Parasympathetic inhibition of sympathetic neural activity to the pancreas

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Received 11 July 2000; accepted in final form 25 October 2000

Benthem, Lambertus, Thomas O. Mundinger, and Gerald J. Taborsky, Jr. Parasympathetic inhibition of sympathetic neural activity to the pancreas. Am J Physiol Endocrinol Metab 280: E378–E381, 2001.—The present study tested the hypothesis that activation of the parasympathetic nervous system could attenuate sympathetic activation to the pancreas. To test this hypothesis, we measured pancreatic norepinephrine (NE) spillover (PNESO) in anesthetized dogs during bilateral thoracic sympathetic nerve stimulation (SNS; 8 Hz, 1 ms, 10 mA, 10 min) with and without (randomized design) simultaneous bilateral cervical vagal nerve stimulation (VNS; 8 Hz, 1 ms, 10 mA, 10 min). During SNS alone, PNESO increased from the baseline of 431 ± 88 pg/min to an average of 5,137 ± 1,075 pg/min (P < 0.05) over the stimulation period. Simultaneous SNS and VNS resulted in a significantly (P < 0.01) decreased PNESO response [from 411 ± 61 to an average of 2,760 ± 1,005 pg/min (P < 0.05) over the stimulation period], compared with SNS alone. Arterial NE levels increased during SNS alone from 130 ± 11 to ∼600 pg/ml (P < 0.05); simultaneous SNS and VNS produced a significantly (P < 0.05) smaller response (142 ± 17 to 330 pg/ml). Muscarinic blockade could not prevent the effect of VNS from reducing the increase in PNESO or arterial NE in response to SNS. It is concluded that parasympathetic neural activity opposes sympathetic neural activity not only at the level of the islet but also at the level of the nerves. This neural inhibition is not mediated via muscarinic mechanisms.

Established concepts, it is usually assumed that the net effect of activation of both the parasympathetic and sympathetic inputs is simply the balance between these two opposing influences at the level of the β-cell.

Previous research by our group (3) revealed a decrease in pancreatic norepinephrine (NE) spillover (PNESO), an index of sympathetic activity to the pancreas, during the early absorptive phase of a meal. This decrease in PNESO was immediately preceded by an increase in the output of pancreatic polypeptide, an index of parasympathetic activity to the pancreas. The timing of these two changes of autonomic activity suggests that parasympathetic activation may inhibit sympathetic activity to the pancreas. Such a direct interaction between the two branches of the autonomic nervous system could be achieved in the integrative centers of the central nervous system. Alternatively, it could be achieved by direct parasympathetic inhibition of sympathetic activity to the pancreas, either at the level of the peripheral sympathetic ganglion or at the level of the peripheral nerve terminals, within the pancreas itself.

Such an interaction between the two branches of the autonomic nervous system has been described for the heart and for vascular smooth muscle [for an excellent review see Vanhoutte (12)]. In these tissues, acetylcholine, released from parasympathetic neurons in the vicinity of adrenergic nerve endings, exerts a profound inhibitory effect on the release of NE into the junctional cleft between the adrenergic nerve terminal and the effector cells (12).

To determine if peripheral parasympathetic activation can inhibit pancreatic sympathetic neural activity, we electrically stimulated the sympathetic nerves above the pancreas [bilateral thoracic sympathetic nerve stimulation (SNS)], alone or simultaneously with stimulation of the peripheral parasympathetic neural activity to the pancreas. To test this hypothesis, we measured pancreatic norepinephrine (NE) spillover (PNESO) in anesthetized dogs during bilateral thoracic sympathetic nerve stimulation (SNS), alone or simultaneously with stimulation of the peripheral parasympathetic neural activity to the pancreas. To test this hypothesis, we measured pancreatic norepinephrine (NE) spillover (PNESO) in anesthetized dogs during bilateral thoracic sympathetic nerve stimulation (SNS), alone or simultaneously with stimulation of the peripheral parasympathetic neural activity to the pancreas.

