Atropine Inhibits the Increase in Gastric Emptying During Hypoglycemia in Humans

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OBJECTIVE — To study the effect of a cholinergic muscarinic blockade on the gastric emptying rate during insulin-induced hypoglycemia in healthy subjects.

RESEARCH DESIGN AND METHODS — In eight healthy subjects, the rate of gastric emptying of an isotope-labeled meal was assessed by a scintigraphic technique during normoglycemia and hypoglycemia with simultaneous infusion of either atropine or saline. Blood glucose concentrations were controlled by an insulin-glucose clamp.

RESULTS — The median time for emptying 50% of the liquid phase from the stomach (T_{50}) was 24.9 min (range 13.9–120.0) during normoglycemia compared with 8.1 min (range 3.6–16.5) during hypoglycemia without atropine infusion (P = 0.0005). The T_{50} for the solid phase was 26.8 min (range 9.7–74.0) and 43.1 min (range 29–57.8), respectively (P = 0.007). During hypoglycemia with atropine infusion, T_{50} was 40.7 min (range 10.0–120.0) for the liquid phase and 111.4 min (range 38.9–120.0) for the solid phase, not statistically different from normoglycemic examinations.

CONCLUSIONS — Cholinergic muscarinic blockade with atropine inhibits the increase in gastric emptying during hypoglycemia. Vagal activity seems to be an important determinant of gastric emptying during hypoglycemia.

The blood glucose concentration affects the gastric emptying rate, a finding of obvious clinical importance in patients with diabetes. Hypoglycemia increases the gastric emptying rate in healthy subjects (1) as well as in patients with insulin-dependent diabetes mellitus (IDDM) (2). Hyperglycemia has the opposite effect on the gastric emptying rate (3–5). The mechanism by which blood glucose affects gastric emptying is unknown.

The aim of this study was to investigate the effect of cholinergic muscarinic blockade using atropine on the increased gastric emptying rate during insulin-induced hypoglycemia in healthy subjects.

RESEARCH DESIGN AND METHODS

Subjects

Eight nonsmoking nondiabetic healthy males agreed to participate in the study. Their mean age was 32.5 ± 5.4 years, and body mass index was 23.3 ± 1.3 kg/m^2. All subjects were examined in a random order on three separate occasions: once during normoglycemia (A) and twice during insulin-induced hypoglycemia with either saline (B) or atropine (C) administered simultaneously. The three examinations were carried out with 1–2 week intervals.

The study was approved by the Örebro Medical Center Hospital Committee on Research Ethics and the Isotope Committee.

Insulin-glucose clamp technique

Subjects were admitted to the hospital at 8:00 A.M. after an overnight fast. One intravenous catheter was inserted into a left antecubital vein for an infusion of insulin, glucose, and atropine/saline, and another was placed in a right dorsal hand vein for blood sampling. This hand was kept heated using an electric pad to arterialize the venous blood (6). An insulin-glucose clamp (7) was started with a continuous infusion of regular human insulin (Actrapid, Novo Nordisk, Copenhagen, Denmark) at a rate of 80 mU · m^{-1} · min^{-1} during the whole study period on all occasions. A 20% glucose infusion was given simultaneously at a varying rate to

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ANOVA, analysis of variance; IDDM, insulin-dependent diabetes mellitus; T_{50}, time for emptying 50% of the content from the stomach.

maintain the blood glucose concentrations within the desired range.

All examinations started with a clamped normoglycemic phase of 1 h before the gastric emptying study. In the control examination (A), normoglycemia (blood glucose 4–6 mmol/l) was maintained throughout the whole study. During the hypoglycemic studies (B and C), the initial normoglycemic phase was followed by induction of hypoglycemia, which was maintained for 15 min before the gastric emptying study. The target for hypoglycemia was a blood glucose concentration of 1.8–2.2 mmol/l. In both hypoglycemic examinations, blood glucose was normalized within 30 min. During examination C, the glucose infusion rate had to be increased to normalize the blood glucose concentration, while in examination B, the ingested food was enough to raise the blood glucose. The blood glucose concentration was then kept within the normoglycemic range for the rest of the experiment.

Atropine administration

Atropine administration was started at the beginning of the normoglycemic phase of the clamp by a bolus injection of 15 μg/kg over 30 s, followed by a maintenance infusion of 4 $\mu g \cdot kg^{-1} \cdot h^{-1}$ (8) until the gastric emptying study was finished. During the control experiment (B), equivalent volumes of saline were infused.

