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The vagus is inhibitory of insulin secretion under fasting conditions

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Blat, S., and C. H. Malbert. The vagus is inhibitory of insulin secretion under fasting conditions. *Am J Physiol Endocrinol Metab* 281: E782–E788, 2001.— The involvement of the vagus in the insulin response during the early phase of absorption of a meal has been demonstrated recently. The extent of this vagal influence was investigated during fasting in an anesthetized porcine model. Portal and systemic insulin were evaluated together with glycemia during cooling and sectioning of both cervical vagal trunks in 12 splanchnicotomized or sham-operated pigs. In sham-operated animals, portal and systemic insulin were significantly and reversibly increased by cooling (173 and 123%, respectively). Portal insulin peaked 20 min after the onset of cooling but declined slowly while cooling was still activated. In contrast, systemic insulin was increased evenly along cooling. Section of the vagus was also associated with a portal and systemic insulin increase (144 and 117%) but to a lesser extent than cooling. In both treatments, portal and systemic insulin increases were either reduced (vagal cooling) or eliminated (vagal section) in splanchnicotomized animals. We conclude that the vagus exerts an inhibitory activity on interdigestive insulin secretion that is partly mediated by the splanchnic nerves.

vagal cooling; splanchnicotomy; interdigestive state; insulin release

IT IS ASSUMED THAT INSULIN secretion is modulated by the vagus mainly during the so-called preabsorptive insulin response, i.e., during presentation and ingestion of food (5). The rationale for this arose from the suppression of the insulin early response in vagotomized compared with sham-operated animals (27, 29) and after muscarinic blockade (6). In contrast, the postabsorptive phase during which insulin outflow rises slowly is mostly the consequence of the increase in arterial glycemia and gastrointestinal hormone concentrations such as gastric inhibitory polypeptide (GIP) and glucagon-like peptide (GLP)-1. Recently, Bentham and co-workers (4) demonstrated that the vagus might also be involved in the postabsorptive phase of insulin secretion. Indeed the sustained insulin response during the early phase of absorption was mediated by parasympathetic stimulation rather than by glucose (4). This militates toward a reevaluation of the role of the para-

sympathetic nervous system in states other than the preabsorptive phase, such as the interdigestive state.

Surprisingly, vagotomy, the key experiment toward a better understanding of the parasympathetic control of insulin secretion, gave conflicting results. In anesthetized dogs, acute section of the cervical vagal trunks decreased portal insulin concentration, whereas blood glycemia remained constant (12). Furthermore, insulin concentration was decreased after acute pancreatic vagotomy in adrenalectomized rats (42). In contrast, basal insulin concentration was unchanged after chronic vagotomy in awake rats (16, 21, 46), dogs (18), monkeys (30), and humans (1, 41), but compensatory effects involving both the sympathetic nervous system and gut hormones cannot be ruled out.

To ascertain the actual role of the vagus toward basal insulin secretion, it is necessary to evaluate the reversibility of insulin changes after removal of vagal influences on the pancreas. Therefore, we performed a reversible cervical vagotomy using vagal cooling in anesthetized pigs. Splanchnicotomy was also performed in one-half of the animals to remove a possible inhibitory splanchnic influence on insulin secretion (3, 45).

METHODS

Experimental protocol. Twelve pigs (35 ± 3.0 kg) were used in this study. They were fasted for 18 h before the experiment. Surgical preparation of the animals was performed in the morning, and experimental procedures were performed in the afternoon. Six animals had their splanchnic innervation (splanchnicotomized group) removed, whereas a sham splanchnicotomy was performed in the six remaining animals (sham-operated group). Each subject underwent the following sequences: 1) 40 min of basal recording (*basal 1*), 2) cooling of the cervical vagal nerves for 1 h, 3) 1 h of return to basal, and 4) section of the vagus nerves and recording for 1 h. For the basal period before section, the last 40 min only immediately before section were taken into account in the analysis (*basal 2*). During the experiment, portal venous and arterial insulin concentrations were evaluated every 10 min. Arterial glycemia was evaluated every 5 min except during the first experimental period, during which the sampling interval was 10 min. Blood was collected in tubes containing

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heparin, samples were centrifuged at 3,000 *g* for 10 min at 4°C, and then plasma was stored at -20°C. One splanchnicotomized animal was removed from the experimental design because its portal insulin levels were not quantifiable.

