Anesthesiology 2009; 111:1044–51

Mechanisms of Impaired Glucose Tolerance and Insulin Secretion during Isoflurane Anesthesia

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Background: **Volatile anesthetics impair insulin secretion and glucose utilization; however, the precise mechanism of action that underlies these effects is unknown. The authors hypothesized that isoflurane inhibits glucose-induced inhibition of adenosine triphosphate–sensitive potassium channel activity in pancreatic β cells, which could result in impaired insulin secretion and glucose tolerance.**

Methods: **Intravenous glucose tolerance tests were performed on 28 male Japanese White rabbits anesthetized with sodium pentobarbital. Glibenclamide (50 μg/kg + 33.5 $\mu g \cdot kg^{-1} \cdot h^{-1}$) or vehicle was administered 75 min before intravenous administration of 0.6 g/kg glucose. Half of the animals (n = 7) in the vehicle and glibenclamide groups received isoflurane at 1.0 minimum alveolar concentration 30 min before administration of glucose, and the other half received a vehicle control. Hemodynamics, blood glucose, and plasma insulin were measured. A cell-attached patch clamp configuration was used to record single channel currents in the pancreas from male Swiss-Webster mice.**

Results: **Isoflurane alone or a combination of isoflurane and glibenclamide inhibited the insulinogenic index to a greater extent than in the vehicle and glibenclamide groups. In the patch clamp experiments, channel activity was significantly decreased as the glucose concentration was increased from 0 to 10 mM. The subsequent application of 0.5 mM isoflurane reversed the effects of glucose on channel activity.**

Conclusion: **These results show that isoflurane impairs insulin secretion and glucose utilization. The mechanism of action responsible for these effects may involve a decrease in glucose-induced inhibition of adenosine triphosphate–sensitive potassium channel activity in pancreatic β cells.**

DIABETES and hyperglycemia are significant predictors of perioperative cardiovascular morbidity and mortality.[1] Several studies[1–4] have shown that there is a strong correlation between blood glucose concentration and outcome in patients with coronary artery disease. These studies have suggested that a decrease in blood glucose concentration results in a lower rate of cardiovascular morbidity and mortality. Volatile anesthetics have been shown to have cardioprotective[5,6] and vasodilative[7–9] effects, which are mediated by activation of adenosine triphosphate–sensitive potassium (K_{ATP}) channels in cardiac myocytes and vascular smooth muscle cells, respectively. Volatile anesthetics have been known since the 1970s to impair insulin secretion and glucose utilization.[10–15] However, the precise mechanism of action responsible for impaired insulin secretion in response to volatile anesthetics remains unclear.

Adenosine triphosphate–sensitive potassium channels are found in cardiac myocytes, vascular smooth muscle cells, and pancreatic β cells.[16] K_{ATP} channels are essential for the regulation of insulin secretion in pancreatic β cells.[16,17] In pancreatic β cells, a high concentrations of ATP resulting from the metabolism of glucose causes K_{ATP} channels to close, leading to exocytosis of insulin-containing granules.[18] Sulfonylureas, which block pancreatic K_{ATP} channels, stimulate insulin release and have been widely used in the treatment of diabetic patients.[19] Patch clamp experiments have shown that isoflurane facilitates the opening of K_{ATP} channels in cardiac myocytes.[20] Data from our laboratory have shown that isoflurane opens K_{ATP} channels in vascular smooth muscle cells through activation of protein kinase A.[21] However, the effects of volatile anesthetics on K_{ATP} channels in pancreatic β cells have not been previously characterized. We hypothesized that isoflurane reverses the inhibition of pancreatic K_{ATP} channel activity induced by glucose or the specific K_{ATP} channel blocker glibenclamide, thereby decreasing insulin secretion and glucose tolerance. Intravenous glucose tolerance tests (IVGTTs) in rabbits and patch clamp experiments in murine pancreatic β cells were used to test this hypothesis.

Materials and Methods

This study was approved by the Animal Investigation Committee of Tokushima University, Tokushima, Japan, and was conducted according to the animal use guidelines of the American Physiologic Society, Bethesda, Maryland.

Intravenous Glucose Tolerance Tests

General Preparation. After fasting for approximately 15 h, male Japanese white rabbits (13 weeks old, 2.5–3.0 kg) were anesthetized with intravenous sodium pentobarbital (30 mg/kg). A surgical plane of anesthesia was maintained by continuous infusion of sodium pentobarbital (17 $mg \cdot kg^{-1} \cdot h^{-1}$) *via* an ear vein catheter.[21] A tracheostomy was performed through a ventral midline incision, and the trachea was cannulated. Rabbits were

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Received from the Department of Anesthesiology, Tokushima University School of Medicine, Tokushima, Japan. Submitted for publication February 24, 2009. Accepted for publication July 29, 2009. Supported in part by a Grant-in Aid for Scientific Research (C, 19591800) from the Japan Society for the Promotion of Science, Tokyo, Japan. Presented at the Annual Meeting of the American Society of Anesthesiologists, San Francisco, California, October 17, 2007.

