Inhibition by Halothane of Glucose-stimulated Insulin Secretion in Isolated Pieces of Rat Pancreas

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In isolated pieces of rat pancreas addition of 300 mg glucose/100 ml bathing medium resulted in a marked and significant increase in insulin secretion. This glucose-stimulated insulin secretion is reversibly inhibited by halothane in a concentration-dependent fashion (0.63, 1.25, and 1.88 MAC halothane produced depressions of 8.4, 18.9, and 37.0 per cent, respectively). Halothane, 1.88 MAC, had no effect on the basal or nonglucose-mediated insulin release. (Key words: Anesthetics, volatile; halothane; Hormones: insulin; Metabolism: halothane.)

In conscious patients glucose infusion results in marked increases in plasma insulin levels. During halothane anesthesia, however, this insulinogenic response to glucose is significantly reduced. The mechanism of this action of halothane is unknown. The possibilities include a direct action of halothane on the pancreas or an indirect pancreatic action due to humoral or nervous alterations induced by halothane. The purpose of this investigation is to determine whether halothane interferes directly with glucose-stimulated insulin release. For this purpose isolated pieces of rat pancreas were incubated in 300 mg/100 ml (16.7 mM) glucose. Under these conditions insulin secretion is nearly maximal.

Methods

Pancreatic tissue was quickly removed from normal fed rats (male, albino, 200–300 g body wt, Sprague-Dawley) and placed in a petri dish containing cold (0 C) substrate-free bicarbonate-buffered incubation medium. Extraneous adipose tissue and lymph nodes were trimmed away and the glandular portion was cut with single-edged razor blades into small pieces (2–4 mg) and rinsed twice with cold buffer. Four pieces were randomly chosen and carefully transferred to small ground-glass-stoppered Erlenmeyer flasks (10 ml) containing cold bicarbonate-buffered incubation medium (2.0 ml) which consisted of glucose (0 or 300 mg/100 ml), bovine serum albumin (1 per cent w/v; bovine albumin, fraction V, Sigma Chemical Co., St. Louis, Mo.), and guinea pig anti-insulin serum (GPAIS, lot 524, prepared in this laboratory). Each flask was equilibrated against oxygen (95 per cent) and carbon dioxide (5 per cent) and incubated for 90 min at 37 C. At the end of the incubation period the pancreatic pieces were gently blotted and weighed.

Immediately prior to incubation, halothane sufficient to achieve partial pressures corresponding to 0.68, 1.25, or 1.88 MAC was added to some of the flasks with a microliter syringe, below the surface of the medium. The medium/gas partition coefficient necessary for this step was determined to be 0.73 ± 0.03 (mean ± SE of seven determinations) at 37 C. The method involved placing a known amount of halothane in a closed system containing a known amount of the medium in question and a known amount of air. After one hour at 37 C the medium was analyzed for halothane using gas chromatography. From the total amount in the system and the concentration determined in the medium, the concentration in air was calculated. The ratio of concentration in medium (54.5 mg/100 ml) to concentration in
TABLE 1. Effect of 300 mg/100 ml Glucose (16.7 mM) on Insulin Secretion

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>aUnits Insulin Secreted/mg Wet Weight/90 Min (±SEM)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose-free (basal)</td>
<td>24</td>
<td>38.1 ± 4.5</td>
<td></td>
</tr>
<tr>
<td>Glucose, 300 mg/100 ml</td>
<td>143</td>
<td>231.1 ± 6.8</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Air (74.6 mg/100 ml) was taken as the partition coefficient. A check of this method using the same saline medium without albumin gave a value of 0.69 ± 0.02 (mean ± SE of seven determinations). This is close to the published value of 0.70 found by Larson et al. In a separate experiment the concentration of halothane in the saline medium after 10 min of incubation was 110 per cent of the value seen at 90 min. This took as evidence of rapid equilibration.

The reversibility studies were conducted by incubating both control and halothane-treated pancreas pieces for an initial period (45 min), followed by carefully transferring the tissue in each flask to different flasks containing freshly-made, halothane-free incubation medium. After gassing, all tissues were again incubated for 45 min. An insulin determination was conducted on a sample drawn at the end of each incubation.

Details of the method used to measure insulin secretion in vitro have been reported. GPAIS was added to the medium to protect secreted insulin against lytic substances released from acinar tissue, as well as to provide an easy means of measuring secreted hormone. Briefly, sufficient GPAIS was added to the incubation medium to bind about twice the amount of insulin which the incubated pieces were expected to secrete.

At the end of the incubation period, a sample (0.2 ml) of medium was taken and the unneutralized antiseraum measured by radioimmunooassay. Insulin secreted into the medium could be calculated from the observed decrease in reactive antibody content during incubation.