Surgical procedure. The pigs were preanesthetized with an intramuscular injection of ketamine (12 mg/kg body wt; Rhône Mérieux, Lyon, France). Suppression of the pharyngo-tracheal reflex was achieved by inhalation of halothane by a facemask (5% vol/vol; Bélamont, Paris, France) immediately before intubation. A venous cannula was inserted in the marginal vein of the ear to infuse pentobarbital sodium (30 mg/kg body wt; Sanofi Santé Animale, Libourne, France), the primary anesthetic agent. The pigs were mechanically ventilated by a Siemens SAL 9000 ventilator with a tidal volume of 15 ml/kg at a respiratory rate of 18 breaths/min. Ventilation was adjusted to obtain normocapnia (end-tidal CO₂ pressure at 35–45 mmHg). Normocapnia was measured with the use of an infrared capnograph (Engström Eliza) with air sampling at the Y piece of the ventilator. Fractional inspired oxygen was adjusted between 20 and 100% using pulse oxymetry (partial oxygen pressure) data supplied by a sensor (Ohmeda, CO, pulse oxymeter) attached to the tail. A catheter inserted in the carotid artery was used to measure heart rate and mean blood pressures. These parameters were logged every 5 min during the whole experimental procedure. A surgical level of anesthesia was maintained throughout the experiment by continuous intravenous infusion of pentobarbital sodium (10 mg·kg⁻¹·h⁻¹). Body temperature was kept at 38.5 ± 0.5°C by a self-regulating heating element placed under the animal.

The abdomen was opened by a midline incision under aseptic conditions. A silicone catheter (Vygon, Ecouen, France) was placed in the portal vein according to a previously described procedure (39). A thoracotomy was performed in all animals at the level of the seventh to eighth intercostal spaces. Section of the thoracic splanchnic nerves (nervus splanchnicus major) was achieved in six animals (splanchnicotomized group), whereas the thoracotomy only was performed on the six remaining pigs (sham-operated group). The thoracic wall was closed afterward using a classical vacuum drainage procedure.

At the cervical level, the two vagal trunks were microdissected to remove the surrounding connective tissue and to split the sympathetic nerve (truncus sympathicus) from the vagus itself. The dissection was completed over 4 cm to allow easy placement of thermodes on the vagal trunks and to position the sympathetic nerve as far as possible from the thermodes.

At the end of the experiment, the animals were killed with an overdose of pentobarbital sodium (Merial, Lyon, France).

Vagal cooling. Two cooling jackets, consisting of a stainless steel tube (2.4 mm OD, 1.8 mm ID) forming a hook and incorporating a temperature probe (590 AD; Analog Devices), were placed around the left and the right cervical vagus. The active part of the thermodes was insulated from tissues surrounding the vagus (namely the sympathetic nerve) by silicon coating. The temperature of each vagal trunk was maintained at 2°C, a temperature inhibiting nerve transmission (13), by a computer-controlled (PID-controller, Labview, National Instrument) pump using -40°C methanol as coolant.

Analytic methods. Arterial whole blood glucose was monitored immediately after blood withdrawal with a glucometer (One-Touch II; Lifescan, Roissy, France).

Plasma portal and arterial systemic insulin concentrations were measured with a specific homologous double-antibody RIA using iodinated porcine insulin (INSULIN-CT; CisBio

International, Gif-sur-Yvette, France) as tracer and porcine monocomponent insulin (Novo Research Institute, Copenhagen, Denmark) for standard curves. Antiserum (Miles Scientific, Naperville, IL) was used at a final dilution of 1:150,000. The quantification limit was 3 μIU/ml, and the intra-assay coefficient of variation (CV) was <5% at 70 μIU/ml.

To quantify the expected weak variations in portal and systemic insulin concentrations during vagal cooling, a more sensitive assay was adapted from the previous method (37). Samples were assayed in triplicate instead of in duplicate. Antiserum final dilution was 1:250,000. Samples were left to incubate with the antiserum for 3 days instead of 1 day before addition of iodinated porcine insulin. Finally, the samples were rinsed two times before radioactivity counting. The quantification limit was lowered to 1 μIU/ml, and intra- and interassay CV were 2.5% at 10 μIU/ml, and interassay CV was 2.0% at 2.5 μIU/ml.