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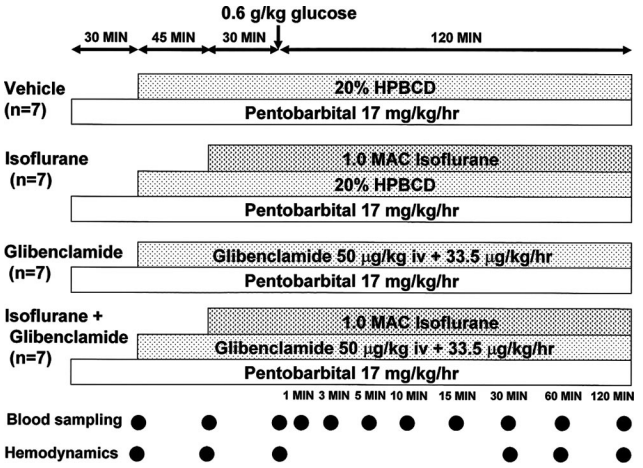

Fig. 1. Schematic illustration of the experimental protocol used for the intravenous glucose tolerance test. HPBCD = hydroxypropyl β cyclodextrin; MAC = minimum alveolar concentration.

ventilated (model 122; New England Medical Instruments, Medway, MA) with positive pressure using 100% oxygen. The ventilator was adjusted as needed to maintain blood pH and arterial carbon dioxide tension within normal physiologic ranges. A heating blanket was used to maintain body temperature at 38.5°C. Heparin-filled catheters were inserted into the right carotid artery and the right jugular vein for the measurement of arterial blood pressure and the administration of drug and maintenance fluid, respectively. Maintenance fluid consisted of lactated Ringer's solution (15 $ml \cdot kg^{-1} \cdot h^{-1}$), which was continuously infused for the duration of the experiment.

Experimental Protocol. Thirty minutes after surgery, rabbits were randomized to receive an intravenous bolus followed by a 195-min intravenous infusion of either glibenclamide (50 $\mu g/kg + 33.5\ \mu g \cdot kg^{-1} \cdot h^{-1}$) or an equivalent volume of vehicle solution (20% hydroxypropyl β cyclodextrin).[22] Forty-five minutes after the infusion was started, 0 or 1 minimum alveolar concentration (MAC) isoflurane was reached and was maintained throughout the duration of the study (fig. 1). Thirty minutes after 0 or 1 MAC isoflurane was reached, a solution of 0.6 g glucose/kg body weight was administered into the right jugular vein. One milliliter of blood was collected to measure levels of blood glucose and plasma insulin at baseline, and again immediately before administration of isoflurane, immediately before administration of glucose, and at 1, 3, 5, 10, 15, 30, 60, and 120 min after administration of glucose (fig. 1). Blood glucose concentrations were measured using a small electrode-type blood glucose meter (ANTSENSE III; HORIBA Ltd., Kyoto, Japan). Plasma was stored at −80°C until immunoreactive insulin (IRI) levels were measured. Plasma IRI levels were determined using a sandwich enzyme-linked immunosorbent assay (Insulin kit; Morinaga Institute of Biologic Science, Yokohama, Japan). The insulinogenic index was calculated by dividing the area under the insulin–time curve by the corresponding area under the glucose–time curve from 0 to 5 min after administration of glucose.

Electrophysiologic Experiments

Cell Preparation. Pancreata from 25–30 g male Swiss-Webster mice were used for patch clamp experiments. The bile duct was cannulated, and 2 mg/ml collagenase was injected into the pancreas, which was then removed and incubated for 5–10 min at 37°C to isolate individual islets. Single-cell suspensions were obtained by gently shaking islets in a low-calcium medium. Cell suspensions were plated onto glass coverslips in 35-mm Petri dishes containing RPMI-1640 medium supplemented with fetal bovine serum, L-glutamate, and penicillin–streptomycin. Petri dishes were kept in a 95% air–5% CO_2 incubator at 37°C. Single cells were fed every other day and incubated no more than 1 week after isolation. For patch clamp experiments, individual coverslips were transferred to the test chamber and placed on an inverted microscope.

Electrophysiologic Recording and Analysis. Cell-attached configurations were used to record currents through single channels using a patch clamp amplifier as previously described.[21] In the cell-attached configuration, the bath solution was composed of 140 mM KCl, 10 mM HEPES, and 1 mM EGTA. The pipette solution contained 140 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM HEPES. The pH of all solutions was adjusted to 7.3–7.4 with KOH. Recordings were made at 36° ± 0.5°C. Patch pipettes were pulled with an electrode puller (PP-830; Narishige, Tokyo, Japan). The resistance of the pipettes filled with internal solution and immersed in Tyrode solution was 5–7 MΩ. Single-channel data were sampled at 5 KHz using a low-pass filter (1 KHz).

