ISOFLURANE INHIBITS INSULIN SECRETION FROM ISOLATED RAT PANCREATIC ISLETS OF LANGERHANS

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SUMMARY

We have investigated the effects of isoflurane on insulin secretion in vitro from rat isolated islets of Langerhans and found a significant, dose-related and reversible inhibition of insulin secretion. Isoflurane 2% decreased insulin secretion stimulated by glucose 20 mmol litre\(^{-1}\) to basal, non-stimulated values. In other studies to identify the stage in the stimulus secretion pathway for insulin at which the anaesthetic may exert an inhibitory action, we have stimulated insulin release using glyceraldehyde and a phorbol ester. Insulin secretion induced by these secretagogues was also blocked by isoflurane. This suggests that the inhibitory effect of the anaesthetic agent may be at a site distal to stimulation of insulin secretion by glyceraldehyde and phorbol esters. (Br. J. Anaesth. 1993; 71: 873-876)

KEY WORDS


Volatile anaesthetic agents used during maintenance of anaesthesia may contribute to the hyperglycaemia associated with surgery by inhibition of insulin release. Halothane and enflurane have been shown to inhibit glucose-stimulated insulin secretion directly from pieces of rat pancreas and from rat isolated pancreatic islets of Langerhans [1,2]. The mechanisms by which these anaesthetics inhibit the stimulus—secretion pathway in insulin-secreting pancreatic islet \(\beta\) cells remain to be elucidated.

Glucose is the major physiological regulator of insulin secretion. After its transport into the \(\beta\) cell, its metabolism triggers the mechanism for insulin release via generation of intracellular second messengers and activation of protein kinases, leading to exocytosis of insulin [3]. A simplified view of the pathway of stimulus—secretion coupling for insulin release from a pancreatic \(\beta\) cell is shown in figure 1. Experimentally, insulin secretion may be stimulated in several ways. Glyceraldehyde is an intermediate in the glycolytic pathway and may be used to stimulate insulin secretion distal to the transport of glucose into the cell and the initiation of glucose metabolism. Diacylglycerol, produced during the turnover of membrane phospholipids, activates protein kinase C and is a potent insulin secretagogue [4]. Tumour-promoting phorbol esters, which mimic diacylglycerol, may also be used \(in vitro\) to stimulate release of insulin.

We have examined the effects of isoflurane on glucose-induced insulin secretion from rat isolated pancreatic islets of Langerhans \(in vitro\), in static incubations and in a perfusion system which allows an assessment of dynamic changes in insulin secretion. We have also investigated the possible site of action of isoflurane using glyceraldehyde and a phorbol ester, phorbol myristate acetate (PMA), to stimulate insulin secretion.

METHODS

Wistar rats were killed by cervical dislocation before excision of the pancreas. Islets of Langerhans were isolated by collagenase digestion [5]. In this technique, the pancreatic duct is cannulated and 5 ml of collagenase solution 1 mg ml\(^{-1}\) injected, which...
digests the exocrine tissue, thus allowing the islets of Langerhans to be picked from the digest using a finely drawn pipette under a dissecting microscope. The islets were pre-incubated for 30–60 min at 37 °C in a bicarbonate-buffered physiological salt solution (pH 7.4) containing glucose 2 mmol litre⁻¹, calcium chloride 2 mmol litre⁻¹ and bovine serum albumin 0.5 mg ml⁻¹. For static incubations, groups of three islets were placed in microcentrifuge tubes containing 100 µl of buffer as above. An additional 500 µl of buffer (Medium 199, Earles salts) containing the test substances of interest was then added to the tubes which were sealed and the preparation incubated in a water bath at 37 °C for 30 min. The islets were pelleted by centrifugation (9000 g, 15 s) and a supernatant sample of incubation buffer was taken and stored at −20 °C until required for subsequent measurement of insulin content by radioimmuno-assay [6]. Basal insulin secretion was measured in groups of islets incubated in buffers containing glucose 5 mmol litre⁻¹. Insulin secretion was stimulated using glucose 20 mmol litre⁻¹, glycerinaldehyde 10 mmol litre⁻¹ or PMA 250 and 500 nmol litre⁻¹. To assess the effects of isoflurane on insulin secretion, incubation media were equilibrated by bubbling with a stream of 100 % oxygen 1 litre min⁻¹ (for 5 min at 20 °C) containing vaporized isoflurane (Ohmeda Mark 3 vaporizer). Control buffers were bubbled with oxygen alone. The required volume of buffer was then added rapidly to the microcentrifuge tubes containing the islets and the preparation incubated as above.

