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Activation of the Parasympathetic Nervous System Is Necessary for Normal Meal-Induced Insulin Secretion in Rhesus Macaques*

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ABSTRACT

Meal-induced insulin secretion is thought to be regulated primarily by absorbed nutrients and incretin hormones released from the gastrointestinal tract. In addition, the parasympathetic nervous system (PNS) is known to mediate preabsorptive, or cephalic phase, insulin secretion. Despite evidence that the PNS remains activated during the absorptive phase of the meal, its role in mediating postprandial insulin secretion has not been established. To study the role of the PNS in absorptive phase insulin release, we measured plasma concentrations of glucose as well as islet hormones and incretins in six healthy rhesus monkeys before and for 60 min after meals while they were infused with saline (control), atropine (muscarinic blockade), or trimethaphan (nicotinic blockade). During the infusion of saline, plasma levels of glucose, pancreatic polypeptide (PP), insulin, glucose-dependent insulintropic polypeptide, and glucagon-like peptide-1 increased promptly after meal ingestion and remained elevated throughout the 60 min of the study. The PP response was nearly abolished in animals treated with trimethaphan, indicating functional blockade of PNS input to the islet, and in contrast to the control study, there were minimal changes in plasma concentrations of glucose, incretin hormones, and insulin. Because trimethaphan inhibited glycemic and incretin stimuli in addition to blocking PNS input to the

islet, it was not possible to discern the relative roles of these factors in the stimulation of insulin secretion. Atropine also significantly decreased PNS transmission to the islet, as reflected by PP levels similar to those observed with trimethaphan. Unlike the trimethaphan study, plasma glucose levels rose normally during atropine treatment and were similar to those in the control study over the course of the experiments (114 ± 22 and 132 ± 23 mmol/L-60 min, respectively). In addition, the rise in plasma glucagon-like peptide-1 following the meal was not suppressed by atropine, and the glucose-dependent insulintropic polypeptide responses were only modestly decreased. Despite the significant increases in circulating glucose and incretins, plasma insulin levels were greatly attenuated by atropine, so that the 60 min responses were more comparable to those during trimethaphan treatment than to those in the control study (atropine, $3,576 \pm 1,284$; trimethaphan, $4,128 \pm 2,616$; control, $15,834 \pm 5,586$ pmol/L-60 min; $P < 0.05$). Thus, muscarinic blockade markedly suppressed the meal-induced insulin response despite normal postprandial glycemia and significant elevations of incretins. These results indicate that activation of the PNS during the absorptive phase of meals contributes significantly to the postprandial insulin secretory response. (*J Clin Endocrinol Metab* 86: 1253–1259, 2001)

NORMAL GLUCOSE tolerance requires a rapid insulin secretory response that is appropriate for the amount of ingested carbohydrate. In healthy humans, insulin levels rise soon after food intake, with the major portion of the β -cell response paralleling the rise in plasma glucose. In addition, plasma insulin levels increase in direct proportion to the quantity of carbohydrate ingested (1, 2). This finely regulated β -cell response is mediated by a system of coordinated inputs to the islet, including stimulation by nutrients (glucose and certain amino acids), gastrointestinal (GI) hormones, and neural signals, collectively known as the entero-

insular axis (3, 4). Although increases in circulating glucose are essential for activation of the β -cell, GI hormones released during nutrient absorption make a substantial contribution to the normal β -cell response to food ingestion. These GI hormones, known as incretins, are secreted in proportion to meal size and increase the responsiveness of the β -cell to glucose, thus regulating both the magnitude and the rapidity of normal postprandial insulin release (3, 4). Of the many candidate GI peptides, only two, glucose-dependent insulintropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), are currently considered to be physiologically important incretins (4–6).

After food intake a small amount of insulin is released even before there are significant increments in plasma glucose concentrations, a phenomenon termed preabsorptive, or cephalic phase, insulin secretion (7–9). Even though preabsorptive insulin release is a relatively minor component of total postprandial β -cell secretion, it appears to be physiologically important, because studies in both rats and humans have shown that loss of the preabsorptive insulin response impairs glucose tolerance (10–13). Preabsorptive insulin se-

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cretion can be prevented by vagotomy or muscarinic blockade with atropine (7–9, 13), indicating that it is mediated primarily by activation of the parasympathetic cholinergic nerves innervating the islet.

