Effect of Acetyl Choline on the Secretion of Glucagon and Insulin from the Isolated, Perfused Canine Pancreas

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SUMMARY

During perfusions with glucose concentrations of 25 and 150 mg./100 ml., the effect of infusions of acetyl choline on glucagon and insulin release was investigated in five perfusion experiments. Acetyl choline at concentrations of 10 to 100 μM. stimulated release of glucagon both at the low glucose strength as well as at the high concentration, which in itself inhibits basal glucagon release. Glucagon was released in a monophasic pattern, resembling the release pattern found after catecholamines and contrasting with the biphasic pattern obtained after stimulation with gastrointestinal hormones and amino acids, as previously reported from our laboratory.

Acetyl choline always stimulated release of insulin in a biphasic pattern at the high glucose concentration, while no consistent effect was obtained at the low glucose concentration. At the termination of the acetyl choline infusions, a rebound increase in insulin was observed during perfusion with both low and high glucose concentrations.

Infusion of atropine at a concentration of 25 μM. completely abolished the stimulatory effect of acetyl choline on both glucagon and insulin release.

The results suggest that the parasympathetic nervous system may play a direct role in the control mechanism of the release of the pancreatic hormones during food ingestion.
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We have recently shown that both the alpha cells and the beta cells of the islets of Langerhans respond to infusions of adrenergic substances, such as epinephrine, norepinephrine and isoproterenol; the studies demonstrate that the adrenergic system—the sympathetic nervous system and/or the adrenal medulla—influences secretion of glucagon and insulin.[1] Thus far, however, it is unknown whether secretion of pancreatic glucagon is influenced by the parasympathetic nervous system. The purpose of the present investigation was to study the effect of acetyl choline upon the secretion of glucagon and insulin from the isolated, perfused canine pancreas.

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METHODS

German dogs weighing 20 to 30 kg. and fed ad libitum with tripe, Purina chow, and water, served as pancreas donors. The animals were fasted overnight, anesthetized with thiopentone, intubated, and ventilated with positive pressure respiration, using oxygen and nitrogen oxide in a ratio of 1:3.

The pancreas was isolated and perfused, using the procedure described in a previous publication.[2] The preparation consists of the pancreas and the proximal 10 cm. of the attached duodenum. The celiac artery and the portal vein are catheterized. In some instances, when the anastomosis between the body and the tail of the pancreas is poorly developed, the splenic artery is also cannulated retrogradely. The pancreas is perfused without recirculation with a synthetic medium, which consists of a Krebs-Ringer buffer modified to the electrolyte concentrations found in dog plasma and contains 4 per cent dextran (commercially available Macrodex Mw 70,000 Pharmacia, Uppsala, Sweden), 0.2 per cent bovine albumin (Ortho Pharmaceuticals Corporation, Raritan, New Jersey), fumarate, pyruvate, and glutamate (Sigma Laboratories, St. Louis, Missouri), each at a concentration of 5 mM. and a glucose concentration of 25 mg./100 ml. The perfusate is equilibrated by a roller screen oxygenator in an atmosphere of 94.4 per cent oxygen and 5.6 per cent carbon dioxide and heated to a temperature of 37 ± 1° C. by use of low wattage heaters with a large surface area. The perfusate is pumped through the gland by means of a roller pump. A constant flow rate of 18 to 20 ml. per minute is achieved with a perfusion pressure of

30 to 40 mm. Hg within five to ten minutes after start of the perfusion. Both parameters are constant throughout the experimental period.

Experimental procedure. Samples were taken every minute from the influx and the efflux. To protect glucagon against degradation, 250 μL. EDTA (30 per cent) was added to the tubes collecting the effluent, resulting in a final concentration of 4 mg. per milliliter.[3] Within half an hour the samples were transferred to a freezer providing a temperature of —20° C.

The substances to be examined were added to the perfusate by means of constant infusion syringes. The infusion pumps were adjusted to speeds which gave from 0.1 to 0.25 ml. per minute to the flow, which was 18 to 20 ml. per minute. Acetyl choline and atropine were dissolved immediately before use in the perfusate buffer.

After the pancreas had been perfused for an equilibration period of twenty to thirty minutes with a glucose concentration of 25 mg./100 ml., the glucagon and insulin response to glucose concentrations of 25, 150, and 350 mg./100 ml. was investigated. The effect of acetyl choline was investigated at a glucose concentration of 25 and 150 mg./100 ml. in the perfusate, while the effect of atropine was investigated only at a glucose concentration of 150 mg./100 ml. At the end of each experiment, the insulin response to a glucose concentration of 350 mg./100 ml. was checked in order to prove that the beta cells were still capable of the characteristic biphasic insulin release.

