Meal-induced insulin secretion in dogs is mediated by both branches of the autonomic nervous system

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Benthem, Lambertus, Thomas O. Mundinger, and Gerald J. Taborsky, Jr. Meal-induced insulin secretion in dogs is mediated by both branches of the autonomic nervous system. Am J Physiol Endocrinol Metab 278: E603–E610, 2000.—We investigated the relationship between autonomic activity to the pancreas and insulin secretion in chronically catheterized dogs when food was shown, during eating, and during the early absorptive period. Pancreatic polypeptide (PP) output, pancreatic norepinephrine spillover (PNESO), and arterial epinephrine (Epi) were measured as indexes for parasympathetic and sympathetic nervous activity to the pancreas and for adrenal medullary activity, respectively. The relation between autonomic activity and insulin secretion was confirmed by autonomic blockade. Showing food to dogs initiated a transient increase in insulin secretion without changing PP output or PNESO. Epi did increase, suggesting β2-adrenergic mediation, which was confirmed by β-adrenoceptor blockade. Eating initiated a second transient insulin response, which was only totally abolished by combined muscarinic and β-adrenoceptor blockade. During absorption, insulin increased to a plateau. PP output showed the same pattern, suggesting parasympathetic mediation. PNESO decreased by 50%, suggesting withdrawal of inhibitory sympathetic neural tone. Therefore, it is possible that sympathoadrenergic stimulation of PNESO, pancreatic polypeptide (PP) output, circulating catecholamines (Epi and NE), and pancreatic insulin output were determined in conscious dogs bearing chronically implanted arterial and pancreatic venous catheters. This was done during the following three phases of the meal: 1) when food was shown to the dogs, without allowing them to eat; 2) during actual eating; and 3) during the early absorptive phase.

PNESO was used as an index for sympathetic neural activity to the pancreas. The measurement of PNESO during hypoglycemia in anesthetized dogs (14, 15), and recently in conscious dogs as well (9), demonstrates the activation of the sympathetic nerves innervating the pancreas during this type of stress. Pancreatic ACh spillover would provide an index for parasympathetic neural activity to the pancreas. However, the extremely rapid degradation of ACh by cholinesterases in plasma and in tissue precludes this option. Measurement of PP output provides an alternative. PP is secreted by pancreatic F cells and is predominantly under vagal control, because muscarinic blockade with atropine as well as vagotomy prevents almost all PP responses (26, 27, 37). PP output has been used as an index for parasympathetic activity to the pancreas and has been correlated with the EIR (26, 37). Finally, circulating Epi serves as an index for

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Experimental Protocols

To avoid novelty stress, the dogs were habituated to the laboratory surroundings and personnel and were trained to stand in a Pavlovian sling before the start of the actual experiments. To minimize external influences, the dogs were habituated to the moderate sound level of a radio. To avoid excitatory visual stimulation during muscarinic blockade, the experimental room was dimly lit.

Experiment 1. The first set of experiments was designed to test whether the adrenal medulla, the pancreatic parasympathetic, or the pancreatic sympathetic nerves were activated during the three phases of a meal. After an overnight fast, the dogs (n = 7) entered the experimental room and were placed in a Pavlovian sling. The arterial catheter was, via a blood pressure transducer (Statham P23Db), connected to a blood pressure monitor (Propac 106 EL; Protocol Systems) for measurement of mean arterial pressure (MAP). The socket of the blood flow probe was connected to the blood flowmeter (T101 ultrasonic bloodflow meter; Transonic Systems) to measure pancreatic venous blood flow. After a cool-down period of ~60 min, during which MAP had stabilized, baseline arterial and pancreatic venous blood samples were taken [time (t) = −10 and −1 min]. At t = 0, food (2 cans of Western Family Chicken Flavored Dog Food (protein >8%, fat >3%, fiber >1%, water <78%, ash <5%) + 50 ml of D50 glucose) was shown to the animals, without allowing them to eat. Blood samples were then taken at t = 2 and 4 min. At t = 5 min, the animals were allowed to eat the meal. In general, the dogs finished the meal within 3 min. When the meal was not finished at t = 11 min, the remaining food was taken away to maintain clear separation between the eating phase and the early absorptive phase. Additional blood samples were taken during eating (t = 7.5 and 10 min) and early absorption (t = 12.5, 15, 20, 25, and 35 min).