Despite convincing evidence that cholinergic signals stimulate insulin secretion, the contribution of the parasympathetic nervous system (PNS) to meal-induced insulin release beyond the preabsorptive phase has received little attention. This is due in great part to methodological difficulties, as interference with vagal transmission, either surgically or pharmacologically, frequently produces plasma glucose profiles different than those of controls (8). This clouds the interpretation of PNS effects during the absorptive phase of insulin secretion when glycemic stimulation has a prominent role. There is currently no consensus on this issue; PNS stimulation of meal-induced insulin secretion beyond the cephalic phase has been suggested by some investigators (14) and dismissed by others (4), but is typically ignored among the important mediators of postprandial β -cell secretion (13, 15–17).

In the experiments described below we sought to gain evidence for a direct role of the PNS to stimulate insulin secretion during the absorptive phase of meal by measuring the effects of cholinergic blockade on the glucose, insulin, and incretin hormone responses to a high carbohydrate, mixed meal in rhesus monkeys.

Materials and Methods

Animals and catheter implantation

Six adult (aged 11–14 yr) male rhesus monkeys (*Macaca mulatta*), weighing 9.1–14.5 kg (mean $\pm$ SEM, 11.5 ± 0.7 kg), were used for these studies. Before selection for the study, a physical examination, complete blood count, and serum biochemistry panel were performed for each animal. The animals had previously been acclimated to several hours of chair restraint with a minimum of 10 training sessions (18). At least 1 week before the first experiment, an iliac or femoral artery was catheterized under ketamine/isoflurane anesthesia with a polyurethane catheter connected to a sc vascular access port (Access Technologies, Skokie, IL) as previously described (18). Animals were housed in the American Association for Accreditation of Laboratory Animal Care-accredited facilities of the California Regional Primate Research Center in accordance with standards established by the U.S. Animal Welfare Act and the Institute of Laboratory Animal Resources. The experimental protocols were approved by the institutional animal use and care committee at the University of California-Davis and the California Regional Primate Research Center and were conducted in accordance with the guidelines of the National Research Council's Guide for the Care and Use of Laboratory Animals.

Feeding protocol

Each animal was studied three times in randomized order: 1) during saline infusion, 2) during atropine infusion, and 3) during trimethaphan infusion. The studies were performed at least 7 days apart. Two animals had the saline infusion, two animals had the atropine infusion, and two animals had the trimethaphan infusion as their first experiment. They were fasted overnight and placed in restraint chairs at least 1 h before the experiment. A cephalic vein was catheterized for infusion of saline, atropine, or trimethaphan. Four baseline blood samples (3 mL each) were drawn from the arterial port at -20 , -10 , -5 , and 0 min. Animals then ingested a meal consisting of one banana, half of an apple cut into four pieces, and three monkey chow biscuits (Purina 5047, Ralston Purina Co., St. Louis, MO). Blood samples were collected 2, 5, 10, 15, 30, 45, and 60 min after the animals began to eat. In each subsequent experiment, the animals were fed the same amount of food that was consumed by that animal during the initial experiment. Therefore, the

amount of energy consumed and the macronutrient composition of the meals did not differ among the treatments. The meals contained an average of 32.7 ± 1.2 g carbohydrate, 6.6 ± 2.1 g protein, and 1.7 ± 0.4 g fat and provided approximately 170 Cal (720 joules) energy. Approximately 76% of the energy in the meals was derived from carbohydrate, 15% from protein, and 9% from fat. The animals were eating for an average of 24 ± 3 min of the 1-h period, and this time period did not differ among the three experimental conditions.

Pharmacological autonomic blockade

To produce muscarinic blockade and impair activation of cholinergic receptors during feeding, animals received a primed continuous iv infusion of atropine sulfate (Elkins-Sinn, Cherry Hill, NJ; 0.1 mg/kg bolus plus 0.001 mg/kg-min) starting 20 min before the meal and throughout the experiment. To produce nicotinic blockade of ganglionic neurotransmission and abolish all parasympathetic postganglionic signaling during feeding, trimethaphan camsylate (Arfonad, Roche, Nutley, NJ) was infused iv (0.1 – 0.4 mg/min) for 30 min before food ingestion and throughout the study. Arterial blood pressure was monitored continuously with a digital blood pressure analyzer (DigiMed, Louisville, KY). The infusion rate of trimethaphan was adjusted to decrease mean arterial blood pressure (MAP) by more than 20 mm Hg without lowering MAP below 80 mm Hg. On the average, MAP decreased from 113 ± 5 before trimethaphan infusion to 92 ± 3 mm Hg at the time the baseline samples were drawn (change, -22 ± 2 mm Hg).

