Effect of Vagotomy and Vagal Stimulation on Insulin Secretion

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SUMMARY

The influence of the vagus nerve on insulin secretion in the dog has been confirmed by studies involving both vagotomy and vagal stimulation. Following vagotomy, a fall in portal vein insulin levels occurs. Following stimulation of both the right and left vagus, insulin levels rise abruptly, peak within five minutes and rapidly return to baseline levels. A small rise in blood glucose, which occurs simultaneously, cannot be blocked by phentolamine, an alpha-adrenergic blocking agent. When given alone, phentolamine stimulates insulin secretion. Atropine inhibits both the glucose and insulin rises following vagal stimulation. Only a portion of releasable insulin appears to be under vagal control. Glucose mediated insulin release and net glucose utilization are not significantly affected by vagotomy. DIABETES *16*:443-48, July, 1967.

The role of the nervous system in the control of blood glucose and of carbohydrate metabolism has been recognized for over 100 years, ever since Claude Bernard demonstrated the phenomenon of piqûre hyperglycemia by stimulating the floor of the fourth ventricle of the dog and producing glycosuria.[1] More recent studies have suggested that certain nuclei of the ventral hypothalamus are also connected with the regulation of blood glucose inasmuch as lesions in this area produced either by electrical or chemical methods result in an obesity associated with hyperglycemia.[2,3]

In the study of the precise pathways by which the central nervous system mediates its influence, the importance of the autonomic nervous system has been long recognized.[4] The role of the sympathetic nervous system is that of producing an increase in blood glucose, and the effects appear to be mediated principally by epinephrine. Interference with the integrity of the sympathetic nervous system by cordotomy[5] or by adrenal denervation[4] results in an impaired recovery from insulin induced hypoglycemia. In a parallel fashion, sympathetic blocking agents impair the metabolic effects attributable to infused epinephrine.[6]

The role of the parasympathetic nervous system has been less well defined. Nearly fifty years ago, a series of histologic studies from different laboratories[7,8] described fibers from the right vagus nerve innervating the pancreas in man, dog, cat and rabbit. Plexuses of such fibers surround the islets, and filaments from these peri-insular networks penetrate the islets and follow the vessels between the cells. No distinction was made between the various types of islet cells in these studies.

In 1927 LaBarre,[9] using the method of pancreatoduodenal-jugular anastomosis in the dog, demonstrated a fall in the blood glucose of the recipient following electrical stimulation of the right vagus nerve of the donor. The period of stimulation and transfusion was fairly prolonged, but the onset of hypoglycemia was not noted until an hour after the onset of stimulation, thus raising the question whether factors other than insulin are involved in the production of hypoglycemia.

More recently, vagus nerve hyperactivity has been mentioned as a possible cause for presumed hyperinsulinemic states such as the reactive hypoglycemia following glucose loading.[10] Yet, the relationship of the vagus nerve to insulin secretion has remained somewhat ambiguous. The present studies were carried out to clarify the role of the vagus nerve on insulin secretion and on the control of blood glucose.

METHODS

The studies were performed on healthy mongrel dogs, weighing from 14 to 23 kg., which had been fasted overnight. Under pentobarbital anesthesia, indwelling catheters were placed in the femoral artery and in the portal vein, beyond the entrance of the pancreatoduodenal vein. Electrocardiographic and femoral arterial blood pressure recordings were obtained throughout the experimental period.

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Plasma insulin was measured on heparinized samples from the portal vein and from the femoral artery by a radioimmunoassay procedure using I-125-insulin[11] and a double antibody precipitation system.[12] Owing to a lack of calibrated source of dog insulin, the values reported are in terms of pork insulin. Blood glucose measurements were made from arterial samples on an Auto-Analyzer (Technicon), using a modification of the ferricyanide method.[13] Insulin-like activity was assayed by the rat epididymal fat pad method.[14] Electrical stimulation was performed from sixty to ninety minutes after vagotomy using direct electrode contact with the tissue to be stimulated. A direct current square wave pulse of 8 milliseconds duration, 5 volt potential, and 60 pulses per second was used for the initial studies. This stimulus generally produced a current of 3mA, and in subsequent studies a constant 3mA current was delivered to the vagus. This required a slight variation in voltage ranging from 4 to 6 volts depending on tissue resistance.