Experiment 2. A second set of experiments was designed to determine which autonomic input mediated the insulin responses to the sight and smell of the food, to eating the food, and early during absorption of the food. To this end, each dog participating in this study underwent a series of four experiments: one with muscarinic blockade, one with β-adrenoceptor blockade, one with combined muscarinic and β-adrenoceptor blockade, and one, as a control, without blockade. Each dog (n = 8) underwent these four experiments in a random order. Muscarinic blockade was induced using methylatropine (0.5 mg/kg; Sigma; see Ref. 23); β-adrenoceptor blockade was induced using timolol (0.2 mg/kg; Sigma; see Ref. 23). Drugs were dissolved in saline and were administered through the cannula in the pulmonary artery. Infusion of saline alone served as the control. The experimental protocol was similar to the one used for experiment 1. A baseline sample was taken at t = −15 min, whereafter blockade was induced and maintained throughout the experiment. Baseline blood samples were then taken at t = −7.5 and −1 min. At t = 0, food was shown to the animals, and blood samples were taken at t = 2 and 4 min. At t = 5 min, the animals were allowed to eat the meal (see above for the procedure). Additional blood samples were taken during eating (t = 7.5 and 10 min) and during early absorption (t = 12.5, 15, 20, 25, and 35 min).

Assays

Blood samples were immediately placed on ice in tubes containing EDTA for determination of immunoreactive insulin, glucose, and PP and in tubes containing glutathione and EGTa for catecholamine determination. Samples were centrifuged (20 min at 2,600 g and 4°C), whereafter the plasma was decanted and frozen at −70°C until assay.
Plasma insulin and PP concentrations were determined using RIAs described previously (6, 36). Plasma NE and Epi concentrations were determined in duplicate with a highly sensitive and specific radioenzymatic assay (10). The intra-and interassay coefficients of variation for the plasma catecholamine assay in this laboratory are 6 and 12%, respectively. Plasma glucose concentration was determined using a glucose oxidase technique.

Data Analysis
Pancreatic insulin and PP output were calculated using the formula

\[
\text{output} = ([\text{SPDV}] - [\text{art}]) \times \text{blood flow}_{\text{SPDV}} \times (1 - \text{hematocrit})
\]

where [SPDV] is SPDV plasma concentration, [art] is arterial plasma concentration, and blood flow_{SPDV} is SPDV blood flow.

Duodenal glucose absorption was calculated as

\[
\text{absorption} = ([\text{glucose}]_{\text{SPDV}} - [\text{glucose}]_{\text{art}}) \times \text{blood flow}_{\text{SPDV}} \times (1 - \text{hematocrit})
\]

where [glucose]_{SPDV} and [glucose]_{art} are the SPDV and arterial plasma glucose concentrations, respectively. PNESO was calculated by the formula

\[
\text{PNESO} = ([\text{NE}]_{\text{SPDV}} - (\text{arterial contribution})) \times \text{blood flow}_{\text{SPDV}} \times (1 - \text{hematocrit})
\]

where [NE]_{SPDV} is the plasma concentration of NE in the SPDV. The arterial contribution was calculated as [NE]_{art} \times ([\text{Epi}]_{\text{SPDV}}/[\text{Epi}]_{\text{art}}), as described by Dunning and colleagues (9), with [\text{Epi}]_{\text{SPDV}}/[\text{Epi}]_{\text{art}} being the actual fractional passage of Epi through the pancreas. The use of this term for the fractional passage of NE through the pancreas assumes that pancreatic extractions of Epi and NE are equivalent (1).