Pancreatic polypeptide and blood glucose measurements

To assess whether adequate cholinergic muscarinic blockade was achieved, plasma levels of pancreatic polypeptide, which is under cholinergic control (9), were measured using an immunoreactive method (10). Blood samples were obtained every 30 min from 120 min before ingestion of the meal to 120 min after the meal. The upper normal limit for pancreatic polypeptide is 40 pmol/l. The blood glucose concentration was measured every 5–10 min by a glucose dehydrogenase technique (HemoCue, HemoCue AB, Ängelholm, Sweden) (11).

Gastric emptying study

Gastric emptying was determined with a scintigraphic method, which uses a double-isotope technique for the simultaneous registration of the emptying of liquids and solid food. Details have been described previously (1,2). However, compared with those studies, the composition of the meal in the present study was changed mainly by increasing the caloric content to ensure a better separation of the liquid and solid phases. The solid phase was an egg omelet of 200 g (380 kcal) labeled with 15 MBq of ^{99m}Tc. The liquid phase consisted of 150 ml of lemonade (70 kcal) labeled with 5 MBq of ^{111}Indium-DTPA (diethylenetriaminepentaacetic acid). The total caloric content of the meal was 450 kcal.

The subjects started with the omelet followed by the lemonade for a maximal time of ingestion of 5 min. The investigation was carried out with the subjects sitting with the collimator placed behind their backs. The time when the subjects started to ingest the study meal was defined as time 0. For the liquid phase, the time it took to empty 50% of the content from the stomach (T_{50}) was calculated. For the solid phase, the duration of the lag period, T_{50}, and the linear emptying rate (%/h) were recorded. The lag period was defined as the time from the ingestion of food until emptying from the stomach began.

Statistical analysis

The results are presented as means ± SD or medians and ranges depending on whether the data were normally distributed or not. Two-way analysis of variance (ANOVA) with factors for subjects and test situations was used (SAS 6.08, SAS Institute, Cary, NC). In case the overall F test for the situation was significant, pairwise comparisons (logarithmic values) between the three test situations of gastric emptying were made.

ANOVA for repeated measurements was applied to the pancreatic polypeptide responses. The hypothesis of parallel lines was tested in an overall F test. In case this test was significant, pairwise comparisons for parallel lines were made.

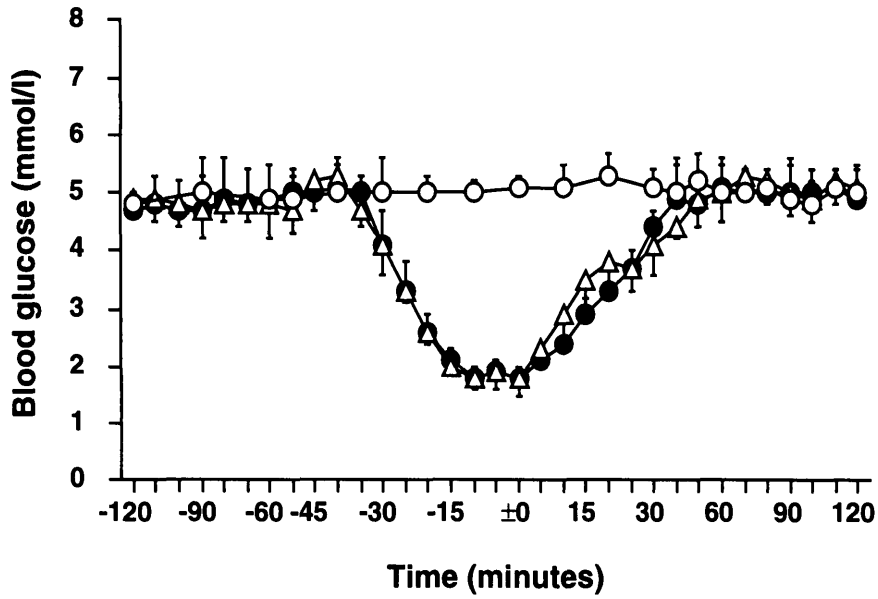

Figure 1—*The mean blood glucose values (± SD) during the different examinations. Time 0 is defined as the time when the gastric emptying study was started. ○, normoglycemia; △, hypoglycemia + saline; ●, hypoglycemia + atropine.*