The partial pressure of isoflurane in the gas space above the buffer in the microcentrifuge tubes was measured by the headspace technique using gas chromatography [7]. The Pye 104 gas chromatograph (GC) used flame ionization detection and a pen recorder produced peaks corresponding to detection of isoflurane. Samples of buffer from duplicate microcentrifuge tubes were taken at intervals throughout the 30-min incubation period during static incubations using a vaporizer setting of 1 % or 2 % isoflurane. The samples of buffer were injected into glass vials sealed with Teflon–silicone liners. The gas headspace in these vials was allowed to equilibrate for 45 min by gentle rotation (20 r.p.m.) at 37 °C in a thermostatically controlled cabinet. A portion of the headspace was then flushed onto the GC column. The buffer sample and headspace were allowed to re-equilibrate and a series of three successive measurements made in duplicate from each buffer sample. For each series, the peak heights produced by the pen recorder were plotted on a logarithmic Y-axis against the equilibration number. The intercept on the Y-axis represented the partial pressure in the gas phase in the original sample. This was calculated using peak heights produced by calibration of the GC with isoflurane of a known partial pressure.

After isolation and preincubation of islets as described above, groups of 50 islets were placed in filter chambers containing nylon mesh filters and perfused simultaneously with buffer (Medium 199, Earles salts) containing the test substances of interest at a rate of 1 ml min⁻¹ in a 37 °C environment. In this way, groups of islets were perfused with a sequence of buffers. The effluent perfusate emerging from the filter chambers was collected at 2-min intervals for measurement of insulin content. After initial perfusion for 10 min with glucose 5 mmol litre⁻¹, a stimulatory concentration of glucose (20 mmol litre⁻¹) was introduced. Subsequently, the effects of isoflurane were examined either by perfusing groups of islets with glucose 20 mmol litre⁻¹ into which isoflurane 2 % was bubbled (experimental group, n = 6), or by continuing to perfuse with glucose 20 mmol litre⁻¹ alone (control group, n = 4).

Insulin secretion was measured by radioimmunoassay [6] using purified rat insulin as standard, guineapig anti-insulin serum and [125I]-labelled bovine insulin. The intra- and inter-assay coefficients of variation for this assay were 5.2 % and 14 %, respectively. Results are described as mean (SEM) insulin secretion (ng/islet 30 min⁻¹). For static incubations, differences between groups were analysed by Student's unpaired t test. In the perfusion experiment, differences between groups and within each group with time were assessed by analysis of variance. P < 0.05 was considered significant.

RESULTS

Effects on basal and glucose-induced insulin secretion in static incubation

Isoflurane had no effect on basal insulin release. In the presence of glucose 5 mmol litre⁻¹, insulin secretion was 1.78 (0.39) ng/islet 30 min⁻¹. With the addition of isoflurane 1 % and 2 %, insulin secretion in the presence of glucose 5 mmol litre⁻¹ was not changed significantly (2.03 (0.31) and 1.97 (0.41) ng/islet 30 min⁻¹, respectively; n = 9 for all groups).