Channel currents were recorded with a patch clamp amplifier (CEZ 2200; Nihon Kohden, Tokyo, Japan) and digitized using an analog-to-digital converter (DigiData 1200; Axon Instruments, Foster, CA) and a personal computer (Aptiva; International Business Machines Corporation, Armonk, NY). pClamp version 7 software (Axon Instruments) was used for data acquisition and analysis. The open probability (P_o) was determined from current amplitude histograms and was calculated using the following equation:

$$P_o = \frac{\left(\sum_{j=1}^{N} t_j \cdot j\right)}{T_d \cdot N}$$

where t_j is the time spent at current levels corresponding to j = 0, 1, 2, N channels in the open state; T_d is the duration of the recording; and N is the number of active channels in the patch. Two- to 3-min recordings were analyzed to determine P_o. Channel activity is expressed

Table 1. Systemic Hemodynamics during Intravenous Glucose Tolerance Tests

				After Glucose		
	Baseline	Before Isoflurane	Before Glucose	30 min	60 min	120 min
HR, min^{-1}						
Vehicle	285 ± 28	284 ± 25	278 ± 20	267 ± 23†	265 ± 23†	269 ± 27†
ISO	296 ± 16	299 ± 21	274 ± 31*	255 ± 35*†	251 ± 35*†	250 ± 35*†
GLIB	283 ± 21	280 ± 22	278 ± 23	278 ± 22†	275 ± 24†	284 ± 28†
ISO + GLIB	293 ± 15	289 ± 18	267 ± 18*	225 ± 21*	213 ± 29*	211 ± 16*
MAP, mmHg						
Vehicle	80 ± 12	81 ± 11	81 ± 9†‡	87 ± 6†‡	89 ± 7†‡	95 ± 10†‡
ISO	94 ± 3	91 ± 5	56 ± 9*	50 ± 10*	48 ± 12*	48 ± 11*
GLIB	86 ± 11	85 ± 13	88 ± 9†‡	89 ± 10†‡	94 ± 11†‡	91 ± 11†‡
ISO + GLIB	87 ± 7	87 ± 9	54 ± 5*	51 ± 8*	46 ± 5*	48 ± 12*

Data are mean ± SD from seven rabbits.

* Significantly ($P < 0.05$) different from baseline. † Significantly ($P < 0.05$) different from the isoflurane (ISO) + glibenclamide (GLIB) group. ‡ Significantly ($P < 0.05$) different from the ISO group.

HR = heart rate; MAP = mean arterial blood pressure.

as NP_o (number of active channels in a patch, N, times open probability, P_o).

Isoflurane Delivery. Isoflurane was delivered as previously described.[21] Briefly, a mixture of concentrated isoflurane and bath solution was prepared in graduated syringes. Isoflurane superfusion was achieved using a syringe pump with a constant flow rate of 1.0 ml/min. A clinically relevant concentration of 0.5 mM isoflurane was used, which is equivalent to 2.4 vol% or 1.8 MAC for a mouse. To verify the concentration of isoflurane in solution, 1.5 ml of the superfusate was collected in a metal-capped 3-ml glass vial at the end of each experiment. Gas chromatography (G-3500; Hitachi, Tokyo, Japan) was used to quantify the concentration of isoflurane in the superfusate, which was 0.50 ± 0.05 mM.

Statistical Analysis

Power analysis revealed a group size of n = 6, providing a power of 0.8 with a minimum detectable difference of means of the insulinogenic index of 0.05, an expected SD of 0.03, and an α value of 0.05. Data are expressed as mean ± SD. Repeated-measures analysis of variance was used for within- and between-group comparisons, followed by Student–Newman–Keuls tests. Changes within and between groups were considered statistically significant when the *P* value was less than 0.05. Analysis was performed on a Macintosh computer with Statview 5.0 statistical analysis software (SAS Institute Inc., Cary, NC).

Results

Hemodynamics during the IVGTTs

There were no statistically significant differences in hemodynamics between groups under baseline conditions or before administration of isoflurane (table 1). Administration of isoflurane significantly decreased heart rate and mean arterial pressure compared with vehicle control. Administration of isoflurane and glibenclamide together resulted in heart rates that were significantly lower than those observed in the vehicle, isoflurane, and glibenclamide groups at 30 min, 1 h, and 2 h after glucose administration.