Data are expressed as means ± SE for experiment 1 and as mean change ± SE from baseline (delta (Δ)) obtained at t = -1 min for experiment 2.Wilcoxon's matched-pairs signed-rank test was used to test for significant changes from baseline at t = -1 min (experiment 1). ANOVA and the Mann-Whitney U-test were used to test for significant differences between responses in the control experiment and those during autonomic blockade (experiment 2). The level of significance was set at P < 0.05.

RESULTS

Experiment 1

Figure 1 shows that insulin output increased when food was shown to the animals, even though they were not yet allowed to eat. This increase was transient; insulin output had already returned to the baseline by t = 4 min. When the animals were allowed to eat (t = 5 min), insulin output increased again (+24.0 μU/min at t = 7.5 min). After this second transient increase, insulin output declined below the baseline (t = 12.5–15 min). Twenty minutes after the onset of the meal (t = 25 min), a third more modest but more sustained increase in insulin output was recognized (P < 0.05 at t = 2, 7.5, 12.5, 15, and 20–35 min).

The arterial insulin concentration increased when food was shown and increased further immediately after the onset of the meal before returning toward the baseline. Twenty minutes after the onset of the meal, arterial insulin increased again and remained elevated throughout the experiment (P < 0.05 at t = 2–12.5 and 20–35 min).

Duodenal glucose absorption was negligible before feeding and was not affected by the sight and smell of the food. Eating had no immediate effect on duodenal glucose absorption. However, duodenal glucose absorption increased significantly at t = 10 min, i.e., 5 min after the start of the meal, and was elevated throughout the remainder of the experiment. It is noteworthy that the increase in duodenal glucose absorption occurred after the second transient increase in insulin output, in fact when insulin output was declining.

The arterial glucose concentration increased progressively above the baseline level when the food was shown to the animals and later when the animals ate the food. Then it declined before another sustained increase occurred. The first increase of plasma glucose occurred before the increase of duodenal glucose absorption; the second increase of plasma glucose occurred during the increase of duodenal glucose absorption.

PP output was not significantly affected by the sight and smell of food (Fig. 2). However, eating the meal did initiate a large increase in PP output that reached a maximal value of 492 ± 128 ng/min at t = 7.5 min before it declined to 135 ± 31 ng/min at t = 15, still significantly above baseline. Thereafter, it increased...
modestly again and remained elevated for the duration of the experiment. After the start of the meal, the time course of PP output was similar to that of insulin output. Arterial PP showed a significant and sustained increase from \( t = 7.5 \) min throughout the remainder of the experiment.

PNESO was not significantly affected by the presentation of the food or immediately by eating the meal. However, at \( t = 10 \) min, PNESO had decreased below baseline and remained suppressed throughout the experiment.

Showing food did not significantly affect SPDV blood flow. However, during eating, SPDV blood flow increased significantly. Thereafter, it gradually declined to reach a value of 83 ± 13 ml/min at \( t = 25 \) min, still significantly above baseline (P < 0.05 at \( t = 7.5-35 \) min).

The arterial Epi concentration (Fig. 3) increased sharply when food was shown. Thereafter, it gradually declined to return to baseline at \( t = 12.5 \) min, where it remained for the rest of the experiment (P < 0.05 at \( t = 2-7.5 \) min). Arterial NE also increased when food was shown, but unlike Epi it increased further when the animals were allowed to eat (P < 0.05 at \( t = 2-10 \) min). NE reached its maximal value at \( t = 7.5 \) min (vs. \( t = 2 \) min for Epi). Thereafter, it declined to reach the baseline at \( t = 12.5 \) min. The time course of MAP was similar to that of NE; MAP increased when food was shown to the animals, increased further when the food was actually eaten, and reached its maximum at \( t = 7.5 \) min (P < 0.05 at \( t = 2, 7.5, \) and 10 min).

**Experiment 2**

The arterial insulin response to showing food to the animals was not reduced by muscarinic blockade (Fig. 4). In contrast, the arterial insulin response to the sight and smell of food was significantly reduced by \( \beta \)-adrenoceptor blockade and by combined muscarinic and \( \beta \)-adrenoceptor blockade.

