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 μg·kg⁻¹·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 both the 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.

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 · kg⁻¹ · h⁻¹) via an ear vein catheter. A tracheostomy was performed through a ventral midline incision, and the trachea was cannulated. Rabbits were
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, 1-glutamate, and penicillin-streptomycin. Petri dishes were kept in a 95% air-5% CO2 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. 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 CaCl2, 1 mM MgCl2, 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 (Po) was determined from current amplitude histograms and was calculated using the following equation:

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Po = \frac{\left( \sum_{j=1}^{N} t_j \cdot f \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
MAP, mmHg
HR

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.
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\textsubscript{ATP} Channels Are Activated by Isoflurane

To investigate whether isoflurane affects K\textsubscript{ATP} channel activity in murine pancreatic \( \beta \) cells, we measured single K\textsubscript{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 \( \mu \text{M} \) diazoxide, a selective K\textsubscript{ATP} channel opener, to the bath solution significantly activated K\textsuperscript{+}-selective channels (NP\textsubscript{o} 0.493 ± 0.157; \( P < 0.05 \) vs. baseline; \( n = 12 \)). The effects of diazoxide were completely blocked by 1 \( \mu \text{M} \) glibenclamide, a specific K\textsubscript{ATP} channel blocker (fig. 5A). A single-channel conductance of 58.6 ± 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.

We also examined the effects of diazoxide on glucose-induced inhibition of pancreatic K\textsubscript{ATP} channels in a cell-attached configuration. As shown in figure 5B, spontaneous K\textsubscript{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 \( \mu \text{M} \) diazoxide to the bath reactivated this channel, resulting in an increase in mean K\textsubscript{ATP} channel activity. Diazoxide-induced K\textsubscript{ATP} channel activity was inhibited by 1 \( \mu \text{M} \) glibenclamide. The changes in NP\textsubscript{o} are summarized in figure 5C.

Representative traces illustrating the effects of 0.5 mm isoflurane on glucose-induced inhibition of pancreatic K\textsubscript{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 \( \mu \text{M} \) glibenclamide to the bath. The changes in NP\textsubscript{o} are summarized in figure 5E. These results demonstrate that isoflurane can mimic the effects of diazoxide by activating pancreatic K\textsubscript{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 showing that volatile anesthetics impair glucose tolerance and insulin secretion. Tanaka et al. reported that isoflurane and sevoflurane
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 KATP channels, membrane depolarization, opening of voltage-dependent Ca2+ channels, Ca2+ influx, an increase in cytoplasmic

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**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 NPo (number of active channels in a patch, N, times open probability, Po). 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 NPo. Each vertical bar represents measurements from 12 patches. *P < 0.05.
Glucose transporter 2 (GLUT2) and the metabolic signaling of ATP/ADP. KATP channel–dependent triggering pathway, elevated blood glucose levels increase glucose metabolism in pancreatic β cells and increase the ratio of [ATP]/[ADP]. This metabolic signal closes KATP 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 KATP channels and affect the coupling of glucose metabolism and insulin secretion. The solid arrow indicates the KATP channel–dependent triggering pathway. The dotted arrow indicates the KATP channel–independent amplifying pathway.

**Fig. 6.** The role of pancreatic adenosine triphosphate–sensitive potassium (K\(_{\text{ATP}}\)) channels in insulin secretion. In the K\(_{\text{ATP}}\) channel–dependent triggering pathway, elevated blood glucose levels increase glucose metabolism in pancreatic β cells and increase the ratio of [ATP]/[ADP]. This metabolic signal closes K\(_{\text{ATP}}\) 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\(_{\text{ATP}}\) channels and affect the coupling of glucose metabolism and insulin secretion. The solid arrow indicates the K\(_{\text{ATP}}\) channel–dependent triggering pathway. The dotted arrow indicates the K\(_{\text{ATP}}\) channel–independent amplifying pathway.

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Free Ca\(^{2+}\) concentration, and activation of the exocytotic machinery (fig. 6).\(^{18-24}\) The second phase involves continuous stimulation of the triggering signal and amplification of the effects of Ca\(^{2+}\) on exocytosis through a K\(_{\text{ATP}}\) 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\(_{\text{ATP}}\) 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\(_{\text{ATP}}\) channel blocker. Patch clamp experiments further showed that isoflurane significantly increased K\(_{\text{ATP}}\) channel activity in the presence of 10 mM glucose. These results strongly suggest that isoflurane-induced activation of K\(_{\text{ATP}}\) 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\(_{\text{ATP}}\) 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 μg · kg\(^{-1}\) · 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. However, isoflurane inhibited the effects of glibenclamide, suggesting that isoflurane-induced activation of K\(_{\text{ATP}}\) 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\(_{\text{ATP}}\) channels,\(^{5,6}\) the results of the current study indicate that isoflurane impairs glucose tolerance and insulin secretion via activation of pancreatic K\(_{\text{ATP}}\) 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 and to impair isoflurane-induced $K_{\text{ATP}}$ channel activation in vascular smooth muscle cells. A recent study has demonstrated that $\beta$-cell function was already impaired in subjects with impaired glucose tolerance and in patients with early type 2 diabetes. 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 and to induce hyperpolarization via $K_{\text{ATP}}$ channel opening in vascular smooth muscle cells.

Volatile anesthetics are thought to activate $K_{\text{ATP}}$ channels in cardiac myocytes and vascular smooth muscle cells. Selective $K_{\text{ATP}}$ 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, suggesting that sarcolemmal and mitochondrial $K_{\text{ATP}}$ channels might play a role in anesthetic preconditioning in cardiac myocytes. Some volatile anesthetics have been shown to enhance sarcolemmal $K_{\text{ATP}}$ Channel currents in cardiac myocytes through facilitation of channel opening after initial activation, to produce coronary vasodilatation through activation of vascular $K_{\text{ATP}}$ channels, and to hyperpolarize vascular smooth muscle through protein kinase A-induced $K_{\text{ATP}}$ channel activation.

Data from mutagenesis patch clamp experiments using expressed sulfonylurea (SUR) 2B/Kir6.1 channels (vascular type $K_{\text{ATP}}$ 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 $K_{\text{ATP}}$ channel activation. In the current study, we extend these findings to show that isoflurane activates $K_{\text{ATP}}$ channel activity in pancreatic $\beta$ cells.

There are some limitations of the current study. We did not determine whether other $K_{\text{ATP}}$ 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 $K_{\text{ATP}}$ channels during hyperglycemia (fig. 5). This may mean that diazoxide-induced activation of pancreatic $K_{\text{ATP}}$ 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 $K_{\text{ATP}}$ channel activity in pancreatic $\beta$ 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 $K_{\text{ATP}}$ channel activation in pancreatic $\beta$ cells.

References