Assays and data analysis

Blood samples for plasma glucose determination were drawn and placed in tubes containing heparin. Blood samples for insulin, pancreatic polypeptide, GIP, and GLP-1 determination were placed in tubes containing ethylenediamine tetraacetate and aprotinin (Sigma, St. Louis, MO). All samples were kept on ice until centrifugation (20 min at 4°C). The plasma was decanted and frozen at -20°C until assayed. Plasma glucose was assayed by the glucose oxidase method with a glucose analyzer (Beckman Coulter, Inc., Fullerton, CA). Plasma immunoreactive insulin was measured by RIA in unextracted plasma with reagents supplied by Linco Research, Inc. (St. Louis, MO). The intra- and inter-assay coefficients of variation for the plasma insulin assay are less than 10% and 14%, respectively. Plasma pancreatic polypeptide (PP), GLP-1, and GIP were measured by RIA using previously described methods (19–21).

Calculations and data analysis

Basal values of glucose, PP, insulin, GIP, and GLP-1 were taken as the mean of the four samples collected before feeding was initiated. The postprandial responses of glucose and hormones were calculated as the area under the curve (AUC) above the basal values using the trapezoidal rule. Both the basal values and the responses to feeding (AUC) were compared among the control, atropine, and trimethaphan studies using ANOVA and Dunnett's test to distinguish differences between groups. In instances where the AUC were different in the control and either atropine or trimethaphan groups, the percent change was computed for each animal, and means of these percentages were computed for each group. Within each experimental condition, plasma values were compared with basal using repeated measures ANOVA with *post-hoc* comparisons made using *t* tests for paired samples. The data are presented as the mean $\pm$ SEM.

Results

Glycemic responses

Arterial plasma glucose responses to the mixed meal in monkeys treated with saline (control), atropine, or trimethaphan are shown in Fig. 1A. Basal (fasting) plasma glucose levels were unaffected by atropine or trimethaphan infusion. In the control study, plasma glucose increased significantly (~ 2 mmol/L) above basal levels 10 min after the start of the meal and remained elevated for the full 60 min

FIG. 1. Plasma concentrations of glucose (A), PP (B), GIP (C), and GLP-1 (D) before, during, and after meals in monkeys infused with saline (circles/solid line), atropine (squares/dashed line), and trimethaphan (diamonds/dotted line). For A and B, the asterisks denote values different from basal in the control and atropine groups ($P < 0.05$). For C and D, the asterisks denote values different from basal in the control group ($P < 0.05$), and # denotes values different from basal in both the control and atropine groups.

TABLE 1. Postprandial responses (AUC) of glucose, pancreatic polypeptide, insulin, GIP, and GLP-1 in monkeys given saline, atropine, or trimethaphan

	Control	Atropine	Trimethaphan
Glucose (mmol/L-60 min)	132 ± 23	114 ± 22	25 ± 8 ^{a,b}
PP (pmol/L-60 min)	8,507 ± 1,872	1,737 ± 1,234 ^a	376 ± 287 ^a
Insulin (pmol/L-60 min)	15,834 ± 5,586	3,576 ± 1,284 ^a	4,128 ± 2,616 ^a
GIP (pmol/L-60 min)	7,464 ± 1,879	3,857 ± 1,035	2,256 ± 1,339 ^a
GLP-1 (pmol/L-60 min)	318 ± 51	357 ± 61	144 ± 65 ^b

^a $P < 0.05$ vs. control.

^b $P < 0.05$ vs. atropine.

of observation. Plasma glucose also increased after meal ingestion in the atropine-treated group, and the increase was comparable to that in the control study; all values from 10 min after meal initiation were significantly greater than basal levels. In contrast, there was a marked diminution, and delay, in the rise of plasma glucose when the monkeys were fed during the infusion of trimethaphan; values were significantly elevated above baseline levels only at 45 and 60 min after initiation of feeding. The glucose AUC above basal after feeding for each of the three treatments is shown in Table 1. The glucose AUCs for the control and atropine studies were

not significantly different from one another, but both were significantly greater than the glucose AUC in the trimethaphan study.