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To determine if peripheral parasympathetic activation can inhibit pancreatic sympathetic neural activity, we electrically stimulated the sympathetic nerves above the pancreas [bilateral thoracic sympathetic nerve stimulation (SNS)], alone or simultaneously with stimulation of the peripheral parasympathetic neural activity to the pancreas.
METHODS

All procedures were approved by the Seattle Veterans Affairs Puget Sound Health Care System Animal Use Committee. All surgical and experimental procedures were performed in the presence of full surgical anesthesia. These experiments were acute terminal procedures. At the conclusion of each day’s experimental protocol, the animals were euthanized with an overdose of an anesthetic, without regaining consciousness.

Animals and Surgical Preparation

After an overnight fast (18 h), adult dogs of mixed breed (28–34 kg) were anesthetized with the ultra short-acting barbiturate thiamylal sodium (thiopental; Park Davis, Morris Plains, NJ). Anesthesia was subsequently maintained with halothane (2.0%) in 100% oxygen, administered from a calibrated vaporizer (Draeger) by mechanical ventilation.

To access pancreatic venous blood, a laparotomy was performed, and an extra corporal Silastic shunt (Dow Corning, Midland, MI) containing a sampling port, an electromagnetic flow probe, and a heparin infusion line was placed between the superior pancreaticoduodenal vein (SPDV) and the portal vein (2). This procedure allows the measurement of hormone output and neurotransmitter spillover from the right lobe of the pancreas, which comprises 35–50% of the whole canine pancreas. This preparation also receives a portion of the venous drainage from the proximal duodenum. The femoral artery (FA) was cannulated for sampling of arterial blood.

To stimulate the sympathetic nerves supplying the upper gastrointestinal tract, bilateral thoracotomies were performed at the seventh intercostal space. The sympathetic trunks were dissected from surrounding tissue along the dorsal rib case, and a bipolar electrode (Harvard Apparatus, South Natick, MA) was placed on each nerve. The nerve trunks were then severed anterior to the electrodes to prevent retrograde stimulation. To stimulate the parasympathetic nerves, the right and left branches of the cervical vagi were isolated from the fascia adjacent to each common carotid artery (9). The vagal trunks were then cut between two ligatures, and the distal segment of each was connected to a bipolar electrode (Harvard Apparatus). Cutting the vagus rostral of the stimulation site prevents stimulation of vagal afferents. The nerve stimulation experiments began 1–1.5 h after the end of surgery.

Experimental Protocols

Experiment 1. In the first experiment (n = 12), the thoracic sympathetic nerves were electrically stimulated (bilateral thoracic SNS) twice for 10 min with square-wave pulses of 1 ms duration and 10 mA current at a frequency of 8 Hz. The stimulations were performed with a stimulator coupled to a stimulus isolation unit (models S-44 and PSIU 6, respectively, Grass Instrument, Quincy, MA). Stimulation parameters were monitored with an oscilloscope. Simultaneously to either the first or the second SNS (random design), the cervical vagal nerves were stimulated (bilateral cervical VNS: 10 min, 1 ms, 10 mA, 8 Hz). Paired blood samples were withdrawn from the FA and from the SPDV at time (t) = −1 min before the start of stimulation at t = 0, and at t = 5, 7.5, and 10 min during stimulation. A reequilibration period of 35 min was allowed between nerve stimulations.

Experiment 2. To test whether muscarinic mechanisms underlay the inhibitory effect of vagal stimulation on sympathetic activation, muscarinic blockade was introduced using atropine (0.25 mg/kg iv + 0.4 µg·kg⁻¹·min⁻¹ iv) after a first SNS (n = 4). This dose of atropine introduces near-total muscarinic blockade in well-anesthetized dogs (1). Twenty-five minutes after introduction of muscarinic blockade, a second SNS was performed. This second SNS was accompanied by a simultaneous VNS. To correct for effects introduced by the order in which stimulations were performed (e.g., fatigue of the nerve), the second SNS was followed by a third SNS without VNS but still in the presence of atropine. Atropine infusion was continued until after the third SNS.