Net glucose utilization was assessed by calculation of the glucose disappearance rate (K) using blood glucose values obtained between ten and forty minutes after intravenous glucose loading.

Insulin secretion following glucose loading was obtained by calculating the area under the femoral artery plasma insulin concentration curve during the first fifteen minutes following glucose loading and is expressed as microunit minutes/ml.

RESULTS

Effect of vagotomy on plasma insulin and blood glucose. Bilateral cervical vagotomy was performed from thirty to sixty minutes after the vagi had been exposed and isolated so as to avoid any stimulation which might occur as a result of the isolation procedure. As illustrated in figure 1, a decline in portal vein plasma insulin levels was observed within fifteen minutes of vagotomy, and this depression persisted for an hour. The mean insulin level prior to vagotomy was 23 μU./ml., and a significant fall was demonstrable at fifteen ($p < .001$), thirty ($p < .01$), and sixty ($p < .05$) minutes. The mean blood glucose prior to vagotomy was 73 mg. per 100 ml. and was unaffected by vagotomy.

Effect of vagal stimulation on plasma insulin and blood glucose. The response to stimulation of the distal segment of the transected vagus for fifteen minutes is shown in figure 2. The mean portal venous insulin rose from 23 μU./ml. to 141 μU./ml. at five minutes and then fell toward prestimulatory levels despite the

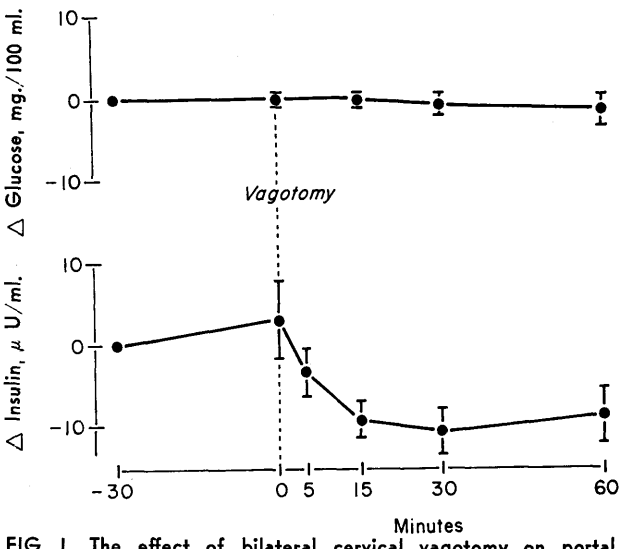

FIG. 1. The effect of bilateral cervical vagotomy on portal venous insulin and arterial glucose levels. Mean values ± S.E.M. are shown (n = 15).

persistence of the stimulation. In a small number of experiments not included in the figure, portal venous samples taken one minute after the onset of stimulation revealed elevated insulin levels, and samples taken at two minutes contained insulin levels nearly twice those observed at five minutes. Thirty minutes following the

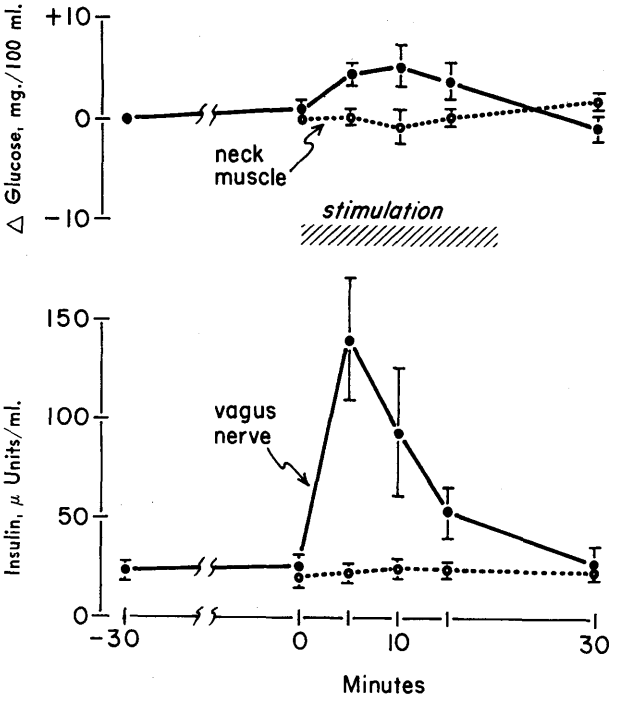

FIG. 2. The effect of vagal (solid line) and neck muscle (broken line) stimulation on portal venous insulin and arterial glucose. Mean values ± S.E.M. are shown (n = 8).