PP

The arterial plasma concentrations of PP during the three studies are shown in Fig. 1B. Treatment with atropine and trimethaphan decreased the basal PP concentrations relative to control levels (35.8 ± 4.3 and 39.4 ± 3.1 , vs. 65.7 ± 16 pmol/L, respectively; $P < 0.05$). In the control study, plasma

PP concentrations increased rapidly after the start of feeding, peaking at 5-fold basal values at 10 min and declining thereafter for the remainder of the study; all postmeal values were significantly greater than basal. This rapid increase in PP during the control study was detectable at the first sample (2 min) after eating commenced and preceded the increase in plasma glucose levels, which did not rise significantly until 10 min. Compared with the control study, plasma PP did not increase after the meal in animals pretreated with either atropine or trimethaphan. As shown in Table 1, atropine and trimethaphan treatment decreased the 60 min PP response to feeding by $84 \pm 9\%$ and $89 \pm 8\%$, respectively ($P < 0.001$ for both). The PP responses in animals given atropine and those in animals given trimethaphan were not different from each other.

Insulin

Figure 2A depicts the arterial plasma insulin values in the monkeys during the three studies. Treatment with atropine or trimethaphan did not significantly alter basal insulin levels relative to saline infusion (78 ± 15 , 95 ± 25 , and 112 ± 22

FIG. 2. A, Plasma concentrations of insulin before, during, and after meals in monkeys infused with saline (circles/solid line), atropine (squares/dashed line), and trimethaphan (diamonds/dotted line). The asterisks denote values different from basal in the control group ($P < 0.05$). B, Cephalic phase insulin secretion in monkeys treated with saline, atropine, and trimethaphan. The vertical bars represent the AUC above basal insulin for the first 10 min after the meal.

pmol/L, respectively; Fig. 2A). In the control study, plasma insulin levels increased significantly within 2 min after the start of the meal and reached a plateau at 30 min; all values after initiation of the meal were significantly greater than baseline levels. The three plasma samples taken in the first 10 min of the meal reflect preabsorptive insulin release, as glucose levels did not increase significantly until the end of this period. The preabsorptive insulin response during the control experiments was 115 ± 44 pmol/L·10 min (Fig. 2B).

As expected, there was no early, preabsorptive, rise in plasma insulin (8 ± 9 pmol/L·10 min) in the monkeys treated with atropine (Fig. 2B). However, there was also a notable effect of atropine on insulin secretion during the absorptive phase of the meal. In these experiments insulin levels did not increase significantly until 30 min after meal ingestion, and the insulin response not only was delayed, but was also greatly attenuated compared with that in the control study (Fig. 2A and Table 1). This inhibition of insulin secretion occurred despite similar increases in plasma glucose in the control and atropine experiments. When the monkeys were treated with trimethaphan, the plasma insulin response in both the cephalic and absorptive phases was also greatly diminished, with no postprandial time point significantly higher than basal. However, in these studies glucose levels did not change from basal in most of the animals, so that glycemic stimulus of the β -cell was minimal. The overall postprandial insulin release during muscarinic or nicotinic blockade was attenuated by $71 \pm 7\%$ and $80 \pm 8\%$, respectively, compared with that in the control study, as shown in Table 1.

Incretins (GIP and GLP-1)

Plasma concentrations of GIP before and after feeding are shown in Fig. 1C. Basal GIP concentrations were not changed by infusion of cholinergic antagonists. After the meal, GIP concentrations rose in the control and atropine groups to levels significantly greater than basal by 5 min in the control and 15 min in the atropine experiments. GIP responses were variably suppressed in five of the six monkeys during atropine treatment, and for the group as a whole the GIP AUC was 66% of the control value (Table 1). During the infusion of trimethaphan the rise in GIP was minimal and did not achieve statistical significance at any of the postprandial time points.

Basal concentrations of GLP-1 were not affected by parasympathetic blockade with either atropine or trimethaphan (Fig. 1D). Meal ingestion caused plasma GLP-1 levels to rise significantly compared with baseline in both the control and atropine-treated animals, and the AUCs were similar during these two experiments (Table 1). In contrast, when the monkeys were treated with trimethaphan, plasma GLP-1 levels did not increase significantly after food ingestion compared with baseline. The mean AUC for GLP-1 during the control and atropine studies were greater than twice the calculated response during trimethaphan treatment.