Glucose and Insulin Levels during the IVGTTs

Mean plasma glucose and IRI concentrations are shown in figures 2 and 3, respectively. Baseline concentrations of glucose and IRI were comparable across groups. In the vehicle and isoflurane groups, concentrations of glucose and IRI measured before administration of isoflurane and before administration of glucose were not significantly different from baseline (figs. 2 and 3). Before glucose administration, glibenclamide decreased blood glucose concentrations and increased IRI levels, whereas isoflurane slightly decreased IRI levels. As a result, blood glucose concentrations measured immediately before glucose administration in the glibenclamide and isoflurane plus glibenclamide groups were significantly lower than those in the vehicle and isoflurane groups. IRI levels measured immediately before glucose

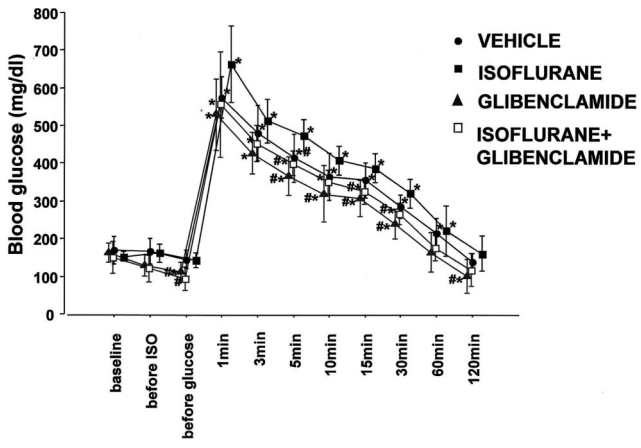

Fig. 2. Changes in blood glucose concentrations before and during the intravenous glucose tolerance test. * $P < 0.05$ *versus* baseline. # $P < 0.05$ *versus* the isoflurane (ISO) group.

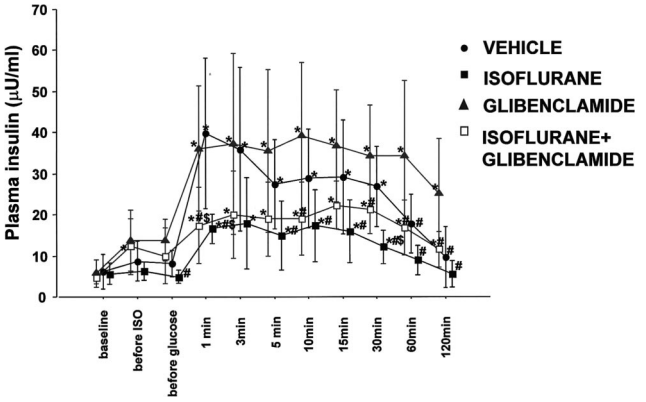

Fig. 3. Changes in immunoreactive insulin levels before and during the intravenous glucose tolerance test. * $P < 0.05$ *versus* baseline. # $P < 0.05$ *versus* the glibenclamide group. $ $P < 0.05$ *versus* the vehicle group. ISO = isoflurane.

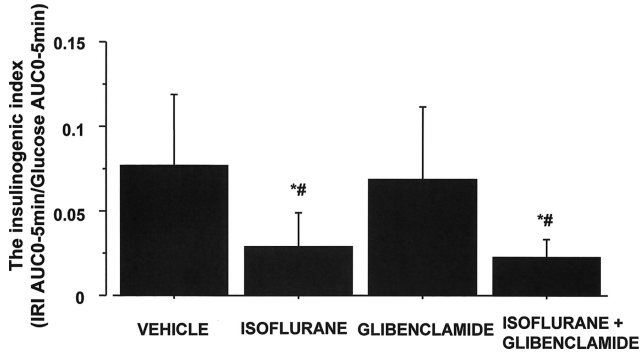

Fig. 4. The insulinogenic index 5 min after glucose administration for each group. The insulinogenic index was calculated by dividing the area under the insulin–time curve by the corresponding area under the glucose–time curve from 0 to 5 min. * $P < 0.05$ *versus* the vehicle group. # $P < 0.05$ *versus* the glibenclamide group. AUC = area under the curve; IRI = immunoreactive insulin.

administration in the isoflurane group were significantly lower than those in the glibenclamide group.

After intravenous administration of 0.6 g/kg glucose, blood glucose concentrations rapidly increased and then returned to baseline levels within 2 h in all experimental groups (fig. 2). Blood glucose concentrations were highest in the isoflurane group at all time points studied and were significantly higher than those observed in the other groups 5 min after glucose administration. IRI levels rapidly increased and then returned to baseline levels in the vehicle group after administration of glucose (fig. 3). In the isoflurane group, IRI levels were significantly increased after glucose administration, but were lower than those in the vehicle or glibenclamide groups at all time points. One minute after glucose administration, IRI levels in the groups that received 1.0 MAC isoflurane (the isoflurane and isoflurane plus glibenclamide groups) were significantly lower than those in the experimental groups that did not receive isoflurane (the vehicle and glibenclamide groups). In the glibenclamide group, IRI levels increased rapidly and remained elevated for 2 h. In the isoflurane plus glibenclamide group, IRI levels were significantly lower than those in the glibenclamide group at 1, 10, 30, 60, and 120 min after glucose administration. The insulinogenic index in the two groups that received isoflurane was significantly lower than in the vehicle control group (fig. 4).

