Influence of the Vagus Nerves on Pancreatic Insulin Secretion

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SUMMARY

The effect of short and long-term faradic stimulation of the vagus nerves on pancreatic immunoreactive insulin (IRI) release was assessed in dogs. In the present study stimulation of the vagus nerves at the level of the diaphragm or in the neck did not enhance pancreatic IRI release. The only significant effect was a transient fall in pancreatic vein IRI values immediately after stimulation was begun. DIABETES *16*:852-57, December, 1967.

The present investigation was prompted by conflicting reports regarding the effect of the vagus nerves on pancreatic insulin secretion. Although early workers almost universally found evidence of enhanced insulin secretion associated with vagus function,[1-4] later investigators autotransplanted the pancreas in experimental animals and demonstrated that innervation was not essential to normal or near normal glucose homeostasis.[5,6] Further studies showing that glucose tolerance was not significantly modified after vagotomy in either experimental animals[7] or man,[8,9] tended to negate the importance of vagal function on pancreatic insulin secretion. Recent investigations by Gellhorn,[10] Kuzuya,[11] and Charbon,[12] utilizing a variety of technics, including the measurement of plasma insulin-like activity, have supported the thesis that vagus nerve stimulation causes an increase in insulin release. Several authorities have accepted this concept of vagal influence on pancreatic endocrine function.[13-15]

The unsettled nature of the question concerning the role of the vagus nerves in insulin secretion is outlined by McLean[16] in a recent review of factors influencing glucose homeostasis. It was anticipated that utilization of a method for measuring immunoreactive insulin (IRI) would obviate the disadvantage of less specific technics which have been applied in the past, and help to resolve the controversy related to this aspect of vagus function.

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METHODS

Healthy adult mongrel dogs weighing between 10 and 22 kg. were fasted fourteen hours overnight before the experiments. Intravenous pentobarbital sodium anesthesia (30 mg./kg. body weight) was used in conjunction with endotracheal intubation and mechanical ventilation with room air using a Harvard respirator. The inferior vena cava was cannulated for repeated blood sampling by passing a polyvinyl catheter cephalad through either femoral vein. A femoral artery was cannulated to monitor arterial blood pressure. Laparotomy was performed, and the major venous channel from the right half of the pancreas was cannulated by passing a nonocclusive polyvinyl tube through the right gastroepiploic vein into the cranial pancreaticoduodenal vein. In order to prevent as far as possible reflux of blood from the portal vein, the tip of the catheter was positioned 1.5 cm. from the point where the gastroduodenal vein, which is the direct continuation of the cranial pancreaticoduodenal, enters the portal vein. Both vagus nerves were isolated from the esophagus just above the diaphragm through a left seventh interspace thoractomy. The animals were then studied after blood pressure, pulse, and respiration had been stable for fifteen minutes. Although every effort was made to minimize stress factors, and there were no significant fluctuations in blood pressure or pulse rate during the operative preparation of the individual dogs, some minor degree of initial shock may have occurred during the surgical manipulations.

In three dogs, the right (dorsal) vagus nerve was doubly ligated and severed between ligatures at the level of the diaphragm. Fifteen minutes later, a 10 volt faradic stimulus was applied to the distal segment of the cut right vagus nerve for ninety seconds with an induction stimulator (Harvard Apparatus Co., Dover, Mass.).

Simultaneous 1 ml. blood samples were obtained from the pancreatic vein and the inferior vena cava one minute before stimulation began and ten seconds before stimulation ended. This sequence of blood sampling and faradic stimulation was repeated three times in each animal at ten-minute intervals.