In another experiment (table I), insulin secretion was increased significantly in the presence of glucose 20 mmol litre⁻¹, from 1.35 (0.60) (n = 7) to 3.52 (0.62) (n = 8) ng/islet 30 min⁻¹ (P < 0.01). This glucose-induced secretion was inhibited in a dose-dependent manner in the presence of isoflurane. With a vaporizer setting of 1 % isoflurane, glucose-induced insulin secretion decreased to 1.70 (0.39) (n = 9) ng/list 30 min⁻¹ (P < 0.05); with a setting of 2 % isoflurane, secretion was 0.97 (0.20) (n = 8) ng/islet 30 min⁻¹ (P < 0.01). Although this latter value was smaller than that with glucose 5 mmol litre⁻¹ alone, the difference was not statistically significant. As the inhibition of glucose-induced insulin secretion in the presence of isoflurane was maximal with a vaporizer setting of 2 % isoflurane, this setting was used for subsequent studies.

Perfusion (fig. 2)

The results of the perfusion experiment showed that, after a change in concentration of glucose in the perfusion medium from 5 mmol litre⁻¹ to a stimulatory concentration of 20 mmol litre⁻¹, there was an immediate increase in insulin release from both control and experimental groups of islets. In
alone, secretion was significantly less from 36 to 46 min (P < 0.05). In these islets, a change of the perifusion medium to glucose 20 mmol litre\(^{-1}\) with isoflurane at 2\% produced a partial pressure of 0.38-0.46 \% isoflurane in the gas phase above the buffer in the microcentrifuge tubes during the static incubations. For the 2\% vaporizer setting, the partial pressure after centrifuge tubes during the static incubations. For the 1\% vaporizer setting for isoflurane, the partial pressure was stable thereafter with a partial pressure of 0.97 \%.

Isoflurane (%)

<table>
<thead>
<tr>
<th>Glucose (mmol litre(^{-1}))</th>
<th>Isoflurane (%)</th>
<th>n</th>
<th>Insulin secretion (ng/islet 30 min(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>—</td>
<td>7</td>
<td>1.35 (0.60) [0.17-2.53]</td>
</tr>
<tr>
<td>20</td>
<td>0.5</td>
<td>8</td>
<td>2.17 (0.59) [1.01-3.33]</td>
</tr>
<tr>
<td>20</td>
<td>1.0</td>
<td>9</td>
<td>1.70 (0.39) [0.94-2.46]</td>
</tr>
<tr>
<td>20</td>
<td>2.0</td>
<td>8</td>
<td>0.97 (0.20) [0.58-1.36]</td>
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Effects on PMA-induced insulin secretion in static incubation (fig. 4)

In this experiment, insulin secretion in response to glucose 5 mmol litre\(^{-1}\) was 0.97 (0.27) ng/islet 30 min\(^{-1}\). Secretion increased significantly—to 3.92 (0.73) ng/islet 30 min\(^{-1}\) (P < 0.01) with glyceraldehyde 10 mmol litre\(^{-1}\), and to 3.67 (0.54) ng/islet 30 min\(^{-1}\) (P < 0.01) with glucose 20 mmol litre\(^{-1}\). Both glyceraldehyde- and glucose-induced secretory responses were suppressed completely by addition of isoflurane at a vaporizer setting of 2\%.

46 min (P < 0.05). The inhibition of insulin secretion by isoflurane in the experimental groups of islets was relieved when the buffer was changed back to glucose 20 mmol litre\(^{-1}\) alone. Insulin secretion in these islets subsequently increased, and from 58 to 70 min was significantly different from the value at 50 min (P < 0.05).

Effects on glyceraldehyde-induced insulin secretion in static incubation (fig. 3)

In this experiment, insulin secretion in response to glyceraldehyde 10 mmol litre\(^{-1}\) was 0.89 (0.20) and 1.12 (0.23) ng/islet 30 min\(^{-1}\), respectively (P < 0.01) (n = 7 in all groups). Significant difference between groups from 36 to 46 min (P < 0.05).

Effects on PMA-induced insulin secretion in static incubation (fig. 4)

In this experiment, insulin secretion in response to glucose 5 mmol litre\(^{-1}\) (0.97 (0.27) ng/islet 30 min\(^{-1}\)) was stimulated significantly by PMA 250 and 500 mmol litre\(^{-1}\), increasing to 1.75 (0.09) and 3.01 (0.83) ng/islet 30 min\(^{-1}\), respectively (P < 0.05). Isoflurane inhibited the insulin secretion enhanced by the presence of both concentrations of PMA, to values of 0.75 (0.13) ng/islet 30 min\(^{-1}\) (P < 0.01) and 1.26 (0.13) ng/islet 30 min\(^{-1}\) (P < 0.05) (n = 7 in all groups).