Glucose-inhibited K_{ATP} Channels Are Activated by Isoflurane

To investigate whether isoflurane affects K_{ATP} channel activity in murine pancreatic β cells, we measured single K_{ATP} channel currents using a cell-attached patch clamp technique. Spontaneous single channel activity was observed in the absence of glucose (fig. 5A). Application of 300 μM diazoxide, a selective K_{ATP} channel opener, to the bath solution significantly activated K^+-selective channels (NP_o 0.493 $\pm$ 0.157; $P < 0.05$ *vs.* baseline; n = 12). The effects of diazoxide were completely blocked by 1 μM glibenclamide, a specific K_{ATP} channel blocker (fig. 5A). A single-channel conductance of 58.6 $\pm$ 4.8 pS (n = 12) was calculated from the current–voltage relation observed between membrane potentials of -80 and $+60$ mV, which is consistent with previous studies.[23]

We also examined the effects of diazoxide on glucose-induced inhibition of pancreatic K_{ATP} channels in a cell-attached configuration. As shown in figure 5B, spontaneous K_{ATP} channel activity was significantly decreased when the glucose concentration in the bath (extracellular) solution was increased from 0 to 10 mM. The subsequent application of 300 μM diazoxide to the bath reactivated this channel, resulting in an increase in mean K_{ATP} channel activity. Diazoxide-induced K_{ATP} channel activity was inhibited by 1 μM glibenclamide. The changes in NP_o are summarized in figure 5C.

Representative traces illustrating the effects of 0.5 mM isoflurane on glucose-induced inhibition of pancreatic K_{ATP} channel activity in a cell-attached configuration are shown in figure 5D. Application of isoflurane to the bath reversed the inhibitory effect of glucose, whereas this channel activity was completely inhibited by subsequent application of 1 μM glibenclamide to the bath. The changes in NP_o are summarized in figure 5E. These results demonstrate that isoflurane can mimic the effects of diazoxide by activating pancreatic K_{ATP} channels.

Discussion

Although 1.0 MAC isoflurane had no effect on blood glucose concentration or IRI values when animals were fasted for 15 h, isoflurane significantly decreased insulin secretion during the early phase of the IVGTT and significantly increased blood glucose concentrations 5 min after glucose administration. These results are consistent with previous studies[10–15] showing that volatile anesthetics impair glucose tolerance and insulin secretion. Tanaka *et al.* [15] reported that isoflurane and sevoflurane