Statistical analysis. Differences in glycemia and in portal and systemic insulin concentrations between experimental treatments in sham-operated and splanchnicotomized animals were determined by two-way (sham section or section was the first factor, experimental treatment was the second factor) ANOVA using the global linear model (GLM) procedure of SAS (SAS 6.1; SAS Institute). The GLM procedure was preferred over the ANOVA procedure because of unequal cell size. Splanchnic innervation [sham section or section (splan)], experimental treatments [*basal period 1*, vagal cooling, *basal period 2*, and vagal section (treat)], and pigs were classification variables. The effect of experimental treatments was tested in each pig. Because the animals were either sham operated or splanchnicotomized, the effect of splanchnic innervation was tested between pigs against the residual variation. For continuous variables, the effect splan and/or the interaction splan times treat was significant ($P < 0.05$). As a consequence, sham-operated and splanchnicotomized animals were studied separately. Differences between treatments were determined by least-square contrasts, since the GLM procedure was used instead of the ANOVA procedure. Data were least square means (LSM) ± SE.

RESULTS

Splanchnicotomy induced a greater systemic but not portal insulin concentration compared with sham-operated animals (systemic insulin = 4.2 ± 0.24 vs. 2.8 ± 0.19 μIU/ml for splanchnicotomized vs. sham operated, $P = 0.0005$; portal insulin = 5.3 ± 0.78 vs. 5.9 ± 0.52 μIU/ml for splanchnicotomized vs. sham operated, $P = 0.827$; see Fig. 2). Similarly, glycemia of splanchnicotomized animals was significantly greater in animals that were sham operated (7.1 ± 0.07 vs. 5.5 ± 0.06 μIU/ml for splanchnicotomized vs. sham operated, $P = 0.0001$).

Vagal cooling. In sham-operated animals, vagal cooling significantly and reversibly enhanced portal (173%) and systemic (123%) insulin concentrations compared with the basal condition (*basal 1*). Immediately after the onset of cooling, portal and systemic insulin rose and peaked within 20–30 min, depending on the animal. Afterward, and while cooling was maintained, portal but not systemic insulin decreased slowly (Fig. 1). At the completion of the cooling procedure, portal insulin was significantly less than during cooling but was greater than during the basal period (5.9 ± 0.52, 10.2 ± 0.35, and 7.6 ± 0.34 μIU/ml before, during, and