The blood sampling schedule and the nerve stimulation parameters were similar to those of experiment 1.

Assays and Calculations

Blood samples were drawn into tubes containing EGTA and glutathione. Samples were placed on ice until centrifugation (20 min at 2,400 g and 4°C). The plasma was then decanted and frozen at −70°C until assayed. Plasma NE was determined in duplicate with a highly sensitive and specific radioenzymatic assay (7). PNESO was calculated by the formula: PNESO = [(NE)SPDV − (arterial contribution)·(bloodflow SPDV)·(1 − hematocrit)], where the arterial contribution was calculated as: [NE]arterial(1 − 0.75), 0.75 being the fractional extraction of NE by the pancreas as determined in anesthetized dogs (9).

Data are means ± SE. Wilcoxon’s matched-pairs signed rank test was used for comparison at any time with the baseline value at t = −2 min. ANOVA and the Mann-Whitney U-test were applied to determine between data obtained during SNS stimulation and data obtained during simultaneous SNS and VNS stimulation. The level of significance was set at P < 0.05.

RESULTS

Experiment 1

Bilateral thoracic SNS increased PNESO from the baseline of 431 ± 88 pg/min to an average of 5,137 ± 1,075 pg/min over the stimulation period (see Fig. 1; P < 0.01). Simultaneous SNS and bilateral cervical VNS resulted in an increase in PNESO from the baseline of 411 ± 61 pg/min to an average of 2,378 ± 445 pg/min over the stimulation period (P < 0.01). This PNESO response to simultaneous SNS and VNS stimulation was significantly smaller than the response to SNS alone (P < 0.05).

A pattern comparable to that of PNESO was observed for arterial NE. Arterial NE levels increased during SNS alone from 130 ± 11 pg/ml to an average of 578 ± 76 pg/ml (P < 0.01) over the stimulation period; during simultaneous SNS and VNS, arterial NE increased from a baseline of 142 ± 17 pg/ml to an average of 330 ± 56 pg/ml over the stimulation period (P <
The latter response was significantly ($P < 0.05$) smaller than the response during SNS alone.

**Experiment 2**

In the second experiment, muscarinic blockade was applied during simultaneous SNS and VNS to test whether the inhibiting effect of VNS on SNS is mediated by muscarinic receptors. In this series, SNS alone increased PNESO from $272 \pm 152$ pg/min to an average over the stimulation period of $2,585 \pm 1,224$ pg/min (see Fig. 2; $P = 0.07$; $n = 4$). Simultaneous SNS and VNS during muscarinic blockade induced an increase in PNESO from the baseline of $289 \pm 135$ pg/min to an average over the stimulation period of $551 \pm 252$ pg/min ($P = 0.06$, $n = 4$). The SNS following combined SNS and VNS, still during muscarinic blockade, did induce an increase in PNESO from the baseline of $228 \pm 132$ pg/min to an average over the stimulation period of $\sim1,700$ pg/min. The difference in the PNESO response between SNS alone and simultaneous SNS and VNS during muscarinic blockade was not statistically different from the difference between SNS alone and simultaneous SNS and VNS in experiment 1, i.e., without muscarinic blockade.

Arterial NE showed the same pattern as PNESO; its response to SNS was decreased during simultaneous VNS. Because PNESO contributes little to the arterial NE concentration (9), the reduced arterial NE response was not caused by a reduced PNESO. Rather, it means that the inhibitory effect of the parasympathetic activation was not restricted to the sympathetic nerves during the stimulation period. During the single SNS following combined SNS and VNS, still during muscarinic blockade, arterial NE increased from a baseline of $150 \pm 41$ pg/ml to an average of $338 \pm 105$ pg/ml ($P < 0.05$). The difference in the arterial NE response between SNS alone and simultaneous SNS and VNS during muscarinic blockade was not statistically different from the difference between SNS alone and simultaneous SNS and VNS in experiment 1, i.e., without muscarinic blockade.

