Ethanol Acutely Stimulates Islet Blood Flow, Amplifies Insulin Secretion, and Induces Hypoglycemia via Nitric Oxide and Vagally Mediated Mechanisms

Zhen Huang and Åke Sjöholm
Karolinska Institutet, Department of Internal Medicine, Stockholm South Hospital, SE-118 83 Stockholm, Sweden

Hypoglycemia induced by alcohol ingestion is a well-known problem in diabetic patients. However, the mechanisms underlying this phenomenon have largely remained elusive. Because insulin secretion in vivo can be rapidly tuned by changes in pancreatic microcirculation, we evaluated the influence of acute alcohol administration on pancreatic islet blood flow (IBF), and dynamic changes in insulin secretion and glycemia in the rat. Ethanol (10%) or saline was iv injected as a bolus into Wistar rats, yielding serum ethanol concentrations of approximately 8 mmol/liter. Measurements of pancreatic blood flow (PBF) were performed by a microsphere technique in combination with a freeze-thawing technique after 10-min injection. Ethanol preferentially and significantly increased pancreatic IBF approximately 4-fold, whereas not influencing whole PBF. The alcohol also augmented late-phase insulin secretion and induced late hypoglycemia upon ip glucose tolerance tests. The nitric oxide synthase inhibitor Nω-nitro-L-arginine methyl ester and atropine prevented the increased pancreatic IBF, enhanced insulin secretion, and hypoglycemia evoked by ethanol. Thus, our findings demonstrate that ethanol acutely exerts substantial influences on pancreatic microcirculation by evoking a massive redistribution of PBF from the exocrine into the endocrine part via mechanisms mediated by nitric oxide and vagal stimuli, augmenting late-phase insulin secretion, and thereby evoking hypoglycemia. This effect may in part underlie the well-known hypoglycemic properties of alcohol in diabetic patients or in alcoholics with hepatic failure. (Endocrinology 149: 232–236, 2008)

Materials and Methods

Animals
Male Wistar rats (ScanBur, Sollentuna, Sweden), weighing 300–350 g, were used in all experiments. The animals had free access to pelleted food (Type R34; ScanBur) and tap water at all times. All experiments were approved by the local animal ethics committee.

Blood flow measurements

The experiments were performed according to a protocol previously described in detail (9). The animals were anesthetized with an ip injection of thiobutabarbital sodium (120 mg/kg body weight, Inactin; Research Biochemicals Intl., Natick, MA) and placed on a heated operating table to maintain body temperature. Polyethylene catheters were inserted into the ascending aorta, via the right common carotid artery, and into the left femoral artery. The catheter in the aorta was connected to a pressure transducer (model PDCR 75/1; Druck Ltd., Groby, Leicestershire, UK) to allow constant monitoring of the mean arterial blood pressure. After the blood pressure was stable, the animals were injected iv with 1 ml 0.9% saline or 1 ml 10% ethanol. Ten minutes later, 1.5–2.0 × 10^6 nonradioactive microspheres (IMT; Stason Labs, Irvine, CA), with a mean diameter of 10 μm, were injected during 10 sec via the catheter with its tip located in the ascending aorta. An arterial blood sample was collected from the catheter in the femoral artery 5 sec before the microsphere injection, and this process was continued for a total of 60 sec.

In a separate series (Table 1) attempting to address whether nitric oxide (NO) and/or vagal mechanisms are involved in mediating the ethanol effects, either the NO synthase blocker Nω-nitro-L-arginine methyl ester (L-NAME) [25 mg/kg body weight (bw); Sigma-Aldrich, St. Louis, MO] or atropine (0.5 mg/kg bw; Sigma-Aldrich) was injected iv 15 min before ethanol injection. Concentrations of L-NAME and atropine increase hepatic blood flow in man (4), baboons (5, 6), and rats (7, 8). The aim of the present study was to investigate the acute effect of alcohol on pancreatic blood flow (PBF), insulin secretion, and glycemia in the rat.

First Published Online October 4, 2007

Abbreviations: AUC, Area under the curve; bw, body weight; IBF, islet blood flow; IPGTT, ip glucose tolerance test; L-NAME, Nω-nitro-L-arginine methyl ester; NO, nitric oxide; PBF, pancreatic blood flow.

