sham groups by measuring specific activities of cytochrome c oxidase (EC 1.9.3.1) as an inner membrane marker enzyme[10] and by electron microscopy.

Ca^{2+}-induced MPT Pore Opening

Mitochondrial permeability transition pore opening was assessed after *in vitro* Ca^{2+} overload. Isolated mitochondria (5 mg protein) were suspended in 100 μl buffer B and added to 900 μl buffer C (150 mM sucrose, 50 mM KCl, 2 mM KH_2PO_4, and 5 mM succinic acid in 20 mM Tris/HCl; pH 7.4) within a 22°C thermostatic Teflon chamber (Veretech, Chaponost, France) equipped with a Ca^{2+}-selective microelectrode, in conjunction with a reference electrode.[11] Mitochondria were gently stirred for 90 s. At the end of the preincubation period, 20-μM $CaCl_2$ pulses were performed every 60 s. Modifications of the extramitochondrial Ca^{2+} concentration were continuously recorded using custom-made Synchronie software (eurosmart, Mane la Vallée, France). After sufficient Ca^{2+} loading, the extramitochondrial Ca^{2+} concentration abruptly increased, indicating a massive release of Ca^{2+} by mitochondria due to MPT pore opening. The amount of Ca^{2+} necessary to trigger a massive Ca^{2+} release was used here as an indicator of MPT pore susceptibility to Ca^{2+} overload.

Electron Microscopy

Electron microscopy was performed either at the end of the preincubation period (*i.e.*, before Ca^{2+} loading) or at the end of the experiment in one animal in the sham 1 group to assess the integrity or the morphologic alterations of the mitochondria after permeability transition, respectively. Samples of mitochondria were fixed for 2 h in 2% glutaraldehyde, 100 mM phosphate buffer (pH 7.4) and postfixed in 1% osmium tetroxide. Dehydration was performed in a series of ethanol and propylene oxide extractions, before sample embedding in Epon.

Chemicals

Cyclosporin A (Novartis Pharma, Rueil Malmaison, France), used *in vitro* (5 μM), was dissolved in ethanol and diluted in buffer C before use to obtain for each dilution the equivalent of 2.5 μl pure ethanol in 1 ml.