Gas chromatography

The results of gas chromatography showed that using the 1\% vaporizer setting for isoflurane produced a partial pressure of 0.38-0.46 \% isoflurane in the gas phase above the buffer in the microcentrifuge tubes during the static incubations. For the 2\% vaporizer setting, the partial pressure after 1 min was 2.0 \%, decreasing to 1.33 \% at 10 min and was stable thereafter with a partial pressure of 0.89 % at 20 and 30 min.

Table 1. Insulin secretion (mean (SEM) [95\% confidence limits]) during static incubation. *P < 0.05 compared with glucose 5 mmol litre\(^{-1}\); **P < 0.01 compared with glucose 20 mmol litre\(^{-1}\) alone.
DISCUSSION

These experiments show clearly that isoflurane inhibited glucose-stimulated insulin secretion from isolated rat pancreatic islets of Langerhans in vitro in static incubations, although basal insulin secretion was not affected significantly. In this respect, our findings are in agreement with earlier results with the volatile anaesthetic agents halothane and enfurane [1,2]. The results of the perfusion experiment show that, after exposure of the islets to isoflurane, the islets remained capable of producing a further insulin secretory response to a stimulatory concentration of glucose. This important finding implies that isoflurane had a reversible inhibitory effect on insulin secretion, rather than impairing the functional ability of the islet cells by a toxic effect of the anaesthetic.

Methods for equilibrating volatile anaesthetics into incubation buffers for biological preparations similar to that used in the present study have been reported previously [8,9]. For clarity, the nominal settings dialled on the isoflurane vaporizer in our experiments have been stated. However, it is apparent that there are losses of isoflurane from the bubbling chamber to the microcentrifuge tubes. Therefore it was necessary to quantify the partial pressure of isoflurane in the vapour phase in equilibrium with the islet preparation. This was done using a standard method with gas chromatography [7] and showed that a nominal 2% vaporizer setting produced partial pressures which decreased initially and then stabilized for the remainder of the experiment.

The results of the present study show that isoflurane did not affect glucose transport across the cell membrane or interfere with the initiation of glucose metabolism, as insulin secretion stimulated at a more distal site by glyceroldehyde was nevertheless inhibited by isoflurane. This is in accordance with previous work using halothane and enfurane which suggested that these agents did not inhibit insulin secretion by interfering with glucose metabolism in the islet cells [2]. Also, the inhibitory effect of isoflurane on the stimulus secretion pathway must occur beyond the activation of protein kinases, as we have shown in the present study that insulin secretion promoted by the activation of protein kinase C by PMA was inhibited by the anaesthetic agent.

The effects of other drugs on secretion of insulin from pancreatic tissue have been documented. Morphine and endogenous opioid receptor agonists show disparate effects on insulin release, depending on the dose and substance used [10]. Clinically relevant concentrations of morphine in the nanomolar range stimulate insulin secretion in vitro [11]. A recent investigation showed that clinical concentrations of midazolam had no significant effect on insulin secretion from isolated rat islets of Langerhans [12].

Isoflurane is used increasingly in clinical practice [13] and has been introduced recently as an agent for sedation in intensive care. As we have shown that, in vitro, isoflurane directly inhibited insulin secretion from isolated pancreatic islets, it is important to know if the findings of the present investigation have any clinical relevance. It is possible that volatile agents may enhance the hyperglycaemia seen during surgery and critical illness. Suppression of basal values of circulating insulin cannot be excluded. A previous clinical study suggested that isoflurane may suppress the insulin response to a glucose load, but the dose of glucose used was excessive and unphysiological, and there was no comparison with a non-volatile anaesthetic [14]. Further studies are required to assess the effects of isoflurane on glucose-induced insulin release in vivo.

REFERENCES