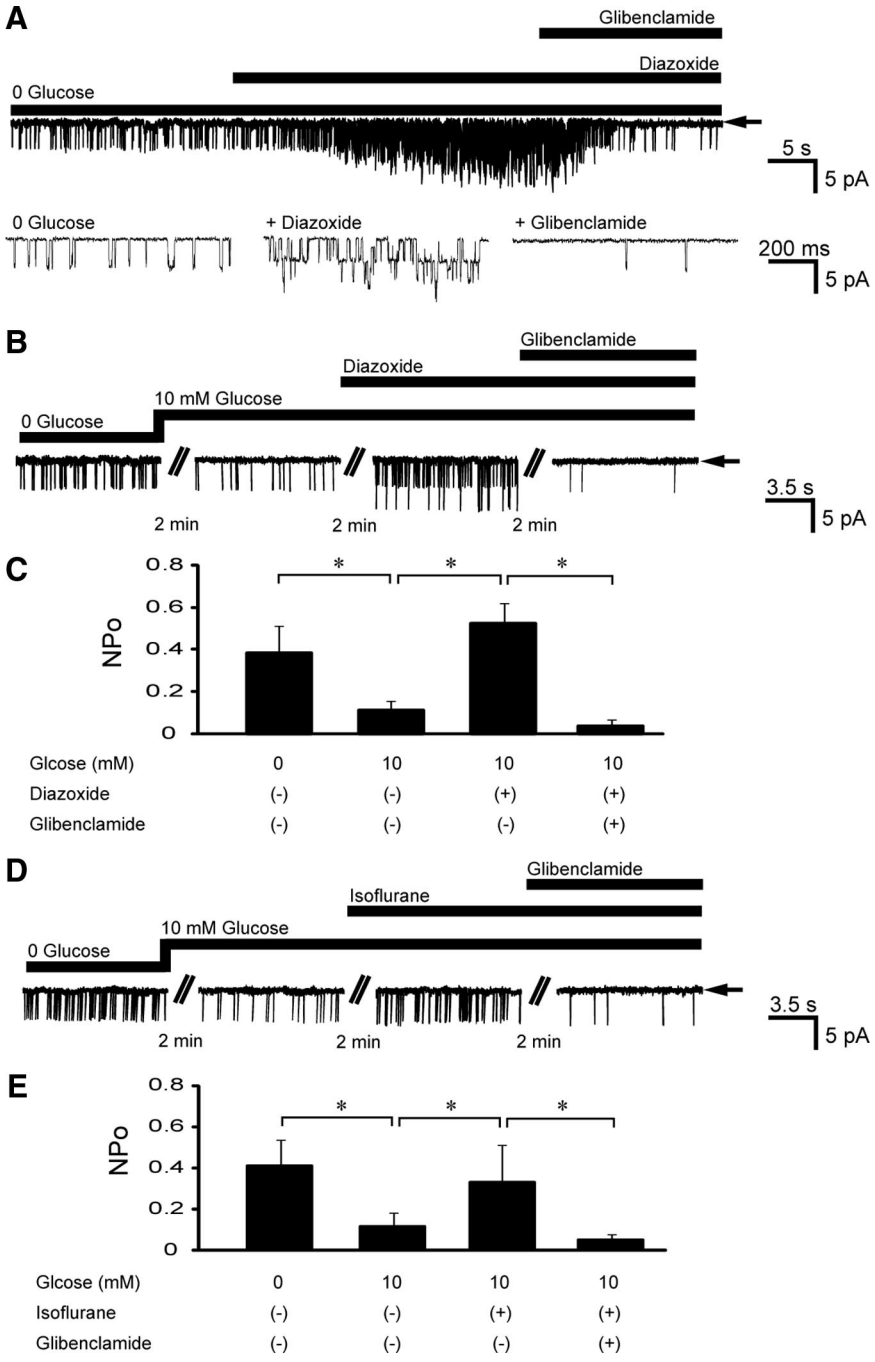

Fig. 5. Effects of isoflurane on adenosine triphosphate–sensitive potassium channel activity in murine pancreatic β cells. (*A*) Single-channel characteristics of pancreatic adenosine triphosphate–sensitive potassium channels in a cell-attached configuration. Glucose level, diazoxide (300 μM), and glibenclamide (1 μM) were superfused into bath solution as indicated by the *horizontal bars*. Membrane potential was clamped at −60 mV. *Horizontal arrow* indicates closed channel state. (*B*) Representative traces of single channel opening in recordings at −60 mV, where the bath solution contained 0 mM glucose, 10 mM glucose, 10 mM glucose + 300 μM diazoxide, or 10 mM glucose + 300 μM diazoxide + 1 μM glibenclamide. (*C*) Summary of changes in NP_o (number of active channels in a patch, N, times open probability, P_o). Each *vertical bar* represents measurements from 10 patches. * $P < 0.05$. (*D*) Representative traces of single channel opening in recordings at −60 mV, where the bath solution contained 0 mM glucose, 10 mM glucose, 10 mM glucose + 0.5 mM isoflurane, or 10 mM glucose + 0.5 mM isoflurane + 1 μM glibenclamide. (*E*) Summary of changes in NP_o. Each *vertical bar* represents measurements from 12 patches. * $P < 0.05$.

anesthesia impair glucose tolerance and insulin secretion to the same extent in humans, an effect that was independent of the dose used. In that clinical study, the authors also showed that the insulinogenic index of individuals in the isoflurane and sevoflurane groups was significantly lower than that observed in a control group.[15] In the current study, the insulinogenic index of the isoflurane groups was significantly lower than in the vehicle control group. These results suggest that isoflurane inhibits insulin secretion during the early phase of an IVGTT.

The normal response of β cells to a rapid and sustained increase in glucose concentration has been reported to involve a biphasic secretion of insulin.[18,24–26] The first phase consists of a rapid but transient (4–8 min) increase in the secretory rate.[26] The second phase consists of an initial decrease followed by sustained or gradually increasing secretion that lasts as long as glucose is present. The first phase involves triggering pathways that initiate the following sequence of events: entry of glucose by facilitated diffusion, metabolism of glucose by oxidative glycolysis, an increase in the ATP-to-adenosine diphosphate ratio, closure of K_{ATP} channels, membrane depolarization, opening of voltage-dependent Ca^{2+} channels, Ca^{2+} influx, an increase in cytoplasmic