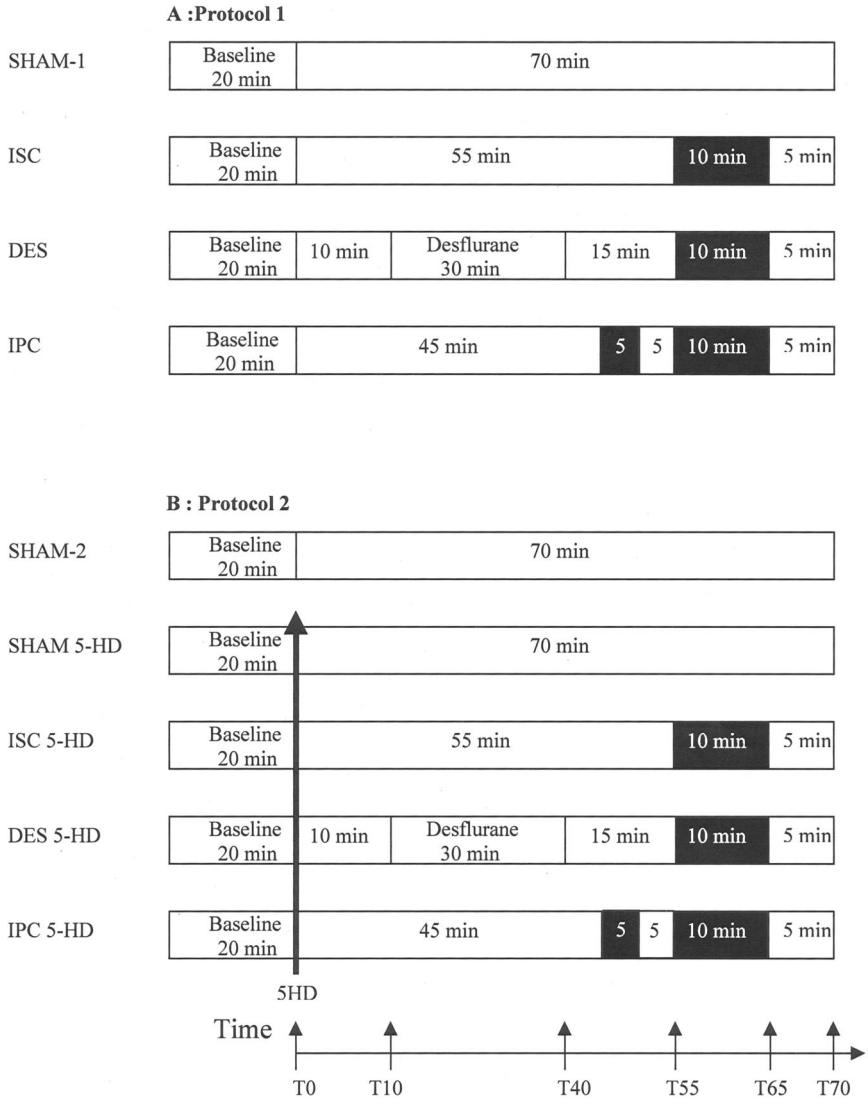

Fig. 1. Experimental design. *Filled bars* = ischemia; *open bars* = perfusion. Desflurane administration consisted of 8.9% end-tidal inhalational administration. In set *B*, 5 mg/kg 5-hydroxydecanoate (5-HD) was injected. DES = desflurane group; IPC = ischemic preconditioning group; ISC = ischemic group; SHAM = sham group.

This amount of pure ethanol had no effect on MTP pore opening (data not shown). Desflurane was purchased from Baxter (Lessines, Belgium), and 5-hydroxydecanoate (5-HD) was purchased from ICN Biochemicals (Aurora, OH). 5-HD was dissolved in 0.9% NaCl.

Experimental Design

Two protocols are presented (fig. 1): the first was designed to investigate whether MPT pore opening is delayed by desflurane-induced preconditioning, whereas the second was aimed at determining whether blockage of mito K_{ATP} by 5-HD can modify desflurane- and ischemia-induced preconditioning modifications of MPT pore opening.

Protocol 1: Effect of Desflurane-induced Preconditioning on Calcium-induced MPT Pore Opening. The rabbits were randomly divided into four groups. After 30 min of stabilization, the desflurane group (n = 12) received 30 min of inhalational desflurane (8.9% end-tidal) followed by a 15-min washout period before 10 min of coronary artery occlusion. The IPC group (n = 12) received one cycle of 5 min of ischemia followed by 5 min of reperfusion before 10 min of ischemia. The sham group (sham 1; n = 12) and the ischemic group (n = 12) underwent either no intervention or 10 min of ischemia, respectively.

Protocol 2: Effect of 5-HD on MPT Pore Opening in Desflurane- and Ischemia-induced Preconditioning. The rabbits were randomly divided into five groups. All animals, except those in the sham 2 group, received a 5-mg/kg intravenous bolus of 5-HD after 20 min of stabilization. Ten minutes after 5-HD injection, the desflurane 5-HD group (n = 12) received 30 min of inhalational desflurane (8.9% end-tidal) followed by a 15-min washout period before 10 min of coronary artery occlusion. The IPC 5-HD group (n = 12) received 5-HD 45 min before the preconditioning cycle. The sham 5-HD group (n = 12) and the ischemic 5-HD group (n = 12) received 5-HD followed by either no intervention or 10 min of ischemia 55 min later, respectively. The sham 2 group (n = 12) received no intervention.