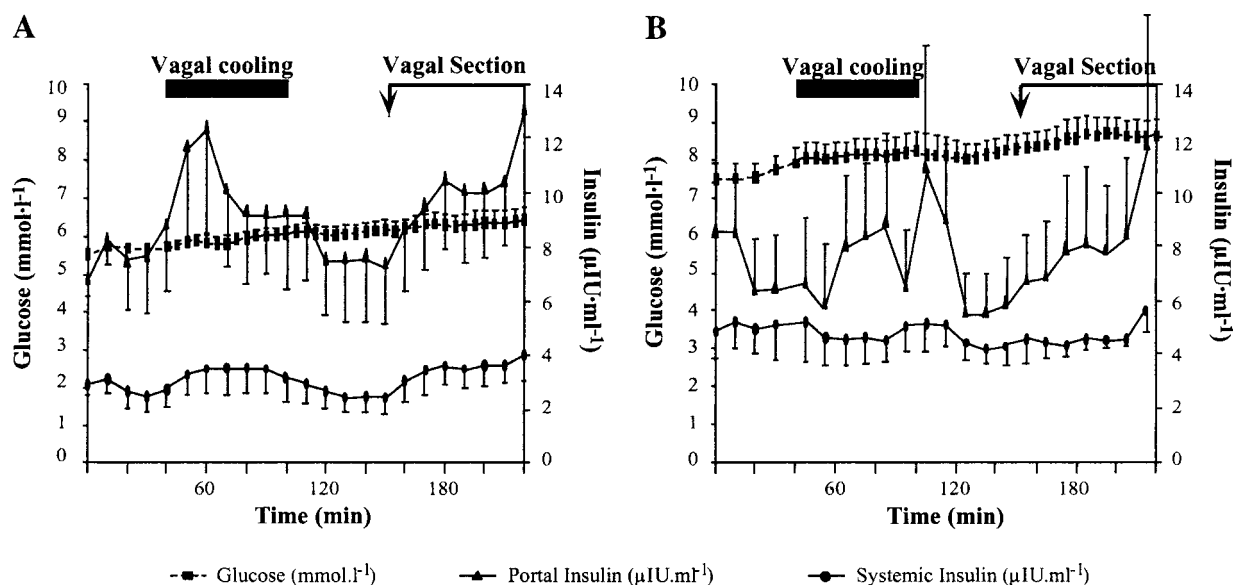


Fig. 1. Portal and systemic insulin and glucose responses to vagal cooling and section in sham-operated (A; $n = 6$) and splanchnicotomized (B; $n = 5$) pigs. Note that, in sham-operated pigs, portal insulin increased within 20 min after the onset of vagal cooling and decreased afterward while cooling was maintained. Vagal section induced a progressive increase in portal insulin. Values are least square means (LSM) \pm SE.

after cooling, respectively; Fig. 2A). In contrast, systemic insulin returned to basal values at the completion of cooling (2.8 ± 0.19 vs. 2.7 ± 0.13 μ IU/ml before vs. after cooling, $P = 0.229$, Fig. 2B). Glycemia increased steadily during cooling, a phenomenon that was not reversed while cooling was stopped (Fig. 2C).

In splanchnicotomized animals, vagal cooling significantly but not reversibly increased portal (144%) and systemic (117%) insulin. Furthermore, this increase was significantly less than in sham-operated animals. Neither portal (7.7 ± 0.61 vs. 7.6 ± 0.56 μ IU/ml during vs. after cooling, $P = 0.962$; Fig. 2A) nor systemic (4.9 ± 0.16 vs. 4.8 ± 0.15 μ IU/ml during vs. after cooling, $P = 0.467$; Fig. 2B) insulin decreased significantly at the completion of cooling. Glycemia was increased during cooling, a phenomenon that was not eliminated upon cooling completion (Fig. 2C).

Vagal cooling induced a significant reduction in mean arterial blood pressure and heart rate that was reversed to basal values within 10 min after the completion of cooling (Table 1). Once reduced, no changes in heart rate (± 4 beats/min) or in mean arterial pressure (± 10 mmHg) were observed during the entire cooling procedure.

Vagal section. In sham-operated animals, portal (7.6 ± 0.34 vs. 9.9 ± 0.34 μ IU/ml before vs. after section, $P = 0.0001$) and systemic (2.7 ± 0.13 vs. 3.6 ± 0.12 μ IU/ml before vs. after section, $P = 0.0001$) insulin concentrations were increased significantly after bilateral section of the vagal trunks. The rate of increase in portal but not systemic insulin was less than that observed during cooling. Although systemic insulin peaked at approximately the same time interval as for cooling, portal insulin went on increasing and did not show any biphasic behavior. Nevertheless, the mean insulin concentrations calculated over the 1-h

vagal cooling and over 1 h after acute vagal section were not significantly different (Fig. 2). Despite the steady increase in glycemia during the entire experiment, the difference in glycemia observed between vagal section vs. *basal 2* (0.341 mmol/l) was not significantly different ($P = 0.964$) from that observed between vagal cooling vs. *basal 1* (0.348 mmol/l).

Neither portal (7.6 ± 0.56 vs. 7.7 ± 0.54 μ IU/ml before vs. after section, $P = 0.921$; Fig. 2A) nor systemic (4.8 ± 0.15 vs. 4.6 ± 0.15 μ IU/ml before vs. after section, $P = 0.341$; Fig. 2B) insulin concentrations were modified after section of the vagus in splanchnicotomized animals compared with *basal 2* before section. Nevertheless, portal and systemic concentrations after section were not significantly different from those observed during vagal cooling. In contrast to sham-operated animals, glycemia changes induced by vagal section (0.266 mmol/l) were significantly less ($P = 0.0416$) than those triggered by vagal cooling.