onset of stimulation, the mean plasma insulin was at the resting level. Changes in femoral arterial insulin levels were not as great as in portal venous samples but generally reached values from one fourth to one third those observed in the portal samples at five minutes.

A similar type of stimulation was applied to the exposed cervical muscles adjacent to the vagus as a control. There was no change in portal venous insulin during or after this stimulation. The differences in insulin values between vagal and cervical muscle stimulation at five, ten, and fifteen minutes are all highly significant ($p < .01$).

Vagal stimulation was accompanied by a small rise in arterial glucose concentration, from five through fifteen minutes following onset of stimulation. The mean maximal rise was 5.3 mg. per 100 ml. at ten minutes. The glucose values following vagal stimulation compared to those following cervical muscle stimulation at five, ten, and fifteen minutes are all highly significant ($p < .01$).

Comparison of effects of left vs right, and of repeated vagal stimulation on insulin secretion. A series of four experiments were performed in which stimulation of one vagus nerve was followed, after a sixty-minute interval, by stimulation of the contralateral vagus. In two experiments the right vagus was stimulated first and, in the other two, the order was reversed. The combined data from the four experiments revealed no difference in effects of right vs left vagal stimulation. The responses in individual animals could not be compared, however, because of the diminished response observed with the second stimulation as compared to the first as shown in figure 3. The mean portal venous insulin five minutes after the first stimulation was 196 μU./ml. whereas after the second stimulation it was only 85 μU./ml. ($p < .05$). This diminished response to the second stimulation occurred regardless of which vagus was stimulated first and despite a sixty-minute interval between stimulations. Sixty minutes following the second stimulation, glucagon (0.2-1.0 mcg./kg., intravenously) elicited a greater rise in portal venous insulin (mean = 168 μU./ml.) than that observed during the second stimulation ($p < .01$).

Comparison of portal venous plasma insulin following vagal stimulation and following glucose. Two samples of portal venous plasma, one obtained following vagal stimulation and one following intravenous glucose loading, were assayed at four different dilutions to check for evidence of possible immunologic dissimilarity. Both plasmas had previously been shown to contain

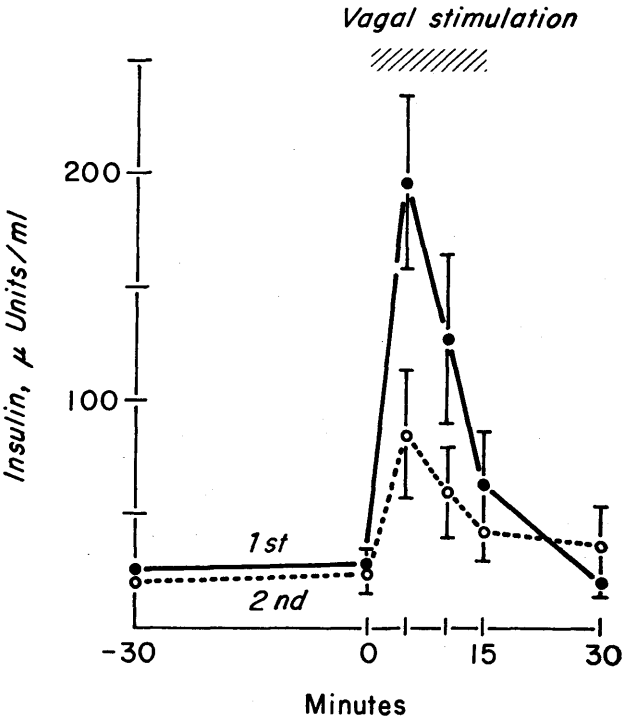

FIG. 3. Comparison of first (solid line) and second (broken line) vagal stimulation on portal venous insulin. Mean values ± S.E.M. are shown (n = 4). In each of four experiments the second stimulation was applied to the contralateral vagus. In two experiments the right vagus was stimulated first and in the other two, the order was reversed.