Table 1. Baseline values for arterial hormone and glucose concentrations in Experiment 2 involving muscarinic blockade (methylatropine), \( \beta \)-adrenoceptor blockade (timolol), or combined muscarinic and \( \beta \)-adrenoceptor blockade (methylatropine + timolol)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Methylatropine</th>
<th>Timolol</th>
<th>Methylatropine + Timolol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin, mU/l</td>
<td>12.2 ± 1.1</td>
<td>10.4 ± 1.6</td>
<td>3.5 ± 0.5</td>
<td>5.6 ± 1.1</td>
</tr>
<tr>
<td>PP, ng/l</td>
<td>210 ± 42</td>
<td>104 ± 18</td>
<td>189 ± 48</td>
<td>98 ± 8</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>98.6 ± 1.9</td>
<td>98.6 ± 1.5</td>
<td>105.6 ± 2.7</td>
<td>100.7 ± 2.3</td>
</tr>
</tbody>
</table>

Values are averages ± SE obtained at time (t) = −1 min. PP, pancreatic polypeptide.
muscarinic blockade (Figs. 5 and 6, respectively; P < 0.05 at t = 2 and 4 min).

The immediate arterial insulin response to eating was significantly reduced, but not totally abolished, after either muscarinic blockade or β-adrenoceptor blockade (P < 0.05 at t = 5 min). Combined muscarinic and β-adrenoceptor blockade, however, totally prevented the eating-induced increase of arterial insulin (P < 0.05 at t = 5 and 7.5 min).

During the early absorptive phase (t = 10–35 min), the insulin response was not affected by β-adrenoceptor blockade (Fig. 5). Muscarinic blockade and combined muscarinic and β-adrenoceptor blockade prevented insulin from increasing during the early absorptive phase (P < 0.05 at t = 25 and 35 min).

No significant differences in the arterial PP responses were observed between the experiment with β-adrenoceptor blockade and the control experiment (Fig. 5). Muscarinic blockade with or without β-adrenoceptor blockade, on the contrary, totally prevented the responses in arterial PP to eating and during the early absorptive phase (P < 0.05 at t = 7.5–35 min).

In the experiment with muscarinic blockade (Fig. 4), the arterial blood glucose response during the early absorption phase was significantly decreased compared with that in the control experiment (P < 0.05 at t = 20–35 min). In the experiment with β-adrenoceptor blockade (Fig. 5) and in the experiment with combined β-adrenoceptor and muscarinic blockade (Fig. 6), the blood glucose response during eating and during the early absorptive phase was significantly increased compared with the glucose response in the control experiment (P < 0.05 at t = 7.5–15 and 25–35 min for the experiment with β-adrenoceptor blockade and P < 0.05 at t = 10–20 min for the experiment with combined blockade).

**DISCUSSION**

We have examined the meal-related insulin responses in dogs and have assessed which branch of the autonomic nervous system mediates these responses. We separated the insulin responses to a meal into the following three phases: 1) the response to the sight and smell of food (when the food was shown to the animals without allowing them to eat (t = 2 and 4 min)), 2) the immediate response to eating the meal (t = 7.5 and 10 min), and 3) the response to the early absorptive phase after eating (t = 12.5–35 min). We sought to characterize the autonomic contribution to each of these insulin responses. We found that showing food to the animals induced a rapid and transient increase in insulin secretion. Surprisingly, the data suggest that this re-
response is not mediated by either glucose or direct parasympathetic stimulation but rather by circulating Epi. When the animals ate the meal, a second rapid and transient insulin response occurred. The data suggest that this response is mediated by combined parasympathetic, muscarinic, and sympathetic $\beta_2$-adrenergic stimulation. During the early phase of absorption, a third, and now sustained insulin response occurred. Surprisingly, the data suggest that this response is mediated by parasympathetic stimulation rather than by glucose. Thus, in dogs, all three early insulin responses to a meal appear to be autonomically mediated.