Discussion

In this study we sought to determine the effect of cholinergic input to the pancreatic islets on the insulin response

during the absorptive phase of a meal. To this end we blocked cholinergic-muscarinic (atropine) or nicotinic ganglionic (trimethaphan) signaling before and for 60 min after the consumption of a high carbohydrate meal and compared the insulin responses to a control study. Plasma levels of glucose and the incretin hormones GIP and GLP-1 were also measured to allow estimates of the degree of β -cell stimulation by these three factors in each of the experimental conditions. The major finding of this study is that muscarinic blockade markedly attenuated the insulin response for the first hour after meal ingestion despite the presence of normal elevations of plasma glucose and GLP-1 and a GIP response that was only modestly reduced. This observation suggests that muscarinic signaling to the islet is essential for the insulin response to the absorptive phase of a meal. If so, the role of the PNS extends beyond its currently accepted function of mediating preabsorptive, or cephalic phase, insulin secretion. This finding indicates that the traditional view that nutrient substrates and GI hormones are the sole mediators of the sustained insulin response during nutrient absorption should be systematically and rigorously reexamined.

The traditional view of meal-induced insulin secretion has been that once nutrient absorption has commenced stimulation of the β -cell is mediated predominantly by circulating glucose and the incretin hormones, and therefore, that the PNS does not contribute significantly to insulin secretion during this period (4, 13, 15–17). This view has persisted despite direct evidence that activation of the PNS input to the islet can markedly stimulate insulin release (22) and indirect evidence that the PNS remains activated during the absorptive phase of the meal (23–25). Part of the problem has been the difficulty in separating direct effects of PNS blockade on the islet from indirect effects due to alterations in GI function and consequently blood glucose. Therefore, studies using surgical or pharmacological methods of denervating the β -cell have also reduced (8) or accelerated (26, 27) gastric emptying and thereby either reduced or accelerated the postprandial glycemic excursions. Because of the variability introduced in the glucose stimulus to the islet, it has not been possible to determine the direct contribution of PNS activation to meal-induced insulin secretion in these studies. For this reason it is widely believed that the major role of the PNS in insulin secretion during meal absorption is indirect, via the regulation of gastric emptying and control of the rate of delivery of nutrients to the intestine. The alternative view, that there are important effects of the PNS to directly stimulate insulin secretion during meal absorption, requires experimental conditions in which PNS blockade has little effect on plasma glucose. Fortunately, in the present study postprandial glucose levels during atropine infusion were similar to those in the control experiments despite clear muscarinic blockade, as indicated by the near-total inhibition of PP secretion. As the insulin response to the meal was also markedly impaired by atropine, this study demonstrates an unambiguous effect of cholinergic signaling to stimulate insulin secretion during the absorptive phase of a meal.

Our findings are supported by the results of two previous studies in other animal models, both of which bypassed the stomach by infusing nutrients directly into the duodenum. Schusdziarra and colleagues showed that atropine signifi-

cantly inhibited insulin secretion during 40 min of enteral nutrient infusion to anesthetized rats (28). Greenberg and Pokol-Daniel showed that cryogenic blockade of vagal neurotransmission during intraduodenal perfusion of mixed nutrients or glucose caused an approximately 50% decrease in the insulin response in the dog (29). In each of these studies, as in our study, the meal-induced glucose levels were not appreciably different between control conditions and those of PNS inactivation, ruling out a diminished glycemic stimulus as the cause of the subnormal insulin release. Taken together, these data support an essential role for the PNS in the composite signal that triggers insulin secretion during the absorptive phase of meals.

Although it is clear that the elevation in plasma glucose that occurs after meals is critical for a normal β -cell response, incretin hormones also play an important role in this process. The incretin effect, defined as the augmentation of insulin secretion seen when glucose is administered orally *vs.* iv, has been attributed primarily to the actions of GLP-1 and GIP, and accounts for as much as 50–70% of postprandial insulin secretion (2, 4). In the present study we have measured the two best-established incretin hormones and can therefore assess their potential to contribute to meal-induced insulin secretion. For example, plasma levels of GLP-1 were not substantially altered by atropine treatment, indicating that muscarinic pathways contribute little to meal-induced GLP-1 release in monkeys. Therefore, diminished stimulation by GLP-1 cannot account for the attenuated insulin response observed during the atropine studies. However, GIP levels were inhibited by one third during atropine treatment, raising the possibility that lower levels of this incretin contributed to the decreased insulin response in these experiments. Previous studies in rats in which GIP action was blocked either by immunoneutralization of circulating GIP or with GIP receptor antagonists have shown an approximately 40% reduction in the insulin response (30, 31). A similar decrement in insulin secretion in the 60 min after an oral glucose load was recently reported in mice with a targeted deletion of the GIP receptor gene (32). In contrast, insulin secretion was decreased by 70% in the atropine studies even though the majority of the GIP response was preserved. Thus, although it is possible that the lower GIP levels during atropine administration explain a portion of the decrement in postprandial insulin release, it is unlikely that impaired incretin stimulation can fully account for the large attenuation of the insulin response that we observed.