Table 1. Hemodynamic Measurements in Different Experimental Groups

	T_0	T_{10}	T_{40}	T_{55}	T_{65}	T_{70}
Systemic blood pressure, mmHg						
Protocol 1						
SHAM-1	78 ± 13	76 ± 14	70 ± 17	70 ± 14	71 ± 13	69 ± 14
Ischemic	79 ± 16	75 ± 15	68 ± 11	68 ± 12	53 ± 8*	64 ± 8
IPC	83 ± 14	79 ± 7	73 ± 11	70 ± 9	50 ± 17*	69 ± 7
Desflurane	66 ± 14	60 ± 17	48 ± 15*	60 ± 13	58 ± 11	67 ± 14
Protocol 2						
Sham 2	74 ± 11	73 ± 12	68 ± 13	66 ± 14	67 ± 13	68 ± 14
Sham 5-HD	89 ± 14	81 ± 9	75 ± 9	72 ± 12	74 ± 7	74 ± 6
Ischemic 5-HD	72 ± 12	66 ± 11	63 ± 10	67 ± 9	54 ± 13	66 ± 8
IPC 5-HD	73 ± 14	68 ± 11	68 ± 15	65 ± 7	53 ± 20	67 ± 13
Desflurane 5-HD	79 ± 9	78 ± 11	47 ± 9*	71 ± 11	60 ± 8	71 ± 11
Heart rate, beats/min						
Protocol 1						
Sham 1	176 ± 27	179 ± 30	175 ± 36	178 ± 35	177 ± 33	179 ± 30
Ischemic	171 ± 39	175 ± 35	173 ± 35	174 ± 27	189 ± 26	165 ± 29
IPC	186 ± 37	183 ± 37	181 ± 32	170 ± 30	174 ± 44	165 ± 38
Desflurane	208 ± 38	198 ± 23	181 ± 22	185 ± 23	167 ± 31	178 ± 32
Protocol 2						
Sham 2	174 ± 27	174 ± 23	180 ± 16	192 ± 20	193 ± 23	192 ± 25
Sham 5-HD	195 ± 27	201 ± 31	201 ± 38	193 ± 27	184 ± 28	185 ± 33
Ischemic 5-HD	212 ± 36	211 ± 36	196 ± 44	184 ± 36	179 ± 32	164 ± 19
IPC 5-HD	195 ± 37	187 ± 34	178 ± 41	167 ± 39	182 ± 31	172 ± 41
Desflurane 5-HD	175 ± 15	180 ± 17	191 ± 26	182 ± 25	183 ± 38	175 ± 26

Values are expressed as mean ± SD.

* $P < 0.05$ compared with the sham from the same protocol.

5-HD = 5-hydroxydecanoate; IPC = ischemic preconditioning; T_0 = baseline; T_{10} = before desflurane; T_{40} = end of desflurane; T_{55} = before ischemia; T_{65} = after ischemia; T_{70} = 5 min after ischemia.

Statistical Analysis

Statistical analysis of hemodynamics was performed with use of two-way analysis of variance with repeated measures on one factor. The concentration of Ca^{2+} necessary to trigger MPT pore opening was analyzed by one-way analysis of variance, and when a significant *F* value was obtained, means were compared using a *post hoc* Tukey test. The effect of 5-HD (comparison of experimental protocols 1 and 2) was analyzed by one-way analysis of variance; means were compared with use of a *post hoc t* test with Bonferroni correction. All values are expressed as mean ± SD. Statistical significance was defined as a value of $P < 0.05$.

Results

One hundred fourteen rabbits were included in the current study: 52 in protocol 1 and 62 in protocol 2. Six rabbits were excluded. Four rabbits were excluded in protocol 1: one because of technical problem during surgery, two because of pump failure during coronary occlusion, and one because of technical problems during evaluation of MPT pore opening. Two rabbits were excluded in protocol 2: one because of sudden death after anesthetic induction and one because of pump failure. Therefore, results are presented for the remaining 108 rabbits.

Hemodynamics

Hemodynamic data, including heart rate and systolic blood pressure, are summarized in table 1. Systolic blood pressure significantly decreased during desflurane administration (protocols 1 and 2) as well as during ischemia (protocol 1) in the ischemic and IPC groups. Heart rate did not differ among groups.