DISCUSSION

This is the first demonstration of a vagal inhibitory influence on interdigestive insulin secretion mediated by the splanchnic nerves. During vagal cooling and after vagal section, portal and systemic insulin concentrations were significantly increased, a phenomenon that was reduced (vagal cooling) or canceled (vagal section) in splanchnicotomized animals.

Systemic but not portal insulin was increased by splanchnicotomy. This result is surprising since splanchnicotomy had no effect on fasting systemic insulin in dogs (18) or monkeys (30). In contrast, an increase in systemic insulin has been reported in splanchnicotomized adrenalectomized rats (3), which was thought to reflect an increased insulin secretion.

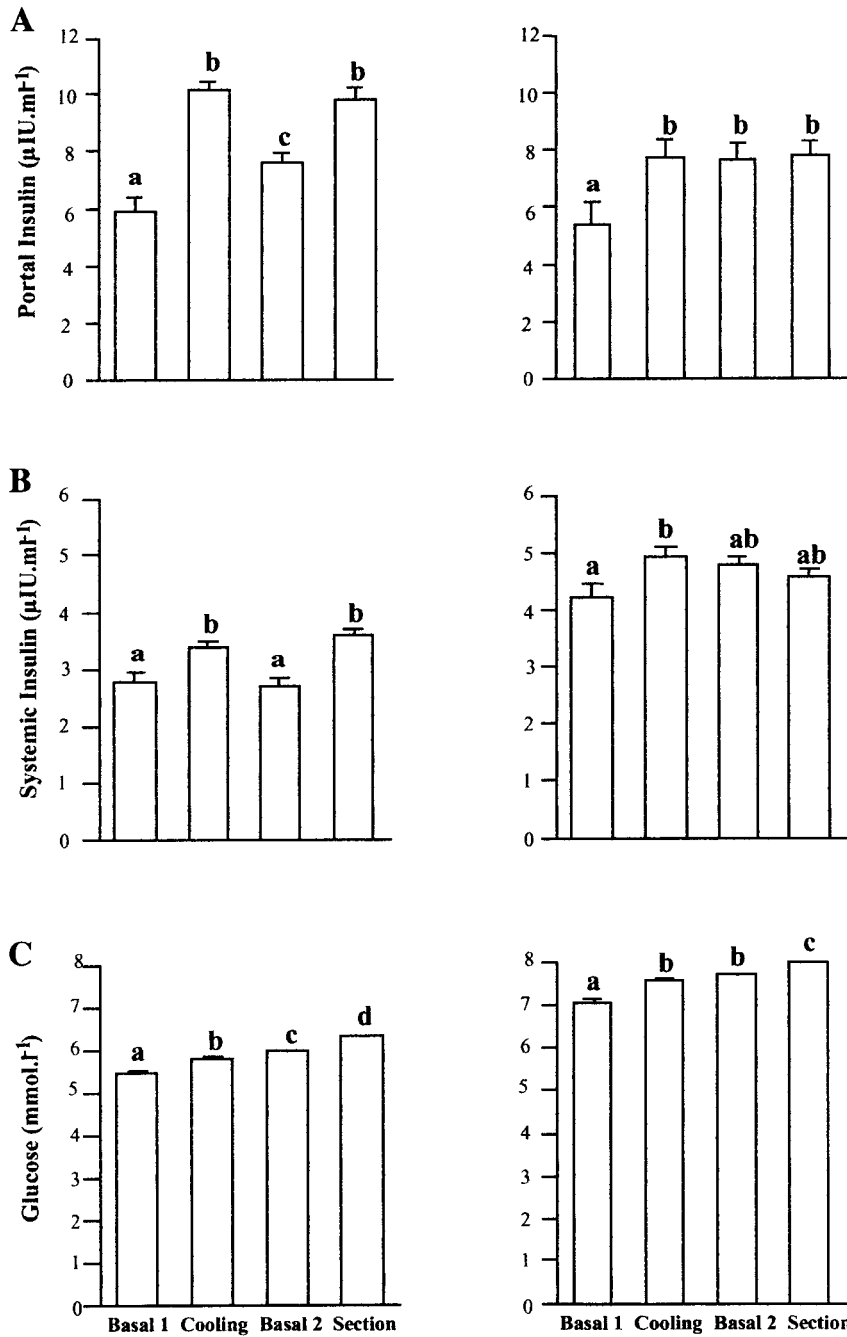


Fig. 2. Portal (A) and systemic (B) insulin concentrations and glycemia (C) before (*basal 1*), during, and after (*basal 2*) vagal cooling and after section of the cervical vagal nerves in sham-operated (*left*, $n = 6$) and splanchnicotomized (*right*, $n = 5$) pigs. *Basal 1* corresponded to a 40-min basal recording before cooling, whereas *basal 2* represented a period of equal duration immediately before section. Values are LSM \pm SE. Different letters indicate significant differences at $P < 0.05$.

However, because in pigs portal insulin was not modified by splanchnicotomy, the increased systemic insulin concentration cannot be explained by an increased insulin secretion. It is not likely the consequence of an increased peripheral resistance to insulin, since spinal cord-injured subjects showed only minor insulin resistance (23). Similarly, it is unlikely that this systemic increase in insulin reflects a decrease in hepatic insulin clearance. Indeed, similar insulin levels were observed during hyperglycemic clamp, whereas C-peptide was increased, suggesting an increased hepatic insulin clearance in liver-transplanted compared with normal subjects (36). In contrast, it is possible that the greater systemic insulin after splanchnicotomy was the conse-

quence of the significantly larger glycemia in splanchnicotomized animals. Indeed, insulin liver extraction is highly variable, ranging from 20 to 80%, and is regulated acutely by portal glucose (38). In an isolated perfused rat liver, glucose perfusion (300 mg/dl) significantly reduced hepatic insulin extraction compared with no glucose perfusion (20). The larger glycemia occurring after splanchnicotomy could therefore decrease insulin hepatic extraction, leading to the observed larger systemic insulin.