Although muscarinic blockade had only limited effects on the secretion of GI hormones, it is likely that atropine delayed gastric emptying to some extent in the present study. Indeed, because of the importance of vagal innervation in the control of gastric motility, blockade of either muscarinic or nicotinic signaling is expected to retard gastric emptying and therefore the delivery of nutrients to the absorptive surface of the small intestine. This, in turn, delays glucose absorption and the stimulation of GI hormone release. For example, we infer from the minimal meal-induced increments of plasma glucose and incretin levels during trimethaphan that gastric emptying was severely impaired during the 60 min of this experiment. We anticipated that muscarinic blockade would have at least a modest effect on gastric emptying based on

previously published studies (8, 33). Although we did not directly measure gastric emptying in the present study, we infer that atropine slowed the gastric delivery of nutrients based on the postprandial GIP profile. Secretion of GIP from upper intestinal K cells is tightly coupled to glucose absorption by the gut mucosa (34, 35), and studies in both humans and animals indicate that atropine does not directly block the release of GIP (29, 36). Therefore the modest reduction in the GIP profiles observed in atropine-treated monkeys probably reflects a modest reduction of glucose delivery to the upper small intestine.

Given the probability that gastric emptying of the meal was modestly delayed by atropine treatment, with decreased rates of glucose absorption, the equivalent plasma glucose excursions in the atropine experiments compared with controls must be explained by other factors. The monkeys were fed meals that were very low in fat, so that the potent effect of ingested lipid to inhibit gastric emptying and add to the action of atropine was minimal. In addition, the 25-min period of meal intake probably mitigated the effects of atropine on gastric motility to some degree. Moran and colleagues have shown that gastric emptying in monkeys is more rapid while intake is ongoing and decreases once feeding has ceased and the stomach is full (37). In our experiments the consumption of the meal over 25 min probably permitted greater rates of gastric emptying than would have occurred if the animals had been given a bolus of food over a shorter time span. Finally, it seems likely that decreased intestinal glucose uptake during atropine was counterbalanced by diminished glucose clearance, resulting in a postmeal glucose profile similar to the control condition. As insulin-stimulated glucose disposal and suppression of hepatic glucose output are essential for normal postprandial glycemia, the lower insulin response in the atropine-treated monkeys probably caused a higher glucose excursion than would have otherwise been seen for the degree of glucose absorption. Whatever the mechanism, the similar plasma glucose profiles in the atropine and control groups coupled with the markedly decreased plasma insulin levels in the atropine-treated group rule out insufficient stimulation by glucose as the cause of the atropine-induced impairment of meal-induced insulin secretion. Furthermore, as the meal given to the monkeys in this study was 80% carbohydrate, it is unlikely that differences in postprandial levels of amino acids or lipids during the control and atropine experiments are responsible for the decreased insulin responses during muscarinic blockade.

In the present study cholinergic blockade did not have a significant effect on fasting insulin levels. We infer from these data that parasympathetic input to the islet does not have a major effect on basal insulin secretion in the fasting state. This inference is consistent with previous studies showing that humans given atropine (38–40) and rats with surgical vagotomies (7) had minimal changes in fasting plasma insulin values. In fact, as it has been shown that atropine does not change the insulin levels in healthy humans receiving iv glucose (36), it appears that PNS stimulation of insulin secretion occurs only when nutrients are ingested. In this regard it is worth considering that the incretin effect, which is calculated as the difference in insulin or C peptide levels stimulated by oral glucose *vs.* an equal glycemic stimulus

from iv glucose (2), may include more than just the effects of insulinotropic GI hormones. As cholinergic, muscarinic signaling is activated by glucose ingestion, but not by glucose infusion, neural signals to the islet may contribute to the generally applied calculation of the incretin effect.

In summary, we have found that muscarinic-cholinergic blockade with atropine inhibited meal-induced insulin secretion in rhesus monkeys. As atropine did not have major effects on the glycemic and incretin stimulation of the β -cell, this finding suggests a direct role of the PNS to mediate the absorptive phase insulin response. These results suggest that neural regulation of postprandial insulin secretion is more important than has been previously believed and emphasize that disrupting islet innervation may reduce the insulin response to meals.

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