Isolated Mitochondria Preparation

In all cases, mitochondrial suspensions exhibited similarly high cytochrome c oxidase–specific activities (*i.e.*, specific mitochondrial marker enzyme). The quality of the preparation was further demonstrated by means of electron microscopy. Figure 2A depicts isolated mitochondria suspended in buffer C, *i.e.*, just before Ca^{2+} loading. Clearly, mitochondria displayed intact membranes and dense matrix space; moreover, there was no contamination by other organelles as lysosomes or plasma membrane vesicles. Comparable morphology was observed at the end of the isolation procedure, indicating that the preparation was not altered during the experiment (data not shown). In contrast, after Ca^{2+}-induced MPT pore opening, most mitochondria underwent dramatic morphologic changes; we observed large swollen mitochondria, with outer membranes appearing ruptured and largely removed (fig. 2B). These results demonstrated the good quality of the fractionation procedure.

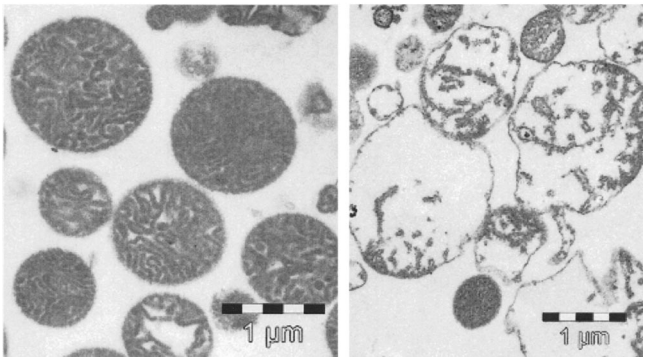

Fig. 2. Electron microscopic views of isolated mitochondria (sham 1 group). (*A*) Electron microscopy of heart mitochondria processed after preincubation in buffer C for 90 s, which showed the integrity and purify of mitochondrial fraction before Ca^{2+}-induced mitochondrial permeability transition pore opening. (*B*) After Ca^{2+}-induced mitochondrial permeability transition pore opening, almost the whole population of the heart mitochondria show massive swelling, with disrupted cristae and damaged matrix.

Ca^{2+}-induced MPT Pore Opening

As depicted in figure 3, each 20-μM $CaCl_2$ pulse was recorded as a peak of extramitochondrial Ca^{2+} concentration. After each pulse, Ca^{2+} was rapidly taken up by the mitochondria *via* the Ca^{2+} uniporter, resulting in a return of extramitochondrial Ca^{2+} concentration to near baseline level. After sufficient Ca^{2+} loading, extramitochondrial Ca^{2+} concentration abruptly increased, indicating a massive release of matrix Ca^{2+} through the MPT pore.

Typical recording in figure 3A shows that mitochondria isolated from sham 1 animals buffered 30 consecutive Ca^{2+} loads (20 nmol · 5 mg protein^{-1} · min^{-1}) before the abrupt release of matrix calcium through the MPT pore. In contrast, in mitochondria isolated from ischemic group hearts (fig. 3B), MPT pore opening occurred after only 12 pulses (*i.e.*, a Ca^{2+} load of 240 μM). Interestingly, mitochondria isolated from preconditioned animals either by desflurane (fig. 3C) or by ischemia (fig. 3D) displayed delayed Ca^{2+}-induced MPT pore opening when compared with ischemic group hearts.

Figure 4 depicts the effect of 5 μM cyclosporin A (a specific MPT pore blocker) added 1 min before the first pulse of $CaCl_2$. The amount of calcium needed to trigger Ca^{2+} release after the addition of cyclosporin A was more than 900 μM for each sample (sham 1 group; n = 6). It confirmed that the release of calcium reflects the opening of the MPT pore.

Figure 5 shows the mean concentration of Ca^{2+} required to open the MPT pore in each group. The calcium load required to open the MPT pore averaged 472 ± 70 and 458 ± 90 μM in sham 1 and sham 2 groups, respectively (P = not significant). In protocol 1, the calcium load required to open the MPT pore was significantly reduced in ischemic group hearts, averaging 268 ± 110 μM. The IPC and desflurane groups needed a significantly higher calcium load (372 ± 74 and 362 ± 84 μM calcium, respectively) to trigger MPT pore opening ($P < 0.05$ *vs.* ischemic group; P = not significant *vs.* sham 1 group; fig. 5A).