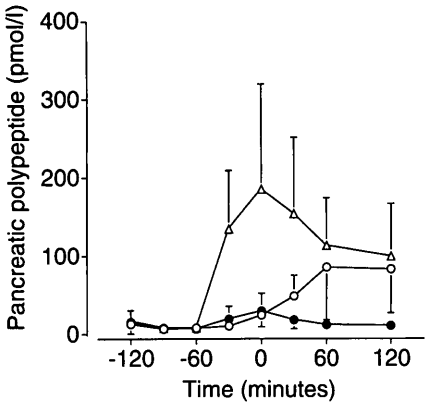

Figure 2—*The mean plasma concentrations of pancreatic polypeptide during the three examinations. Time 0 is defined as the time when the gastric emptying study was started. The differences between the three curves were highly significant in pairwise comparisons (P = 0.0001) using ANOVA for repeated measurements. ○, normoglycemia; △, hypoglycemia + saline; ●, hypoglycemia + atropine.*

Table 1—*The gastric emptying rate of liquids and solid food during the three different experimental conditions*

	Normoglycemia (A)	Hypoglycemia + saline (B)	Hypoglycemia + atropine (C)
Liquid phase			
T_{50} (min)	24.9 (13.9–120)	8.1 (3.6–16.5)‡	40.7 (10.0–120)
Solid phase			
Lag phase (min)	26.8 (9.7–74.0)	13.9 (2.4–30.0)†	21.5 (14.4–46.0)
T_{50} (min)	64.7 (50.9–120)	43.1 (29–57.8)†	111.4 (38.9–120)
Linear emptying rate (% h)	44.8 (14.7–52.8)	62.5 (48.3–85.5)‡	22.3 (8.1–57.1)*

Data are medians (ranges). *$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$ compared with normoglycemia.

RESULTS— The study protocol was well tolerated by all subjects, although hypoglycemia gave rise to characteristic symptoms. Subjects were not informed whether they were given atropine or saline during the hypoglycemic studies. However, atropine administration was followed by side effects such as tachycardia and dry mouth in all subjects.

The mean blood glucose concentrations were within the target ranges for all experiments (Fig. 1).

The plasma concentrations of pancreatic polypeptide at baseline did not differ among the three examinations. At time 0 during hypoglycemia with saline (B), a peak plasma pancreatic polypeptide concentration of 184.5 ± 135.2 pmol/l was observed, significantly higher than that during normoglycemia (A) (23.5 ± 14.4 pmol/l, $P < 0.001$) and that during hypoglycemia with atropine (C) (29.1 ± 22.6 pmol/l, $P < 0.001$) (Fig. 2).

During hypoglycemia and saline infusion (B), the gastric emptying rate of both liquids and solid food was significantly increased compared with that during normoglycemia (A) (Table 1). In contrast, during hypoglycemia and atropine infusion (C), the gastric emptying rate was not increased compared with that during normoglycemia. Instead, there was a tendency toward a slower emptying rate during atropine infusion, with a weak significance for the linear phase of the emptying of solids (Table 1). The individual T_{50} responses for both phases for each subject are presented in Fig. 3.

CONCLUSIONS— There are two main findings in this study. First, the results confirm our previous observations that hypoglycemia increases the gastric emptying rate in healthy subjects (1). In the present study, we used a meal composition that enabled a better separation of the gastric emptying for liquid and solid phases compared with that in previous studies of healthy subjects and patients with IDDM (1,2). Therefore, it is now possible to conclude that the gastric emptying rates of both liquids and solid food are increased by hypoglycemia.