Results

Secretion of insulin by isolated pieces of rat pancreas was markedly stimulated by addition of 300 mg glucose/100 ml bathing medium (table 1). Halothane produced concentration-dependent inhibition of glucose-stimulated insulin secretion (table 2). Statistically significant inhibition was seen with 1.25 and 1.88 MAC halothane. An identical number of controls was run at the

TABLE 2. Effect of Halothane on Glucose-stimulated (300 mg/100 ml) Insulin Secretion in Isolated Pieces of Rat Pancreas

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>aUnits Insulin Secreted/mg Wet Weight/90 Min (±SEM)</th>
<th>Per Cent Inhibition</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>143</td>
<td>231.1 ± 6.8</td>
<td>8.4</td>
<td>N.S.</td>
</tr>
<tr>
<td>Halothane, 0.63 MAC (0.14 mM)</td>
<td>48</td>
<td>211.8 ± 14.5</td>
<td>13.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Halothane, 1.25 MAC (0.28 mM)</td>
<td>47</td>
<td>187.5 ± 11.7</td>
<td>37.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Halothane, 1.88 MAC (0.41 mM)</td>
<td>48</td>
<td>145.6 ± 9.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TABLE 3. Reversibility of Halothane (1.88 MAC) Inhibition of Glucose-stimulated (300 mg/100 ml) Insulin Secretion (Values are Means ± SEM)

<table>
<thead>
<tr>
<th></th>
<th>First Period (45 Min)</th>
<th>Conditions</th>
<th>aU Ins/mg/45 Min</th>
<th>Second Period (45 Min)</th>
<th>Conditions</th>
<th>aU Ins/mg/45 Min</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>Control</td>
<td>120.5 ± 12.8</td>
<td>Control</td>
<td>126.8 ± 8.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>Halothane, 1.88 MAC</td>
<td>64.3 ± 9.9 *</td>
<td>Halothane removed</td>
<td>124.2 ± 11.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* P < 0.001 compared with first-period control.
same time that each halothane experiment was conducted. Control values were 225.4 ± 12.8, 236.9 ± 13.4, and 230.9 ± 8.9 μ Units insulin secreted/mg wet weight/90 min. These values were not significantly different from each other and thus were pooled to give the value of 231 ± 6.82 μU found in table 2. Inhibition of insulin secretion in pancreatic pieces incubated in the presence of halothane for 45 min could be completely reversed when the pieces were transferred to a second flask free of halothane and incubated a further 45 min (table 3). The low basal level of insulin secretion seen in glucose-free medium was unaffected by halothane (table 4).

Discussion

Glucose is known to stimulate insulin secretion by incubated pieces of rat pancreas,3,5 maximum stimulation being observed at glucose concentrations of 300 mg/100 ml or more. The present results clearly demonstrate that halothane in clinically effective concentrations (1.25–1.88 MAC) produces significant and reversible inhibition of this stimulant effect of glucose.

The mechanism of this inhibitory action is not known. It must involve some effect of halothane upon the action of glucose, since in the absence of glucose, halothane has no effect on insulin release (table 4). The effect observed here is also unlikely to have been the result of any stimulant action which glucose has upon insulin biosynthesis; newly-formed insulin is not released until after incubation for at least 45 minutes to one hour,4 and halothane is capable of suppressing insulin secretion for the first 45 minutes of incubation (table 3). It could be acting at some stage in the action of glucose on the secretory process. Thus, it could be interfering with glucoreceptors which are postulated to be necessary for glucose action3,4 or it could inhibit glucose oxidation, without which this sugar has no stimulant effect.9

Functional studies in isolated rat16–18 and human atria and biochemical studies in isolated rat atria18 and cerebral cortex slices16 have demonstrated a halothane-induced block of glucose metabolism. The site of blockade has been localized to an early step(s) in glycolysis. At present, the most likely site appears to be the glucose phosphatase isomerase step, necessary for the conversion of glucose-6-phosphate to fructose-6-phosphate. This might also be the case in the inhibitory action of halothane upon glucose-stimulated insulin secretion, but no evidence is presently available to confirm this.

In their clinical studies, Merin et al.1 showed that the marked increases in serum insulin concentration induced by glucose infusions in conscious patients were significantly reduced during halothane anesthesia; but that glucose disappearance from the blood was not significantly affected. They used only a small group of patients (five), some of whom showed reduced glucose assimilation during anesthesia. It is possible, therefore, that halothane may also interfere with actions of insulin on peripheral tissues. This does not appear to be the case, for Merin17 has shown that insulin has a positive inotropic effect and increases glucose uptake in halothane-depressed hearts of intact dogs. In our laboratory we have shown that insulin stimulates oxidation of glucose to CO₂ by isolated rat atria from 680 ± 28 to 917 ± 24 nmoles glucose/g dry weight/hour under normal conditions.18 Halothane (3.25 MAC) suppresses basal CO₂ production to 380 ± 38 nmoles, but added insulin is still able to increase CO₂ production under these conditions to 690 ± 98 nmoles, an effect of insulin (+ 280) which is very similar to that obtained with normal atria (+ 237).

From these results we conclude that halothane can inhibit glucose-stimulated insulin secretion by a direct effect on the pancreas. This inhibitory effect may contrib-
ute to the clinical picture of reduced insulinogenic response to glucose during halothane anesthesia.

References


Neonatology

FETAL EEG Fetal electroencephalography (FEEG) was monitored during the second stage of labor. When the vertex was delivered spontaneously, FEEG remained unchanged, independent of fetal heart rate decelerations with head compression or maternal “pushing.” During forceps application, and traction, FEEG changed rapidly from low-voltage irregular activity to a flat line. After removal of the forceps, FEEG once returned in appearance to that seen in neonates delivered without forceps. (Rosen, M., Scibetta, J., and Hochberg, C.: Fetal Electroencephalography. IV. The FEEG during Spontaneous and Forceps Births. Obstet Gynecol 42: 283–289, 1973.)