In protocol 2, 5-HD had no significant effect on sham 2 hearts (fig. 5B). The calcium load required to open the MPT pore in ischemic 5-HD hearts averaged 292 ± 102 μM calcium ($P < 0.05$ *vs.* sham 2 group; P = not significant *vs.* ischemic group). However, the calcium load required to open the MPT pore averaged 270 ± 104 and 238 ± 96 μM in the IPC 5-HD and desflurane 5-HD groups ($P < 0.05$ *vs.* sham 2 group; $P < 0.05$ *vs.* corresponding groups in protocol 1).

Discussion

In the current study, we report that mitochondria isolated from *in vivo* desflurane-preconditioned rabbit hearts display a delayed MPT pore opening when exposed to a Ca^{2+} overload. This pattern of Ca^{2+}-induced MPT pore opening is comparable with that of ischemia-preconditioned mitochondria. In addition, the mito K_{ATP} blocker 5-HD abolished this improved tolerance of the MPT pore to Ca^{2+} overload, induced by both desflurane and IPC.

It has been shown that most volatile anesthetics can induce preconditioning.[1,2] Desflurane has been reported to be the most potent volatile anesthetic agent,[12] possibly because it also involves intramyocardial adrenergic stimulation.[13] Underlying mechanisms of volatile anesthetic preconditioning have been actively investigated. Adenosine receptors and K_{ATP} channels have been proposed as important mediators of volatile anesthetic–induced cardioprotection.[14] Piriou *et al.*[15] recently reported that 5-HD, a potent blocker of mito K_{ATP} channels, inhibits isoflurane-induced preconditioning.

The pivotal role of mitochondria, and more specifically of permeability transition, in cell death is now well recognized.[16,17] MPT is due to opening of a large pore in the inner mitochondrial membrane, which causes matrix swelling, inner membrane potential ($\Delta\Psi_m$) collapse, uncoupling of the respiratory chain, efflux of Ca^{2+}, and release of small proteins, such as cytochrome c.[18] After ischemia–reperfusion, MPT is mostly induced by Ca^{2+} overload, associated with adenine nucleotide depletion, increased inorganic phosphate concentration, and oxidative stress.[19] MPT pore opening is inhibited by the immunosuppressive drug cyclosporin A, which can protect the heart from ischemia–reperfusion in *in vitro* models.[20,21,22] MPT seems to be a critical event in the transition from reversible to irreversible myocardial injury after an ischemic insult.

Recent evidence suggests that IPC may modify MPT pore opening and thereby limit infarct size and reduce cardiomyocyte apoptosis. Through the measurement of