Showing food to the dogs, without allowing them to eat, induced a rapid and transient insulin response. Such an early insulin response to the sight and smell of food has been observed in a variety of species, including humans (24, 28, 29, 42). It has been suggested to be mediated by a conditioned increase in parasympathetic activity (24, 29, 42). However, in the experiments presented here, OP output did not increase when food was shown, suggesting that vagal activity to the pancreas had not increased at that moment. This conclusion was confirmed by the failure of methylatropine to block or even reduce the arterial insulin response to showing food. Thus, in dogs, the insulin response to the sight and smell of food cannot be attributed to increased parasympathetic cholinergic stimulation.

Although the plasma glucose concentration rose slightly when food was shown, it is unlikely that this increase triggered the insulin response. First, at that time, there was no increase in glucose absorption from the duodenum (see Fig. 1). Second, the arterial insulin response to showing food was still present after muscarinic blockade, even though plasma glucose did not increase.

Another possible mediator of the insulin response to the sight and smell of food could be withdrawal of inhibitory sympathetic neural tone. Such tone is apparently present in rats, since $\alpha$-adrenergic blockade increases plasma insulin concentrations (3, 31). However, in the present experiments, PNESO, an index for sympathetic neural activity to the pancreas (9, 14, 15), did not decrease significantly when food was shown, suggesting that any inhibitory sympathetic neural tone was still present. Alternatively, because our measurement of NE spillover includes both pancreas and attached duodenum, one could argue that NE spillover from the proximal duodenum might have increased, masking a potential decrease of NE spillover from the pure pancreatic tissue. However, duodenal dilution of pancreatic blood is estimated at only 24% (9), making the above explanation unlikely.

Ruling out parasympathetic stimulation, withdrawal of sympathetic tone and stimulation by plasma glucose as the mediators of the insulin response to showing food leaves sympathoadrenal $\beta_2$-adrenoceptor (18) stimulation as the likely mediator. Arterial NE is increased in response to showing food, despite unchanged PNESO, indicating that the sympathetic nerves to other organs beside the pancreas, for example the heart, are activated. This observation confirms the poor relation between circulating NE and sympathetic drive to individual organs, in particular the pancreas (8). Arterial Epi is also increased in response to showing food, indicating that the adrenal branch of the sympathetic system was activated as well. Feeding-associated activity of the sympathoadrenal system has been reported for dogs (7) and for rats (30) and has been related to the increased energy expenditure noticed during the cephalic phase of feeding (7). A direct link to the stimulation of feeding-associated insulin secretion, however, has to the best of our knowledge not been made before. Still, we would argue that the increase in insulin secretion is due to $\beta_2$-adrenoceptor-mediated stimulation, with the arterial insulin response to the sight and smell of food being blocked by the $\beta$-adrenoceptor antagonist timolol, despite the higher arterial glucose concentration present during $\beta$-blockade.

The $\beta_2$-adrenoceptor-mediated stimulation of insulin secretion is most likely to be mediated via Epi rather than via NE, because $\beta_2$-adrenoceptors have a much higher affinity for Epi than for NE (17). This means that Epi at low concentrations, such as present in the current experiments, has profound $\beta_2$-adrenoceptor-stimulating properties and can stimulate insulin secretion (2). Epi is released primarily in situations having...
excitatory emotional components (12), a situation clearly reflected in the behavior of the animals when food was presented without allowing them to eat. β-Adrenoceptor-mediated stimulation of insulin secretion is suggested to prevent total α-adrenoceptor-mediated suppression of insulin release during sympathetic (neural) activity, thus securing a minimal release of insulin (35).

Eating induces a rapid and transient insulin response similar to that produced by just showing the food. This early insulin response to eating has been observed in a variety of species, including humans (4, 7, 11, 16, 30–35, 36, 39). Like the insulin response to showing food, the early response to eating has been attributed to a neural reflex, because it is also observed in response to sham feeding conditions (4, 5, 38) or taste stimulation (20, 21). This reflex has been associated with hypothalamically mediated parasympathetic stimulation of the pancreas (22). In rats, the insulin response to eating can be prevented by vagotomy (19) or atropinization (34), confirming the parasympathetic mediation in that species.