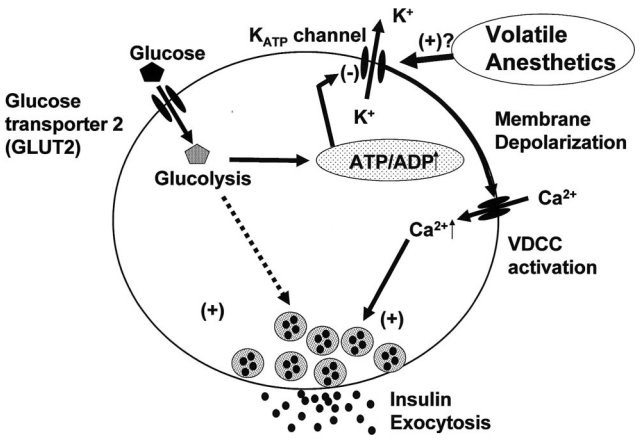

Fig. 6. The role of pancreatic adenosine triphosphate–sensitive potassium (K_{ATP}) channels in insulin secretion. In the K_{ATP} channel–dependent triggering pathway, elevated blood glucose levels increase glucose metabolism in pancreatic β cells and increase the ratio of $[ATP]/[ADP]_i$. This metabolic signal closes K_{ATP} channels, resulting in depolarization, activation of voltage-dependent Ca^{2+} channels (VDCCs), Ca^{2+} entry, and exocytosis of insulin. Volatile anesthetics might facilitate the opening of K_{ATP} channels and affect the coupling of glucose metabolism and insulin secretion. The *solid arrow* indicates the K_{ATP} channel–dependent triggering pathway. The *dotted arrow* indicates the K_{ATP} channel–independent amplifying pathway. ADP = adenosine diphosphate; ATP = adenosine triphosphate.

free Ca^{2+} concentration, and activation of the exocytotic machinery (fig. 6).[18,24–26] The second phase involves continuous stimulation of the triggering signal and amplification of the effects of Ca^{2+} on exocytosis through a K_{ATP} channel–independent mechanism. The molecular mechanisms underlying this amplification are still unknown.[25] Our results have shown that isoflurane inhibits insulin secretion during the early phase of an IVGTT, suggesting that isoflurane inhibits the triggering pathways through a K_{ATP} channel–dependent mechanism.

In the current study, we demonstrated that isoflurane inhibits insulin secretion induced by administration of glucose and by administration of a K_{ATP} channel blocker. Patch clamp experiments further showed that isoflurane significantly increased K_{ATP} channel activity in the presence of 10 mM glucose. These results strongly suggest that isoflurane-induced activation of K_{ATP} channels in pancreatic β cells mediates the effects of isoflurane on insulin secretion and glucose utilization. It has been known since the 1970s that volatile anesthetics can impair insulin secretion and glucose utilization.[10–15] However, the precise mechanism underlying these effects is unknown. We are the first to suggest that isoflurane-induced inhibition of insulin secretion is mediated by the isoflurane-induced opening of K_{ATP} channels in β cells of the pancreas.

Glibenclamide treatment in the glibenclamide alone and glibenclamide plus isoflurane groups consisted of a 50-μg/kg bolus of glibenclamide followed by 33.5 $\mu g \cdot kg^{-1} \cdot h^{-1}$. In the glibenclamide (alone) group, IRI levels were markedly increased after glucose administration and remained elevated for 2 h. Interestingly, isoflurane significantly decreased insulin secretion during the early and late phases of the IVGTT in the glibenclamide plus isoflurane group compared with the glibenclamide group. The continuous infusion of glibenclamide resulted in high IRI levels during the early and late phases of the IVGTT. The inhibitory effect of glibenclamide on K_{ATP} channels in pancreatic β cells would be expected to induce and maintain high levels of insulin secretion during the early and late phases of the IVGTT. However, isoflurane inhibited the effects of glibenclamide, suggesting that isoflurane-induced activation of K_{ATP} channels inhibits glibenclamide-induced insulin secretion.

Glibenclamide (45 min after administration) increased insulin levels from 5.6 ± 3.3 to 13.7 ± 7.5 μU/ml and from 4.6 ± 1.5 to 12.3 ± 6.9 μU/ml in the glibenclamide and glibenclamide plus isoflurane groups, respectively. Thirty minutes after administration of 1.0 MAC isoflurane, insulin levels were decreased (although not significantly) to 10.0 ± 6.7 μU/ml. In the glibenclamide group, insulin levels remained unchanged (13.7 ± 5.1 μU/ml) when isoflurane was not administered. Likewise, there were no significant differences between the glibenclamide and glibenclamide plus isoflurane groups in blood glucose levels at baseline, before administration of isoflurane, or before glucose administration. These results suggest that the relatively modest effects of isoflurane on insulin secretion in the absence of administration of glucose are unlikely to have clinical relevance.