The effect of prolonged vagal stimulation was assessed in twelve animals. In ten dogs the right vagus nerve was ligated and severed as described. Fifteen minutes after the nerve had been severed, continuous 10 volt faradic stimulation was applied to the cut distal nerve for twenty minutes. Simultaneous 1 ml. blood samples were obtained from the pancreatic vein and the inferior vena cava one minute before stimulation began; at 1, 3, 5, 10, 15, and 20 mins. during the stimulation period, and at 5, 10, and 15 min. intervals after stimulation ceased. In two other animals, the 10 volt faradic current was applied to the cut end of the left (ventral) vagus nerve. Four control animals were prepared as described in the preceding group, except that no vagal stimulation was used. In these dogs 1 ml. blood samples were obtained from the pancreatic vein and the inferior vena cava at fifteen minute intervals for ninety minutes.

An additional group of four animals was studied as described by Frohman et al.[17] Thiopental anesthesia was used, but thoracotomy was not done. Blood samples were collected from the proximal portal vein rather than the pancreatic vein, and a 5 volt faradic stimulus was applied for ten minutes to the cut distal end of the right vagus nerve in the neck. Simultaneous 1 ml. portal vein and inferior vena cava blood samples were obtained after isolation of the vagus nerve in the neck; fifteen minutes after the nerve had been ligated and severed and immediately before stimulation was begun; at 1, 3, 5, and 10 mins. during stimulation, and five minutes after stimulation had been stopped. In two animals, a repeat ten-minute period of stimulation was applied fifteen minutes after all vagal fibers had been severed below the diaphragm through the laparotomy incision. The second sequence of blood sampling was the same as during the first stimulation.

All blood samples were collected in heparinized tubes. Protein-free filtrates for plasma glucose were made immediately after the blood samples had been obtained. Plasma glucose was determined by a semimicro glucose oxidase technic.[18] Plasma insulin was determined by a modification[19] of the Yalow and Berson radioimmunoassay procedure using insulin I-125 and separating free from bound insulin on Sephadex G-75.

In order to demonstrate the pancreatic insulin responsiveness of our animal preparation, five dogs (numbers 1,256, 162, 232, 233, and 226) were given glucose (0.5 gm./kg.) intravenously after the sampling sequence of the experiments described above had been completed. In each of these five dogs, a five-minute postglucose pancreatic vein plasma sample was assayed for IRI. The values were all higher than our highest standard (10 μU.) which, with the dilution factor, gave values in excess of 1,000 μU./ml. These values were all considerably higher than the pancreatic vein plasma IRI value determined just prior to the glucose injection in each animal (table 2).

RESULTS

Short-term faradic stimulation (ninety seconds) caused no consistent change in pancreatic vein IRI or inferior vena cava plasma glucose values (table 1).

TABLE 1

Inferior vena cava glucose and pancreatic vein insulin responses in three dogs subjected to repeated short-term (ninety-second) 10 volt faradic stimulation of the right vagus nerve at level of the diaphragm

Dog			Before stimulation	During stimulation‡
	First Stimulation	Glucose	128*	140
		Insulin	444†	348
199	Second Stimulation§	Glucose	143	139
		Insulin	372	426
	Third Stimulation	Glucose	144	139
		Insulin	480	432
	First Stimulation	Glucose	92	91
		Insulin	240	136
181	Second Stimulation	Glucose	103	99
		Insulin	120	112
	Third Stimulation	Glucose	107	104
		Insulin	144	112
	First Stimulation	Glucose	114	125
		Insulin	126	264
275	Second Stimulation	Glucose	121	120
		Insulin	180	120
	Third Stimulation	Glucose	129	137
		Insulin	90	102

*mg./100 ml.
†μU./ml.
‡Samples obtained ten seconds before end of ninety-second stimulation.
§Stimulation periods repeated at ten-minute intervals.

There was no significant change in pancreatic vein IRI or inferior vena cava plasma glucose during or after prolonged vagal stimulation (twenty minutes) except for a transient decrease after stimulation had begun (table 2, figure 1).