The increase in insulin secretion observed after both vagal cooling and vagal section in sham-operated animals is partially contradictory with others, since 1) chronic vagotomy is not accompanied by significant

Table 1. Effect of cervical vagal cooling and section on arterial pressure and heart rate

	Sham Operated		Splanchnicotomized	
	Mean Arterial Pressure, mmHg	Heart Rate, beats/min	Mean Arterial Pressure, mmHg	Heart Rate, beats/min
<i>Basal 1</i>	99.8 ± 1.77*	110 ± 2.2*	113.3 ± 2.65*	147 ± 1.4*
Vagal cooling	75.1 ± 1.32†	95 ± 1.6†	80.2 ± 1.87†	111 ± 1.1†
<i>Basal 2</i>	93.3 ± 1.38‡	99 ± 1.6‡	94.4 ± 1.87‡	120 ± 1.0‡
Vagal section	68.2 ± 1.32§	89 ± 1.4‡	74.4 ± 1.87§	99 ± 1.0§

Values are least square means ± SE; $n = 6$ sham-operated and 5 splanchnicotomized animals. *Basal 1* corresponded to 40-min basal recording before cooling, whereas *basal 2* represented a period of equal duration immediately before section. Different superscripts indicate a significant difference at $P < 0.05$.

changes in basal insulin concentration (18, 30) and 2) vagal stimulation triggers insulin secretion (19). However, it is possible that chronic vagotomy induced compensatory mechanisms that could mask a vagal inhibitory influence toward insulin secretion. GLP-1 is a strong candidate, since its secretion is controlled vagally (40). GIP could also be involved, although vagal regulation of GIP secretion is less obvious (14, 22, 31). On the other hand, peripheral vagal stimulation that mimics the meal-induced increase in insulin secretion recruits the efferent neurons only, whereas both afferent and efferent neurons are depressed during vagal cooling. Finally, because of the neuronal diversity present in the vagus, similar but not opposite effects have been elicited for gastrointestinal function by both stimulation and section in pigs (28).

Vagal cooling and vagal section increased glycemia in both sham-operated and splanchnicotomized animals. A similar increase after vagotomy has been previously reported in dogs (18), whereas others failed to identify such a phenomenon (12). This might relate to a low basal glycemia (4 mmol/l; see Ref. 8) compared with higher values (5.9 mmol/l in Ref. 13 and 5.5 and 7.0 mmol/l for sham-operated and splanchnicotomized, respectively, in our own study). This larger glycemia is a powerful stimulant for the tonic glycogenetic influence of the vagus toward the liver (25, 34). Because section of the vagus in these high-glycemia conditions suppressed this tonic influence, hepatic glycogen deposition was then reduced, and glucose release from liver was enhanced.