170 μU./ml. As illustrated in figure 4, the dilution curves of the two samples were identical.

Another pair of portal vein plasma samples obtained under similar conditions were assayed for insulin-like activity by the rat epididymal fat pad method. The plasma samples chosen had been previously shown to contain almost identical insulin values by radioimmunoassay, as shown in table 1. The bioassay values for insulin were also similar to one another.

Effect of parasympathetic and sympathetic blocking agents on the glucose and insulin changes following vagal stimulation. The effect of parasympathetic blockade was studied by giving atropine (2 mg., intravenously) to a group of dogs prior to a second vagal

TABLE 1
Comparison of insulin immunoassay and bioassay on plasma obtained after glucose and after vagal stimulation

	Insulin (μU./ml.)	
	Immunoassay	Bioassay
Intravenous glucose	172	702
Vagal stimulation	170	968

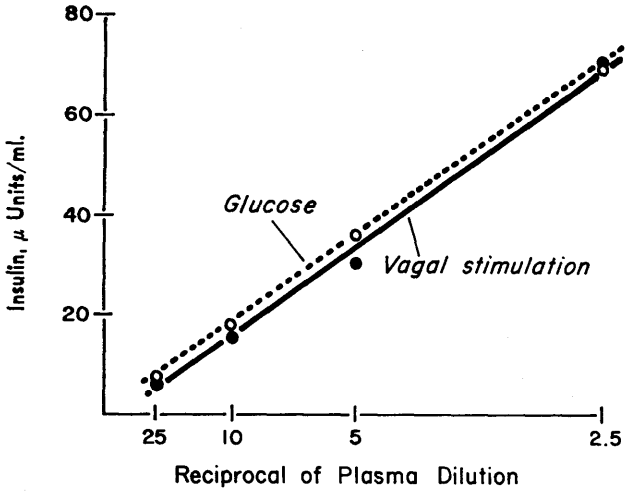

FIG. 4. Comparison of the effects of dilution on the insulin radioimmunoassay of portal venous plasma obtained after glucose (broken line) and after vagal (solid line) stimulation.

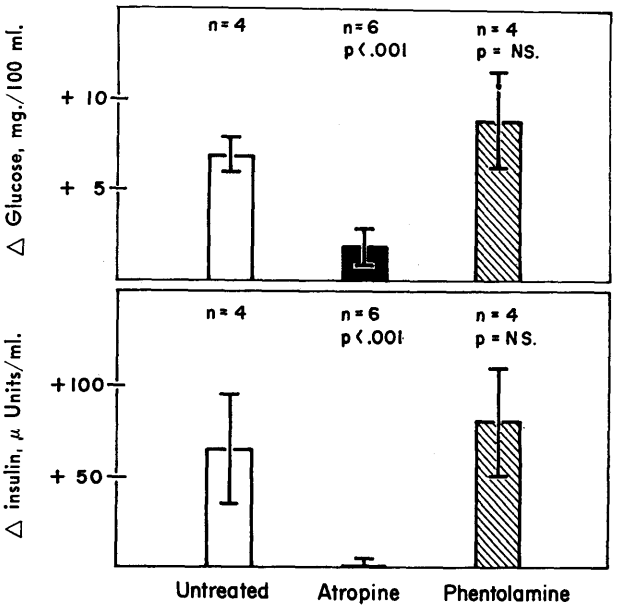

FIG. 5. The effect of atropine and phentolamine on the rise in portal venous insulin and arterial glucose five minutes following onset of vagal stimulation. In each group the mean change ± S.E.M. is shown. In every animal the values obtained were during a second vagal stimulation. An initial vagal stimulation was performed one hour earlier to serve as a positive control.

stimulation. Atropine alone did not significantly change insulin or glucose values. The cardiovascular effects of vagal stimulation (asystole of fifteen to twenty seconds duration, followed by sinus bradycardia) were completely blocked by atropine. As is shown in figure 5, atropine eliminated the rise in portal venous insulin and in arterial glucose observed five minutes after vagal stimulation. Neither the mean changes in glucose or insulin values were significantly different from zero.