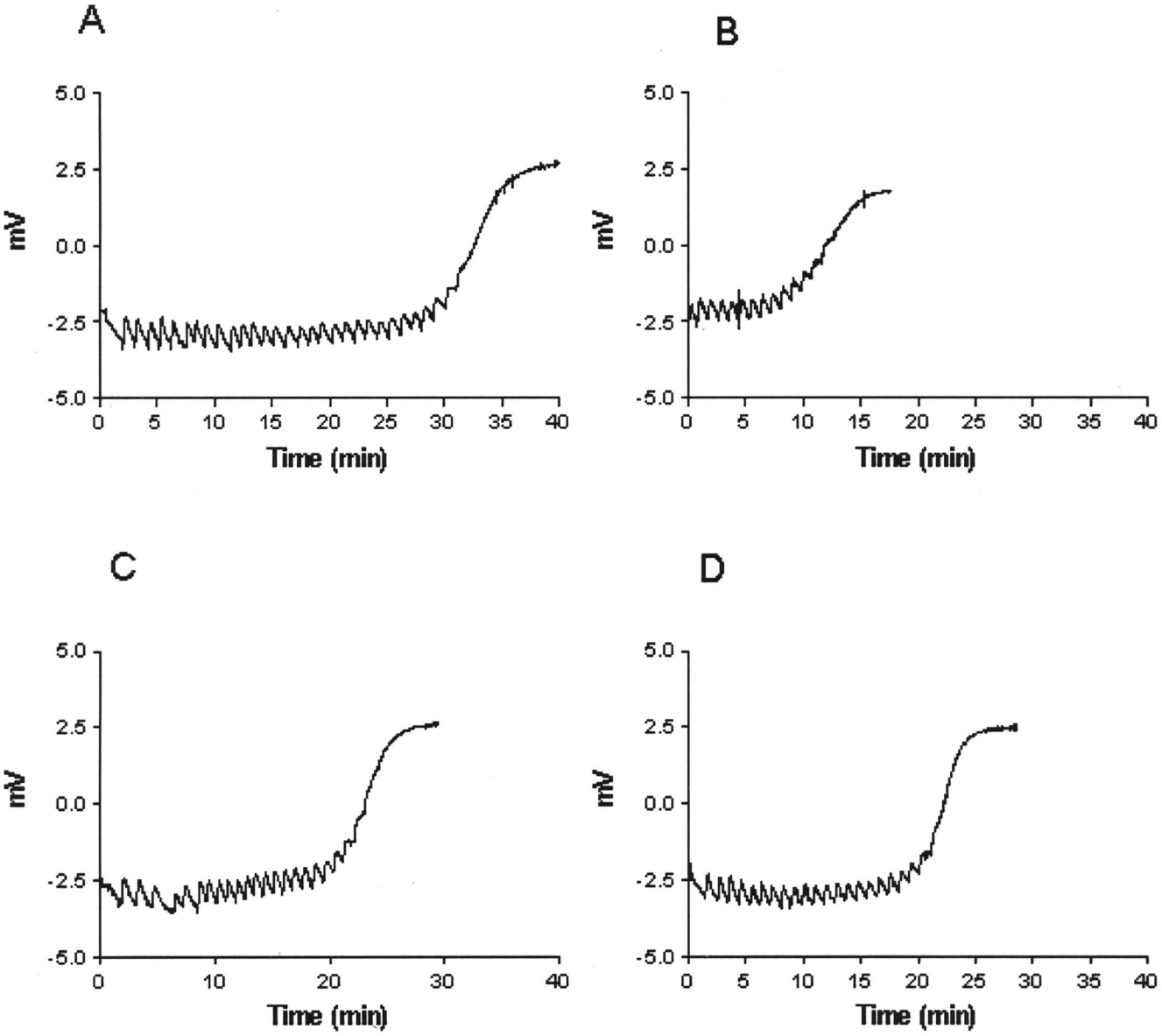

Fig. 3. Typical examples of measurement of Ca^{2+}-induced mitochondrial permeability transition pore opening on isolated heart mitochondria (protocol 1). The calcium uptake by mitochondria (5 mg protein in 1 ml) was measured in the presence of 5 mM succinate by using a specific calcium electrode. Equivalent pulses of $CaCl_2$ (4 nmol Ca^{2+}/mg protein = 20 μM) were added to mitochondria every 1 min until a spontaneous release of Ca^{2+} occurred. Mitochondrial preparations were from the sham 1 (*A*), ischemic (*B*), desflurane (*C*), and ischemic preconditioning (*D*) groups.