TABLE 2

Inferior vena cava glucose and pancreatic vein insulin responses in twelve dogs subjected to long-term (twenty-minutes) 10 volt faradic stimulation of the right or left vagus nerve at the level of the diaphragm

Dog	Vagus stimulated		Baseline	During stimulation 1	3	5	10	15	20 minutes	Post stimulation 5	10	15 minutes
1,220	Right	Glucose	132*	132	119	124	131	124	119	127	128	128
		Insulin	305†	228	198	168	198	264	240	492	528	618
1,224	Right	Glucose	114	111	104	104	106	107	106	113	106	106
		Insulin	204	165	84	63	150	—	63	—	—	96
273	Right	Glucose	109	107	107	107	92	98	101	101	106	108
		Insulin	81	36	41	48	24	36	—	168	192	222
277	Right	Glucose	97	90	90	82	87	88	94	100	101	105
		Insulin	51	141	132	102	168	168	—	25	60	59
1,256	Right	Glucose	127	135	133	131	131	135	136	142	135	129
		Insulin	428	408	384	392	576	736	472	664	788	—
162	Right	Glucose	120	124	126	125	125	127	126	124	136	140
		Insulin	108	192	96	144	214	222	330	42	114	234
232	Right	Glucose	92	92	95	99	92	99	99	100	97	92
		Insulin	225	132	—	120	—	252	264	234	141	63
233	Right	Glucose	95	92	94	90	97	97	95	98	105	99
		Insulin	354	186	198	228	246	306	306	222	—	264
226	Right	Glucose	101	104	104	99	102	98	101	106	119	106
		Insulin	168	180	156	180	270	270	342	624	324	—
2,158	Right	Glucose	152	153	147	141	144	141	143	141	138	144
		Insulin	978	828	870	552	888	888	960	580	876	—
209	Left	Glucose	95	101	98	96	96	97	103	99	100	101
		Insulin	210	168	300	168	165	300	243	228	288	300
2,156	Left	Glucose	94	90	90	93	94	92	88	92	90	94
		Insulin	126	78	90	78	144	—	156	114	252	—

*mg./100 ml.
†μU./ml.

In four control animals that underwent sham operations without vagal stimulation, there were no significant changes except that late in the study period, both the inferior vena cava glucose and pancreatic vein IRI values rose slightly (table 3, figure 2).

In the last series of experiments using thiopental anesthesia and faradic stimulation of the vagus nerve in the neck as described by Frohman et al.,[17] no significant change in either portal vein IRI or inferior vena cava glucose during vagus stimulation was observed (table 4). Portal vein IRI values are lower than those found in the pancreatic vein, presumably because of the admixture of venous blood from portal areas aside from the pancreas.

DISCUSSION

In the present study vagal activity did not enhance pancreatic insulin release. A slight and transient fall in pancreatic vein IRI shortly after the initiation of faradic vagus stimulation was the only significant change noted in these studies.

In this report emphasis has been placed on discerning

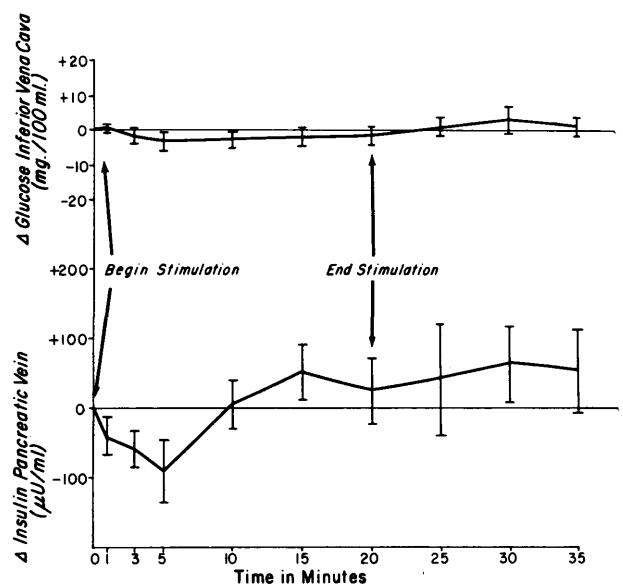

FIG. 1. Inferior vena cava glucose and pancreatic vein immunoreactive insulin responses in ten dogs subjected to prolonged 10 volt faradic stimulation of the right vagus nerve at the level of the diaphragm. Values represent mean ± S.E.M.