**DISCUSSION**

The first set of experiments was designed to test whether increased parasympathetic activity could suppress sympathetic activity to the pancreas. To this end, we applied bilateral thoracic SNS above the pancreas either alone or simultaneously (random design) with bilateral cervical VNS. During stimulation, PNESO was measured as an index for sympathetic activity to the pancreas. We found that the PNESO response to SNS was significantly reduced during simultaneous VNS. This result clearly demonstrates that parasympathetic stimulation can modulate pancreatic sympathetic activity.

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that innervate the pancreas. Sympathetic nerves that innervate other abdominal organs, like for example the kidneys, which are also innervated by sympathetic fibers originating from the celiac ganglia (8,11), seem to be inhibited by parasympathetic activity as well. This phenomenon may not be restricted to the abdomen, because Levy and Blattberg (10) observed a reduction in NE overflow into coronary sinus blood during simultaneous stimulation of the stellate ganglion and the vagus nerve in anesthetized dogs, compared with stellate ganglion stimulation alone (10). Thus parasympathetic activation apparently can inhibit the sympathetic outflow to several organs, including, as demonstrated here, the pancreas.

After concluding that parasympathetic nervous activity can mediate sympathetic activity to the pancreas, we sought to explore the mechanism by which parasympathetic nervous activity suppresses pancreatic sympathetic nervous activity. Parasympathetic modulation of sympathetic activity has been described in heart and blood vessel walls (12). In these tissues, there are complex adrenergic-cholinergic prejunctional interactions, at the level of the autonomic nerve terminal, as well as postjunctionally, at the level of the responding cells themselves. The principal prejunctional interaction in heart and blood vessel walls appears to be an inhibition of the release of NE from adrenergic nerve terminals by the acetylcholine liberated from nearby cholinergic nerve endings (12). To test whether such an interaction could play a part in the autonomic control of pancreatic function, we applied muscarinic blockade before and during simultaneous stimulation of the stellate ganglion and the vagus nerve in anesthetized dogs, compared with vagus nerve stimulation alone (10). Thus parasympathetic activation apparently can inhibit the sympathetic outflow to several organs, including, as demonstrated here, the pancreas.

An alternative mechanism to account for the effects of parasympathetic nerve stimulation to impair sympathetic neural responses involves parasympathetic-sympathetic interactions at the level of the sympathetic ganglia. Berthoud and Powley (4, 5) provided anatomical evidence that parasympathetic fibers travel into the celiac ganglia of rats and synapse on sympathetic neurons. Others have demonstrated that sympathetic ganglia neurons are innervated by peptidergic fibers whose neurotransmitters have traditionally been associated with the parasympathetic nervous system (6). Based on those studies, one could hypothesize that stimulation of the cervical vagi might activate inhibitory parasympathetic fibers innervating prevertebral sympathetic ganglia, thereby suppressing sympathetic transmission across these ganglia, thus reducing sympathetic outflow.

With respect to the studies presented, we conclude that, in dogs, parasympathetic activity can inhibit the activity of sympathetic nerves to the pancreas. Surprisingly, this inhibition is not mediated by the classical neurotransmitter acetylcholine acting on muscarinic receptors.

The authors thank Richard Chang, Rix Kuester, Hong Nguyen, and Jira Wade for expert technical assistance.

This research was supported by the Medical Research Service of the Department of Veterans Affairs and by the National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-12829, DK-12047, and DK-50154. L. Benthem was supported by an Albert Renold Fellowship awarded by the European Association for the Study of Diabetes and by a fellowship grant from the Diabetes Foundation Netherlands.

REFERENCES