In dogs, the mechanism underlying the immediate increase in insulin during eating appears to be more complex. As in other species, there is activation of the parasympathetic nervous system, as indicated by a rapid increase of the arterial PP concentration. This parasympathetic activation does contribute to the meal-induced increase of the arterial insulin concentration, since methylatropine abolishes the PP response and reduces the insulin response to eating. However, some of the eating-induced insulin response remains during muscarinic blockade (see Fig. 4). This remaining insulin response is not due to a decrease in pancreatic sympathetic nervous activity, since PNESO dropped only after the insulin response to eating had already peaked. Rather, it is due to β-adrenergic stimulation of the β-cell, because the addition of β-adrenergic blockade to muscarinic blockade abolished the insulin response to eating. This total suppression of the eating-induced insulin response occurs despite the higher glucose levels present during combined blockade. This observation excludes the small increase in plasma glucose, which is observed immediately at the start of eating, as a mediator of the immediate insulin response. However, the present experiments do not rule out the alternative possibility that the effect of the small rise of plasma glucose to contribute to eating-induced insulin secretion is inhibited by the unopposed β-adrenergic effect of catecholamines during β-adrenergic blockade.

During eating in the control experiment, a small increase in glucose can be recognized, although glucose absorption has not yet increased. This small increase in glucose in the control experiment is probably mediated by direct or indirect (via glucagon) sympathoadrenal stimulation of hepatic glucose production (13). Epi, a potent stimulus for glucagon, is still increased during eating. The elevated glucose concentrations during eating after combined blockade were most likely allowed by the decrease in baseline insulin, resulting from the combined withdrawal of β-adrenoceptor and muscarinic stimulation of the β-cell, leaving unopposed α-adrenoceptor-mediated inhibition of insulin secretion.

After the immediate response to eating, insulin output fell rapidly below the baseline and then rose slowly. This slow rise occurred during increasing glucose absorption and is traditionally thought to be due to the rising arterial glucose level. However, the importance of the small increase in circulating glucose for stimulation of insulin secretion can be argued. Based on a vast number of in vitro studies, it is beyond dispute that glucose directly stimulates insulin secretion. However, it is also true that most of the in vitro studies challenged the β-cells with glucose concentrations that by far exceed the small increase of the arterial glucose concentration observed in the present study.

Alternatively, stimulation of insulin secretion during the early absorption phase could be due to parasympathetic stimulation of the β-cell, either directly or indirectly via interaction with gastrointestinal hormones, because PP remains elevated during this early absorptive phase. The blockade of the PP response and the lowering of the insulin level by methylatropine during this phase are consistent with this hypothesis. However, muscarinic blockade also lowers the glucose level, probably by impairing glucose absorption. Therefore, it is unclear from these data if it is the decrease in parasympathetic stimulation or the decreased glucose level that causes the decreased insulin response during early absorption in the experiments with muscarinic blockade. However, combined muscarinic and β-adrenoceptor blockade totally abolishes the insulin response during absorption, despite higher glucose levels (see Fig. 6). Therefore, it is our current opinion that the insulin response during absorption is mediated by increased parasympathetic activity rather than by the small rise in glucose level. One cannot exclude, however, that small increases in glucose may still play a role in potentiating the β-cell response to parasympathetic stimulation. Another possible contributor to this insulin response could be the withdrawal of inhibitory pancreatic sympathetic neural tone, since PNESO appears to be decreased throughout this time period (see Fig. 2).

Based upon these results, we conclude that, in the dog, 1) the insulin response to showing food is mediated by the β2-adrenergic effects of Epi; 2) the immediate insulin response to eating is mediated both by parasympathetic cholinergic stimulation and by the β2-adrenergic effects of Epi; and 3) the insulin response during early absorption is mediated via parasympathetic cholinergic stimulation, with a possible contribution of withdrawal of sympathetic neural tone.

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