To test the possibility that the small rise in blood glucose following vagal stimulation was due to epinephrine release, the alpha-adrenergic blocking drug phentolamine was given to another group of dogs prior to vagal stimulation. This drug has previously been shown to inhibit epinephrine induced hyperglycemia.[6] The blockade was confirmed at the end of each experiment by showing an absence of the characteristic rise in blood pressure following the injection of epinephrine. Phentolamine (2 mg. intravenously) had no effect on the rise in arterial glucose following vagal stimulation as illustrated in figure 5. The mean portal venous insulin concentration during vagal stimulation while under phentolamine blockade was no different than that during the control stimulation. The effect of phentolamine on vagus-stimulated insulin release is difficult to assess since phentolamine alone (2 mg. intravenously) produced an increase in portal venous insulin concentration (figure 6). This effect was unaltered by vagotomy.

Effect of vagotomy on glucose-mediated insulin release and on glucose utilization. The mean glucose disappearance rate (K) following the rapid injection of either 0.2 gm./kg. or 0.5 gm./kg. in seven animals was slightly decreased after vagotomy, but because of the large variation in individual experiments, the change was not significant. Vagotomy resulted in no significant alteration of the mean glucose-mediated insulin secretion as calculated from arterial insulin levels. These data are shown in table 2.

DISCUSSION

The present studies have confirmed the presence of a vago-insulin axis with the demonstration that the secretion of insulin can be influenced by both the right and left vagus nerves. The insulin secretory response to vagal stimulation is prompt and, under the conditions of the experiments described, short lived, peaking before five minutes despite the persistence of the stimulation. Less insulin is secreted in response to a second stimulation than to an initial stimulation, suggesting a tachyphylactic phenomenon. The ability of the beta cells to respond to exogenous glucagon stimulus following repeated vagal stimulation with a greater release of insulin than during the second stimulation implies a different mechanism of action of these two stimuli.

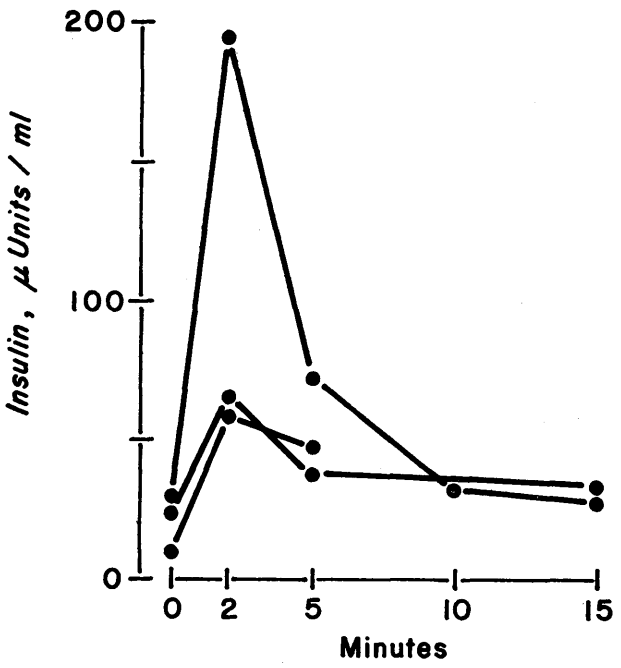

FIG. 6. The effect of phentolamine on portal venous insulin levels. Phentolamine, 2 mg., was given as a single intravenous injection. The curves represent results of individual experiments.

No changes in pancreatoduodenal venous blood flow were observed by Britton[4] during intra-abdominal vagal stimulation in cats. In the present studies no attempt was made to measure blood flow, but it was assumed that no increase in pancreatoduodenal venous blood flow occurred in light of the systemic circulatory changes. Thus, it appears unlikely that the increase in portal venous insulin levels was due to increased pancreatoduodenal venous blood flow.