Endocrinology is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.
TABLE 1. L-NAME and atropine block the ethanol effects on IBF, serum insulin concentrations, and blood glucose levels

<table>
<thead>
<tr>
<th>Treatment</th>
<th>IBF (% of control)</th>
<th>Serum insulin concentration (% of control)</th>
<th>Blood glucose (% of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (saline)</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Ethanol</td>
<td>191 ± 22</td>
<td>174 ± 19</td>
<td>79 ± 3.0</td>
</tr>
<tr>
<td>Ethanol + L-NAME</td>
<td>50 ± 7.1</td>
<td>103 ± 8.4</td>
<td>90 ± 7.5</td>
</tr>
<tr>
<td>Ethanol + atropine</td>
<td>130 ± 16</td>
<td>91 ± 6.7</td>
<td>107 ± 4.8</td>
</tr>
<tr>
<td>L-NAME</td>
<td>89 ± 9</td>
<td>109 ± 7.6</td>
<td>89 ± 6.1</td>
</tr>
<tr>
<td>Atropine</td>
<td>87 ± 33</td>
<td>77 ± 5.5</td>
<td>91 ± 3.4</td>
</tr>
</tbody>
</table>

After iv injection of 10% of ethanol or saline into normal rats, rates of blood perfusion in the pancreatic islets were measured using a microsphere technique. In some groups the NO synthase blocker L-NAME (25 mg/kg bw) or atropine (0.5 mg/kg bw) was injected iv 15 min before ethanol/saline injection. Serum insulin concentrations were measured with ELISA, and blood glucose levels were measured with test reagent strips during a simultaneous IPGTT over 120 min. Changes in the insulin and glucose responses were calculated as AUC integrated over 120 min. Values represent mean ± SEM for five independent experiments.

\[ Q_{\text{org}} = Q_{\text{ref}} \times \frac{N_{\text{org}}}{N_{\text{ref}}} \]

Where \( Q_{\text{org}} \) denotes organ blood flow (ml/min), \( Q_{\text{ref}} \) denotes withdrawal rate of the reference sample (ml/min), \( N_{\text{org}} \) denotes number of microspheres in the organ, and \( N_{\text{ref}} \) denotes the number of microspheres in the reference sample.

Intraperitoneal glucose tolerance test (IPGTT)

The animals were injected ip with a 30% (wt/vol) p-glucose solution (2 g glucose/kg bw). Ethanol and the other test substances were administered a few seconds after the ip glucose bolus. Blood samples were drawn from the tail vein immediately before, and 10, 30, 60, and 120 min after glucose administration. Area under the curve (AUC) for the IPGTT was determined by computerized image analysis.

Measurement of ethanol, glucose, and insulin concentrations

Ethanol concentrations in serum were determined by the clinical chemistry laboratory in our hospital, using the enzymatic alcohol dehydrogenase method with spectrophotometric measurements of reduced nicotinamide adenine dinucleotide as end product. Blood glucose concentrations were measured with test reagent strips (Medisense, Solna, Sweden) and serum insulin concentrations with ELISA kit (Rat Insulin ELISA; Mercodia, Uppsala, Sweden).

Statistical analysis

All values are given as means ± SEM. Statistical comparisons were made with the Student’s unpaired t test. \( P < 0.05 \) was deemed statistically significant.

Results

Neither whole PBF (1.65 ± 0.1 compared with saline 1.35 ± 0.1 ml/min/g tissue) nor adrenal blood flow (13.9 ± 13 compared with saline 13.2 ± 0.1 ml/min/g tissue) was affected by 10% ethanol (n = 8). In contrast, a marked enhancement of islet blood flow (IBF) was noted after giving 10% ethanol, both in absolute terms (Fig. 2A) and when expressed as a fraction of the whole PBF (Fig. 2B). Renal blood flow was also modestly increased after administration of 10% ethanol (Fig. 3). Serum ethanol concentrations averaged 8.0 ± 1.1 mmol/liter (n = 5).

None of the treatments influenced nonstimulated serum insulin levels significantly (Fig. 4A). Blood glucose concentrations also did not differ between any of the treatment groups in the nonstimulated state (Fig. 5A). In contrast, during an IPGTT, late-phase insulin secretion was substantially augmented (Fig. 4, A and B) and glucose tolerance was significantly improved (Fig. 5A) in animals treated with ethanol. At the end of the IPGTT (120 min after the 10-sec bolus of ethanol), hypoglycemia was induced in ethanol-treated rats (Fig. 5A), with blood glucose levels averaging 2 mmol/liter (40 mg/dl). Separate calculations of the total amount of insulin secreted during the 120-min IPGTT (AUC) revealed that insulin secretion in rats treated with ethanol was significantly higher than in control rats receiving solvent only (Fig. 4B). As shown in Fig. 5B, there was also a decrease in post-load glycemia, expressed as AUC. No effects on mean arterial blood pressure (averaging 110 mm Hg) were detected after ethanol administration (data not shown).

In a separate series (Table 1) attempting to address
whether NO and/or vagal mechanisms are involved in mediating the ethanol effects, either the NO synthase blocker l-NAME (25 mg/kg bw) or atropine (0.5 mg/kg bw) was injected iv 15 min before ethanol injection. Both these inhibitors blocked the increased pancreatic IBF, enhanced insulin secretion, and hypoglycemia evoked by ethanol.