Second, the increase in the gastric emptying rate during hypoglycemia is reversed by atropine. The mechanisms be-

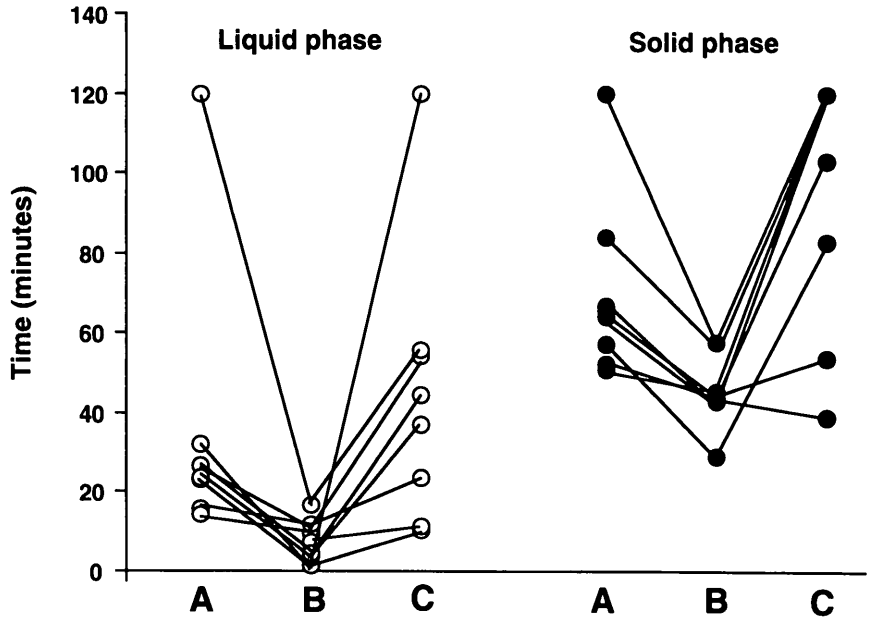

Figure 3—*The individual T_{50} values for the liquid (○) and solid food (●) in the three examinations. A, normoglycemia; B, hypoglycemia + saline; C, hypoglycemia + atropine.*

hind the increased gastric emptying rate during hypoglycemia are thus far undefined. Hormonal responses occurring during hypoglycemia as well as sympathetic and parasympathetic nervous system activation (12,13) can influence the gastric emptying rate. Our results suggest that cholinergic stimulation is of major importance for the increased gastric emptying rate during hypoglycemia. Atropine is a universal inhibitor of the cholinergic neurotransmitter acetylcholine and can block the effect of vagal nerve stimulation. The fact that plasma pancreatic polypeptide concentrations were unchanged during hypoglycemic experiments with atropine (C) as compared with during normoglycemia suggests that a complete inhibition of the cholinergic transmission was achieved (9,14). Whether the present results can be taken as evidence of a direct vagally mediated parasympathetic stimulation by hypoglycemia or whether vagal activity is in turn stimulated by one of the hormones activated during hypoglycemia cannot be concluded from these experiments. It is likely, however, that vagal activity is an important determinant of the varying rate of gastric emptying at different glycemic levels, although it is not yet known if decreased vagal activity has a role in the slowing of gastric emptying found during severe hyperglycemia (4,5). Atropine is known to retard gastric emptying in normal subjects (15). In our study, the gastric emptying rate during hypoglycemia with atropine (C) tended to be even slower than during normoglycemia (A), which is in accordance with these findings.

The pancreatic polypeptide response in the atropine-treated subjects resembles that of diabetic patients with autonomic neuropathy who are exposed to insulin-induced hypoglycemia (16). It has been shown that vagal disturbances are frequent in diabetic patients with autonomic neuropathy (17), which can lead to a reduction in the stimulated gastric acid output (18). A failure to increase gastric emptying in response to hypoglycemia would be of obvious disadvantage when ingestion of oral carbohydrates is used to restore normoglycemia. Diabetic patients with autonomic neuropathy could be at increased risk for severe hypoglycemia.

The influence of glycemia on vagal activity and gastric emptying rate probably alters the optimal time interval between insulin injections and postprandial increases in the blood glucose concentration in patients treated with short-acting insulin at mealtime. This may cause variations in blood glucose concentrations. Further, it is possible that drugs with anticholinergic effects, e.g., antidepressants, may negatively influence the gastric emptying rate during hypoglycemia in diabetic patients.

In conclusion, the present study confirms our previous findings that both liquid and solid phases of gastric emptying are significantly increased during hypoglycemia and that the main mechanism for this increased gastric emptying is mediated by cholinergic stimulation, probably by means of increased vagal activity. The findings underscore the fact that the current blood glucose concentration is one important determinant of the gastric emptying rate. In addition, vagal disturbances in patients with diabetic autonomic neuropathy may impair their ability to adjust the gastric emptying rate to variations in the blood glucose concentration.

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