TABLE 3

Inferior vena cava glucose and pancreatic vein insulin responses in four dogs subjected to sham operation without vagal stimulation

Dog		Baseline	15	30	45	60	90
				minutes			
1,107	Glucose	106*	112	131	132	120	128
	Insulin	176†	176	192	352	288	432
1,104	Glucose	127	138	147	—	144	162
	Insulin	96	120	130	—	130	272
1,115	Glucose	103	114	109	109	123	129
	Insulin	96	92	160	184	216	—
1,122	Glucose	119	116	123	119	113	139
	Insulin	280	160	208	176	240	282

*mg./100 ml.
†μU./ml.

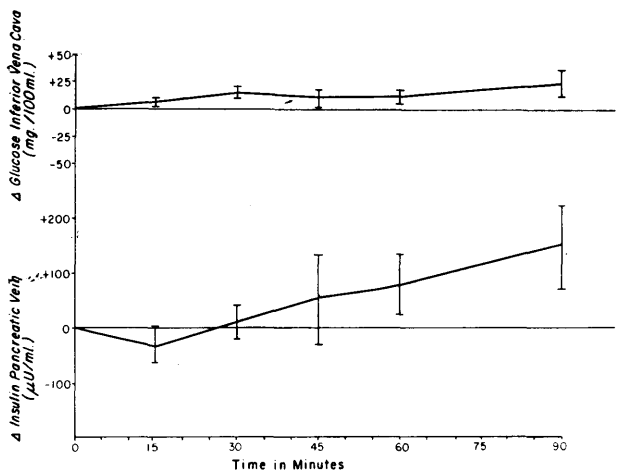

FIG. 2. Inferior vena cava glucose and pancreatic vein immunoreactive insulin responses in four sham-operated dogs without vagal stimulation. Values represent mean ± S.E.M.

the effects of stimulation of the right vagus nerve on pancreatic IRI secretion. In dog, as in man, the grossly demonstrable vagal fibers which reach the pancreas come from the right vagus nerve.[20] We have confirmed this in dogs by postmortem dissections.

In all but one group of animals we chose to stimulate the distal end of the severed vagus nerve at the level of the diaphragm. This obviated the marked cardiorespiratory response to stimulation of either vagus nerve in the neck. An example of this effect can be seen in the characteristic blood pressure, pulse, and respiration changes of a dog recorded on an E&M Physiograph recorder in association with a 5 volt faradic stimulation of the distal cut end of the right vagus nerve in the neck (figure 3). Although such a marked disruption in respiratory and cardiovascular function might indirectly affect pancreatic endocrine function, we failed to find consistent changes in portal vein IRI using this technic

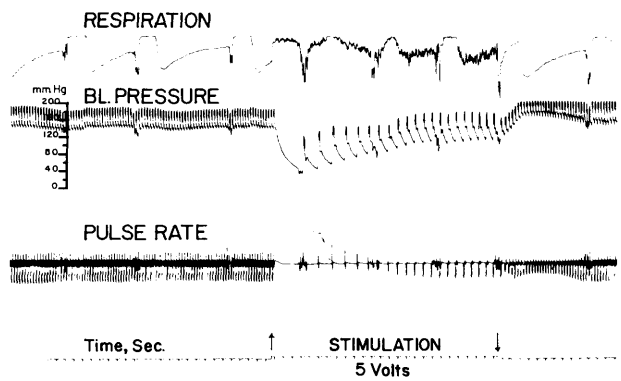

FIG. 3 Respiration, blood pressure and pulse rate responses in a dog subjected to 5 volt faradic stimulation of the right vagus nerve in the neck.