Discussion

The results of this study indicate that low concentrations of ethanol elicit a substantial stimulation of IBF, augmenting late-phase insulin secretion, and also modestly enhance kidney blood flow (KBF). Ethanol is known to cause vasodilation. However, the effects noted in our current work do not seem to reflect a generalized response in the splanchnic bed because perfusion in other abdominal organs, such as the exocrine pancreas and adrenals, was not altered. This action of ethanol may be mediated, at least in part, by NO. Our results indicating that the NO synthase inhibitor l-NAME can block the ethanol effects corroborate this assumption. Ethanol consumption is known to increase plasma concentrations of NO (13, 14). Because islet blood perfusion is extremely sensitive to NO, a local increase in NO production would be expected to increase preferentially IBF, rather than total pancreatic blood perfusion (11, 15). Whether direct NO effects on β-cells impact insulin secretion positively (16), negatively (17–19), or not at all (20) remains highly contro-

FIG. 2. Ethanol markedly and preferentially enhances IBF. After iv injection of 10% ethanol into normal rats, rates of blood perfusion in the pancreatic islets (A) and the fraction of total PBF contributed by islets (B) were measured using a microsphere technique. Bars represent means ± SEM for eight independent experiments. ***, P < 0.001 for a chance difference vs. controls using the Student’s unpaired t test.

FIG. 3. Ethanol increases renal blood flow. After iv injection of 10% ethanol into normal rats, rates of blood perfusion in the kidneys were measured using a microsphere technique. Bars represent means ± SEM for eight independent experiments. **, P < 0.01 for a chance difference vs. controls using the Student’s unpaired t test.

FIG. 4. Ethanol augments late-phase insulin secretion. A, After iv injection of 10% ethanol into normal rats, serum insulin concentrations were measured with ELISA during a simultaneous IPGTT over 120 min. B, Changes in the insulin response are expressed as AUC integrated over 120 min. Values represent means ± SEM for five independent experiments. **, P < 0.01 and ***, P < 0.001 for chance differences vs. controls using the Student’s unpaired t test.
ethanol intake may lead to the development of insulin resistance and impaired glucose disposal, characterized by elevated fasting blood glucose levels (27, 28) and nonfasting hyperglycemia (29). However, not all reports arrive at that conclusion; several studies show a decreased incidence of new-onset diabetes by alcohol (30, 31), and alcohol has long been known for its predominantly hypoglycemic effects when given acutely (32, 33). At variance with this, recent studies found that ethanol exposure may increase hepatic glucose production, associated with hepatic insulin resistance, and decreased glucose utilization rates (34, 35). In our present observations, late-phase hypoglycemia occurred, and we speculate that this was caused by the sustained insulin secretion evoked by the heightened IBF elicited by the alcohol.

Our results may also have relevance for the derailed metabolic situation in diabetic subjects. Alcohol intake may provoke sustained hypoglycemia in type 2 diabetic patients treated with commonly used hypoglycemic sulfonylureas, such as glibenclamide (36). This may pose special problems, considering the long biological half-life of many sulfonylureas. In addition, many alcoholics are malnourished and/or have liver cirrhosis such that they may not be able to mount a gluconeogenic response to hypoglycemia. We have been unable to find any reports that ethanol in any way can influence insulin clearance. In contrast, it has been well known for many years that hepatic glucose production is suppressed by ethanol, and we cannot exclude the possibility that such an effect could have in part contributed to our results. However, because the increase in insulin evoked by alcohol was proportionally much more pronounced than the decrease in glyceemia, it seems likely that any such suppressive influence of ethanol on hepatic glucose production was by far quantitatively outnumbered by a counter-regulatory response (involving glucagon, adrenaline, cortisol, etc.) mounted that would involve (among other mechanisms) heightened hepatic glucose production. On the other hand, alcohol might delay the recovery from hypoglycemia because there is evidence that ethanol might impede the counter-regulatory hormonal response to hypoglycemia, in as much as cortisol, glucagon, and GH responses were attenuated by ethanol (37).

In summary, our findings demonstrate that ethanol exerts substantial influences on pancreatic microcirculation by evoking a massive redistribution of blood flow within the pancreatic gland, redirecting it into the endocrine part, evoking sustained insulin secretion and hypoglycemia. This mechanism, which seems to involve NO and vagal pathways, may in part underlie the well-known hypoglycemic properties of alcohol in diabetic patients or in alcoholics with hepatic failure.

Acknowledgments

Received May 15, 2007. Accepted September 27, 2007.

Address all correspondence and requests for reprints to: Åke Sjöholm, Karolinska Institutet, Department of Internal Medicine, Stockholm South Hospital, SE-118 83 Stockholm, Sweden. E-mail: ake.sjoholm@sodersjukhuset.se.

This work was supported by the Alcohol Research Council of the Swedish Alcohol Retailing Monopoly, the Janne Elgqvist Family Foundation, the Swedish Society of Medicine, the Sigurd and Elsa Golje

References


Endocrinology is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.

Huang and Sjöholm • Mechanism of Alcohol Hypoglycemia

Downloaded from endo.endojournals.org at Karolinska Institutet Biblioteket on December 23, 2007