Hyperglycemia, severe hyperglycemia in particular, is associated with increased morbidity and mortality in a variety of patient populations.[27] Although volatile anesthetics have been reported to have cardioprotective effects mediated by activation of cardiac K_{ATP} channels,[5,6] the results of the current study indicate that isoflurane impairs glucose tolerance and insulin secretion *via* activation of pancreatic K_{ATP} channels. How, then, should anesthesiologists respond to these conflicting effects of volatile anesthetics in the operating room? Several randomized clinical trials of volatile anesthetics in patients undergoing coronary artery surgery indicate that volatile anesthetics decrease troponin release, duration of stay in intensive care units, and late cardiac events, and enhance left ventricular function compared with the nonuse of volatile anesthetics.[28] These trials formed the basis for the suggestion in the American College of Cardiology–American Heart Association 2007 guidelines on perioperative cardiovascular evaluation and care for noncardiac surgery that it can be beneficial to use volatile anesthetic agents during noncardiac surgery for maintenance of general anesthesia in hemodynamically stable patients at risk for myocardial ischemia (class IIa, level of evidence B).[28] These data suggest that volatile anesthetics have beneficial cardioprotective effects that outweigh the adverse effects of decreased insulin secretion. However, hyperglycemia has been reported to at-

tenuate isoflurane-induced cardioprotection[29] and to impair isoflurane-induced K_{ATP} channel activation in vascular smooth muscle cells.[30] A recent study has demonstrated that β-cell function was already impaired in subjects with impaired glucose tolerance and in patients with early type 2 diabetes.[31] In this study, we have shown in healthy rabbits that exposure to isoflurane by itself without glucose injection produced insulin and blood glucose values comparable to the vehicle group. However, isoflurane by itself without glucose overload may decrease insulin secretion in these subjects. Taken together, to induce the beneficial cardiovascular effects of isoflurane, control of blood glucose concentration using insulin may be required during general anesthesia with volatile anesthetics, because isoflurane inhibits insulin secretion, as shown in the current study. Insulin has been reported to protect the myocardium against ischemia and reperfusion injury[32] and to induce hyperpolarization *via* K_{ATP} channel opening in vascular smooth muscle cells.[33]

Volatile anesthetics are thought to activate K_{ATP} channels in cardiac myocytes and vascular smooth muscle cells. Selective K_{ATP} channel antagonists such as HMR-1098 (selective for sarcolemmal channels) and 5-hydroxydecanoate (selective for mitochondrial channels) abolish the protective effects of desflurane in response to ischemia and reperfusion injury in dogs,[34] suggesting that sarcolemmal and mitochondrial K_{ATP} channels might play a role in anesthetic preconditioning in cardiac myocytes. Some volatile anesthetics have been shown to enhance sarcolemmal K_{ATP} channel currents in cardiac myocytes through facilitation of channel opening after initial activation,[20,35] to produce coronary vasodilatation through activation of vascular K_{ATP} channels,[7,8] and to hyperpolarize vascular smooth muscle through protein kinase A–induced K_{ATP} channel activation.[9] Data from mutagenesis patch clamp experiments using expressed sulfonylurea (SUR) 2B/Kir6.1 channels (vascular type K_{ATP} channels) from our laboratory have shown that protein kinase A–mediated phosphorylation of Kir6.1 and SUR2B subunits plays a pivotal role in isoflurane-induced vascular type K_{ATP} channel activation.[21] In the current study, we extend these findings to show that isoflurane activates K_{ATP} channel activity in pancreatic β cells.

There are some limitations of the current study. We did not determine whether other K_{ATP} channel agonists, such as diazoxide, have a similar effect to isoflurane on insulin secretion. We could not determine the appropriate concentration of diazoxide to inhibit insulin secretion during IVGTTs in rabbits. We demonstrated in the patch clamp experiments that isoflurane can mimic the effects of diazoxide on activation of pancreatic K_{ATP} channels during hyperglycemia (fig. 5). This may mean that diazoxide-induced activation of pancreatic K_{ATP} channels also inhibits insulin secretion. We also did not determine whether other volatile anesthetics inhibit insulin secretion. Therefore, it is unclear whether this is an agent-specific phenomenon or whether it is a common property of the volatile anesthetics. Further studies are required to answer this question.

In conclusion, the results of the current study demonstrate that 1.0 MAC isoflurane decreases glucose-induced insulin secretion and increases blood glucose concentrations during the early phase of an IVGTT in rabbits. Isoflurane also attenuates glibenclamide-induced insulin secretion during the late phase of an IVGTT. Patch clamp experiments indicate that 0.5 mM isoflurane increases K_{ATP} channel activity in pancreatic β cells in the presence of 10 mM glucose. Taken together, these results suggest that isoflurane impairs insulin secretion and glucose utilization. These results further indicate that the inhibitory effects of isoflurane on glucose utilization might be mediated by isoflurane-induced K_{ATP} channel activation in pancreatic β cells.

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