mitochondrial 2-deoxy[^{3}H]glucose entrapment, Javadov *et al.*[7] showed that IPC inhibits MPT pore opening during reperfusion. By studying calcein fluorescence in isolated rat heart mitochondria, Hausenloy *et al.*[23] demonstrated that opening the mito K_{ATP} channel by diazoxide inhibits MPT pore opening. Our current data further extend information provided by those studies. We report here that the volatile anesthetic desflurane reverses the decreased resistance of MPT pore to Ca^{2+} overload induced by ischemia–reperfusion. This beneficial effect was similar to that observed in heart mitochondria that underwent IPC. In the current study, Ca^{2+}-induced MPT pore opening was assessed after 10 min of ischemia and 5 min of reperfusion. We purposely chose such a reversible ischemic injury to avoid isolating mitochondria from heterogeneously infarcted tissue with a mixture of dead and still-viable cardiomyocytes. If ischemia had been more prolonged and had caused irreversible tissue damage, surviving myocytes (*i.e.*, mitochondria) would come not only from the least ischemic part of the area at risk in control hearts, but also from a larger and more severely ischemic region in the myocardium salvaged by preconditioning. Those two populations of mitochondria would not be truly comparable. In fact, our results are in close agreement with other investigators who used more prolonged coronary artery occlusions.[7,23]

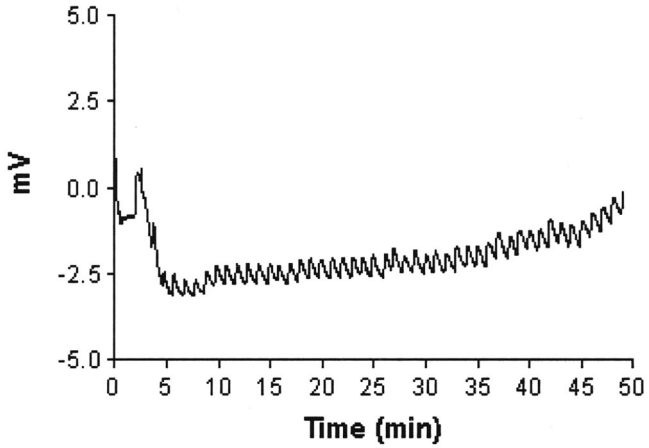

Fig. 4. Example of measurement of Ca^{2+}-induced mitochondrial permeability transition pore opening on isolated heart mitochondria in presence of 5 μM cyclosporin A. Cyclosporin A, 5 μM, was added in buffer C just before the addition of 5 mg mitochondria prepared from a sham 1 animal. Note the resistance induced by cyclosporin A to mitochondrial permeability transition pore opening.