TABLE 4

Inferior vena cava glucose and portal vein insulin responses in four dogs subjected to 5 volt faradic stimulation of the right vagus nerve in the neck for ten minutes. In two dogs values are also shown during repeat ten-minute stimulation periods.

Dog		Vagus intact	Vagus cut in neck	During stimulation 1	3	5	10	5-min. post stimulation	Vagi cut at diaphragm	During stimulation 1	3	5	10	5-min. post stimulation
				minutes						minutes				
4,198	Glucose	90*	88	90	94	88	92	95	—	—	—	—	—	—
	Insulin	34†	44	14	42	50	50	44	—	—	—	—	—	—
4,211	Glucose	143	114	110	116	119	138	129	—	—	—	—	—	—
	Insulin	60	46	72	64	48	84	72	—	—	—	—	—	—
2,173	Glucose	83	88	85	83	89	84	92	98	95	96	91	100	103
	Insulin	32	33	36	34	38	32	32	32	28	37	39	36	44
4,236	Glucose	129	123	119	119	124	129	122	138	131	136	136	130	143
	Insulin	18	15	8	12	14	17	10	16	4	8	13	18	20

*mg./100 ml.
†μU./ml.

of vagal stimulation.

We can only speculate why other investigators have found vagus stimulation to augment insulin secretion. Many previous studies have sought to evaluate the role of the vagus nerves on pancreatic insulin release by assessing changes in blood glucose or in glucose tolerance after vagus section.[3,21-24] In one instance, anesthetic drugs now known to affect glucose metabolism were used to facilitate sample collection in the experimental animals.[1] Later investigators carried out studies which incorporated the measurement of plasma insulin-like activity.[10,11] It is possible that their results were influenced by the lack of specificity of the insulin assay. In addition, since many previous experiments have not specifically been designed to keep stress at a minimum, the recently recognized inhibiting effects of the catecholamines on insulin release might have influenced the findings.[25,26] The demonstration by Porte et al.[25] of a rebound increased insulin release following the fall of elevated catecholamine values is especially noteworthy in this respect.

Although the findings reported in this study negate the importance of the vagus nerves in mediating insulin secretion from the pancreas, there was a constant early fall in pancreatic vein IRI after initiation of vagal stimulation. The explanation for this is not known, but it is possible that it results from an indirect humoral response to vagus activity. Stimulation of the vagus nerves releases acetylcholine, which mediates the release of epinephrine. Increased circulating epinephrine would in turn suppress insulin release. The transient nature of this effect can be explained on the basis of exhaustion of acetylcholine stores at the peripheral endings of the vagus nerve with continued stimulation. Whether acetylcholine is too rapidly inactivated in the circulation to cause this effect is a point which also must be considered. It also is possible that a direct effect of vagal stimulation on pancreatic blood flow could account for our findings.

The gradual rise in plasma glucose and plasma IRI in the later stages of the long-term studies does not appear to be a specific result of any experimental manipulation. We believe this represents a nonspecific response to prolonged anesthesia and operative trauma.

Since stimulation of the sympathetic nervous system tends to raise blood sugar, a counterbalancing effect to lower blood sugar would be expected from parasympathetic activity. The stimulation of glycogenolysis and the inhibition of insulin release by catecholamines illustrate the role of the sympathetic nervous system in elevating blood sugar, but no such well-documented effect of parasympathetic function to lower blood glucose is known. To the contrary, one of the well-known effects of hypoglycemia is the activation of parasympathetic vagal activity. The Hollander insulin-hypoglycemia test[27] for completeness of vagotomy is based on this observation. During the Hollander test, insulin hypoglycemia stimulates vagus nerve activity resulting in an increase in gastric acid secretion if gastric vagal fibers are intact. Since insulin hypoglycemia stimulates vagus function, it seems inappropriate for vagal activity to augment insulin release further and thus perpetuate the hypoglycemic state.

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