During vagal cooling, portal but not systemic insulin peaked and then was reduced ~30 min after the onset of the cooling procedure, while cooling was still effective on other physiological functions, namely cardiovascular ones. The partial escape of the islets from the removal of vagal innervation cannot be explained by a transient recovery of vagal conduction. Indeed, during the cooling procedure, both heart rate and mean arterial pressure were consistently and permanently lowered. Furthermore, it is unlikely that the reduction in insulin output during the late vagal cooling procedure was the consequence of the exhaustion of the insulin store present in the islets. Indeed, portal insulin con-

centration was only one-tenth of that observed after a meal or during vagal stimulation (19). Similarly, the reduction in insulin output is unlikely the result of vasomotor consequences of vagal cooling. Cold block of the cervical vagi increased the transpancreatic electrical conductance, indicating that vasodilatation had occurred (15).

The transient increase in insulin concentration observed during vagal cooling might be masked or amplified by the pulsatile nature of insulin secretion. Insulin is released cyclically in numerous species with a period of 5–10 min (44) and an amplitude in the range of 1 μ IU/ml (17, 24). However, this period is shorter than the duration of vagal cooling (1 h), and the amplitude of the pulses is smaller than the differences observed when the insulin peaked, i.e., 20–30 min after the onset of the cooling procedure.

There were differences in insulin secretion induced by vagal cooling vs. vagal section in sham-operated animals. The decrease in portal insulin while cooling was still active contrasted with the steady increase in portal insulin after section. The greater blood glucose before section compared with before cooling might be causative for this. Portal hyperglycemia reduces the vagal hepatic afferent activity (33) that is supposed to be responsible for insulin secretion through a depression of the inhibitory tone of sympathetic pancreatic efferents (9). Alternatively, vagal neurons with different thermal sensitivity toward vagal cooling have been demonstrated as follows: unmyelinated neurons are blocked at temperatures lower than for myelinated neurons, i.e., 2–4°C vs. 6–7°C, respectively (10, 11, 35, 43). However, unmyelinated neurons with lower blocking temperature, i.e., 0°C, have been described in the lung (7, 35). It is therefore possible that a temperature of 2°C left conduction for a minority of unmyelinated neurons.

With the use of an isolated perfused but innervated preparation in rats, a direct tonic inhibition of glucose-stimulated insulin secretion involving probably a vagal component has been suggested (8, 9). In contrast, a recent study demonstrated a vagal stimulation of insulin secretion during the early phase of meal absorption (4). Pancreatic polypeptide spillover was used in this study as an indicator for vagal efferent recruitment. Because pancreatic polypeptide secretion is under other regulatory mechanisms (2), this last result must be considered with caution. Using vagal cooling, we further validate the hypothesis of an inhibitory influence of the vagus on insulin secretion under fasting conditions. In both basal and glucose-induced insulin secretion, vagal inhibition could be the result of the following two mechanisms: 1) the vagus nerves are composed of efferent pancreatic neurons that inhibit insulin secretion; or 2) the vagus nerves contain afferent neurons that transmit information to the brain, which then reflexively inhibits insulin release via sympathetic efferents to the pancreas. Both mechanisms could also occur simultaneously. Our experiment allows validation of one of the former possibilities, since splanchnicotomy canceled the increase in insulin in-

duced by vagal cooling and section. Therefore, an inhibitory vagal input is driven to the pancreas by its sympathetic supply in the fasting state. The origin of the inhibitory vagal inputs cannot be determined from our experiment. However, it is possible that they originate from the liver. Indeed, hepatic inhibitory vagal inputs have been described extensively in the rat (26, 32, 33).

In conclusion, our results demonstrate a vagal inhibitory activity on fasting insulin secretion, mediated partly by the sympathetic nerves to the pancreas. Although this mechanism prevents hypoglycemia in the interdigestive state, its physiological relevance and intensity still remain to be investigated in other physiological and pathological conditions.

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