The inhibition of vagal effect by atropine indicates that acetylcholine is the chemical mediator of this effect. Confirmatory in vitro studies demonstrating the stimulatory effect of carbamylcholine on rat pancreas have recently been reported by Malaisse et al.[15]

The small but significant fall in plasma insulin levels following vagotomy indicates that a portion of basal insulin secretion is under vagal influence. The lack of change in blood glucose following vagotomy would appear to indicate a compensatory change in other factors controlling blood glucose.

The absence of hypoglycemia and, in fact, the slight rise in blood glucose associated with vagal stimulated insulin release raised the question whether the immunoreactive insulin secreted following vagal stimulation was in any way different from that secreted following glucose. Neither by immunologic methods nor by bioassay could any difference be found. The possibility that a concomitant sympathetic stimulation occurred was considered, since early experiments leading to the vago-insulin axis theory by LaBarre[9] and Feldman et al.[16] required adrenal denervation or adrenomedullectomy to demonstrate hypoglycemia. Phentolamine, an alpha-adrenergic blocking agent, given in a dose sufficient to inhibit the effects of exogenously administered epinephrine, was ineffective in blocking the small rise in blood glucose associated with vagal stimulation. This compound has recently been shown by Porte[6] to inhibit the hyperglycemic effects of epinephrine as well as to counteract the epinephrine inhibition of insulin secretion. The ineffectiveness of phentolamine in preventing the glucose rise makes it unlikely that it is due to the liberation of endogenous epinephrine. It is possible that the vagus may influence glucagon secretion in a manner similar to that of insulin, or that the high intensity stimulus resulted in an activation of the alpha cells which would not have occurred under physiologic circumstances. If so, this could explain both the blood glu-

TABLE 2

Effect of vagotomy on net glucose utilization and glucose-mediated insulin secretion

Dose glucose	K* Prevagotomy	K* Postvagotomy	Per cent change	Insulin secretion Prevagotomy (μU.-min./ml.)	Insulin secretion Postvagotomy (μU.-min./ml.)	Per cent change
0.2 gm. per kg.	1.69	1.70	+ 0.6	1,340	1,242	— 7.3
	2.04	0.95	−53.4	2,352	1,434	−39.0
	1.83	1.38	−24.6	934	1,226	+31.3
0.5 gm. per kg.	1.02	0.55	−46.1	727	925	+27.1
	1.16	1.58	+36.2	1,781	1,633	− 8.4
	3.75	4.08	+ 8.8	1,550	1,026	−33.8
	3.55	3.75	+ 5.6	1,218	1,655	+35.9
Mean per cent change			−10.4			+ 0.8

*K is defined as the glucose disappearance rate (per cent per minute) observed between ten and forty minutes after intravenous glucose loading.

cose rise following vagal stimulation and the lack of hyperglycemia following vagotomy.

The rise in plasma insulin following phentolamine appears to represent a direct stimulatory effect of this agent rather than a release of the beta cells from the inhibition of endogenous epinephrine. The peak insulin levels following phentolamine are four to six times control values while insulin levels following cervical cordotomy, a procedure which blocks epinephrine response to hypoglycemia, are not increased.[17] In addition, Wright[18] has shown a stimulatory effect of phentolamine on the rat pancreas in vitro. The inability of phentolamine to stimulate insulin secretion in man[6] may be due to a species difference.

The lack of effect of vagotomy on net glucose utilization confirms the earlier studies of Clark[19] and Phillips[20], and the lack of change in glucose-mediated insulin secretion following vagotomy indicates that the vagus has little if any effect on insulin secretion in response to glucose loading. Consequently, the present studies do not support the concept that vagal hyperactivity is responsible for reactive hypoglycemia.

The physiologic stimulus for vagal stimulation of insulin secretion remains to be defined. Studies by Zunz and LaBarre[21] and by Gellhorn et al.[22] suggest that hypothalamic stimulation may result in activation of the vago-insulin axis, but the precise mechanisms are still unknown. Reciprocal changes in blood glucose levels following stimulation of the ventromedial and ventrolateral hypothalamus in the rabbit have been shown by Shimazu et al.[23] In addition, Kosaka and associates[24] have suggested an inhibitory influence on insulin secretion by cerebellar centers. The mechanism of this inhibition, however, has not been clarified.

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