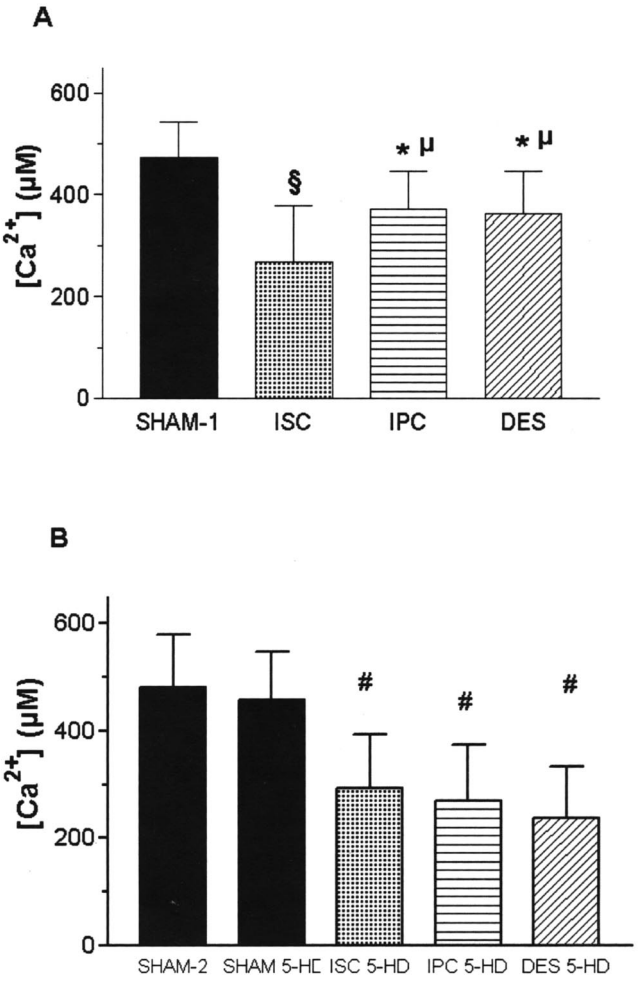

Fig. 5. Ca^{2+} overload required for mitochondrial permeability transition pore opening during ischemia–reperfusion. After Ca^{2+} loadings, the Ca^{2+} concentration necessary to trigger the massive release of Ca^{2+} by mitochondria due to mitochondrial permeability transition pore opening was depicted for protocol 1 (*A*) and protocol 2 (*B*). * $P < 0.05$ compared with ischemic (ISC) or sham 1 group. # $P < 0.05$ compared with sham 2 or sham 5-hydroxydecanoate (5-HD). § $P < 0.05$ compared with sham 1 group. μ $P < 0.05$ compared with the group receiving the same experimental protocol with 5-HD. DES = desflurane group; IPC = ischemic preconditioning group.

We further demonstrated in the current study that this modification of mitochondrial transition permeability by desflurane, as well as IPC, was abolished by the mito K_{ATP} channel blocker 5-HD. Taken together, these results indicate that desflurane- and ischemia-induced cardioprotection are triggered by activation of mito K_{ATP} and mediated by MPT pore opening. Despite this important information, the link between mito K_{ATP} activation and inhibition of MPT pore opening remains elusive. Recent investigations have highlighted the role of oxygen-derived free radicals on one hand and Ca^{2+} on the other hand. Scavengers of reactive oxygen species (ROS) can block the cardioprotection afforded by isoflurane preconditioning.[24] Inhalational anesthetics induce the generation of a small amount of ROS, acting as a trigger of preconditioning.[24,25] This results in a decrease in ROS production during reperfusion after sustained ischemia,[26,27] thereby limiting ischemia–reperfusion injury, possibly by the prevention of MPT pore opening and cell death. ROS could act as a second messenger by activating protein kinase mediators, such as protein kinase C.[28] Mito K_{ATP} channels might act as mediators of preconditioning,[3] controlling free radical generation within the mitochondria through potassium entry in the mitochondrial matrix[29,30] and rendering mitochondria more resistant to calcium overload.[31] Another possibility would be that ischemic as well as volatile anesthetic preconditioning reduces cytosolic calcium loading after sustained ischemia.[32] Riess *et al.*[5] demonstrated that sevoflurane pretreatment decreases mitochondrial calcium overload during ischemia and reduces ischemia–reperfusion injury. Limiting the matrix concentration of Ca^{2+}, the major activator of permeability transition, would delay or attenuate cell death. 5-HD reverses this effect of volatile anesthetics on mitochondrial calcium overload, suggesting a role for mito K_{ATP} channels.

Although there is increasing evidence that mito K_{ATP} channels are involved in this protection and functionally connected to the MPT pore, one cannot rule out that nonspecific effects of pharmacologic modulators of these channels may play a confounding role. Recently, Hanley *et al.*[6] reported in pig heart mitochondria that volatile anesthetics can inhibit (at least partly) the mitochondrial respiratory chain at the level of complex I. The same authors reported that diazoxide and 5-HD can directly inhibit the respiratory chain, independent of any effect on mito K_{ATP} channels.[6] However, in the current study, isolated mitochondria were energized with use of succinate, a complex II–linked substrate that bypasses

complex I. Therefore, in our preparation, the observed effects of desflurane and 5-HD cannot be related to an effect at the level of complex I. However, further studies are needed to assess the role of inhibition of complex I in preconditioning, in part because complex I is a major site of ROS production.

In conclusion, the current study demonstrated that both ischemia and desflurane-induced preconditioning improve the resistance of the MPT pore to ischemia–reperfusion. This MPT pore opening inhibition was prevented by 5-HD preadministration, suggesting a link between mito K_{ATP} and the permeability transition pore. Further studies are needed to understand the underlying mechanisms of volatile anesthetic–induced cardioprotection.

The authors thank Elisabeth Pinelli (Secretary, Service d'Anesthésie Réanimation, Centre Hospitalier Lyon Sud, Pierre Bénite, France).

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