Biochemical methods. Glucagon and insulin were measured by radioimmunoassay using minor modifications of the wick chromatography method described by Orskov[4,5] and outlined previously.[6] During infusion of acetyl choline and/or atropine and five minutes before infusion of these substances, the hormone concentrations were measured in duplicate determinations. The hormone concentrations in the rest of the experiment were measured in a single determination. There was no effect upon the standard immunoassay curve for glucagon or insulin by either acetyl choline or atropine.

Glucose was measured using a glucose oxidase method (Glox Kabi Reagents, Stockholm, Sweden). There was no interference from the high dextran concentrations.

RESULTS

Effect of glucose upon the secretion of pancreatic glucagon and insulin. The response of the pancreas to glucose concentrations of 25, 150, and 350 mg./100 ml. is seen in figures 1 and 2. During perfusion with a glucose concentration of 25 mg./100 ml., a mean glucagon concentration of 460 pg./ml. (SEM ± 90, N = 6) was obtained. The insulin concentration at this glucose concentration was 10 to 25 μU./ml. The sudden increase in glucose to 150 mg./100 ml. immediately suppressed the glucagon output to a mean value of 160

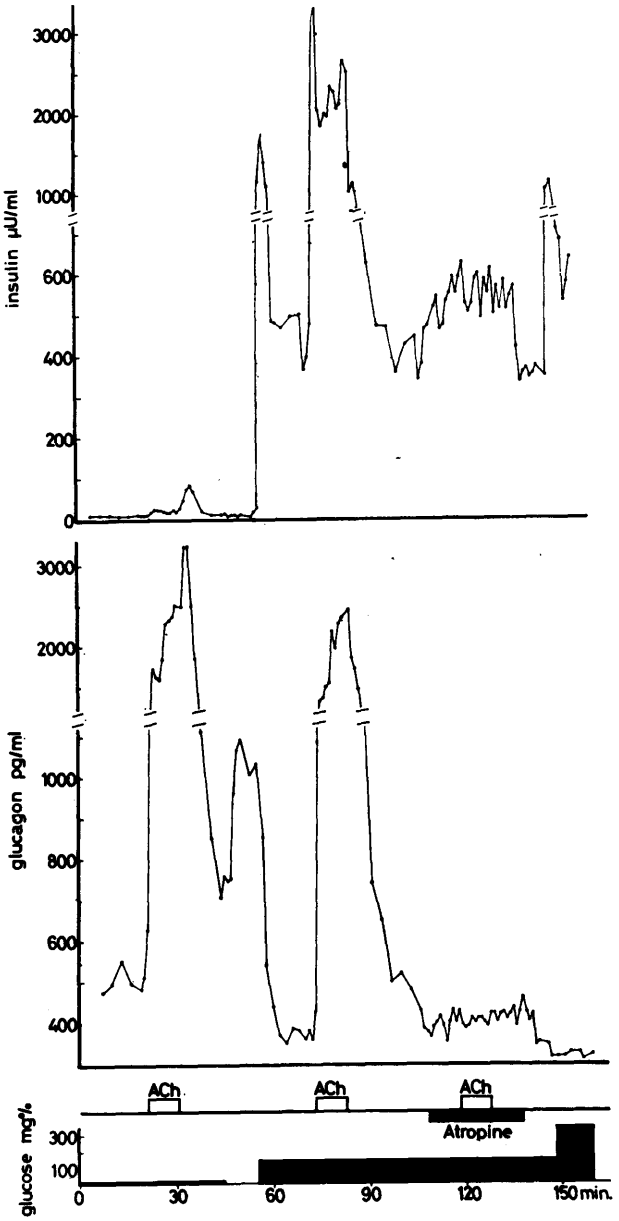

FIG. 1. Effect of acetyl choline at a concentration of 100 μM. on the secretion of pancreatic glucagon and insulin during perfusions with glucose concentrations of 25 and 150 mg./100 ml. and the effect of atropine upon that response.

pg./ml. (SEM ± 40). Glucose concentrations of 150 mg./100 ml. elicited the well known biphasic pattern of insulin release, a prompt rise in insulin during approximately two to three minutes followed by a rapid decline; values leveled off to about half the peak value and after that remained almost constant. When the glucose concentration was changed back to 25 mg./100 ml., the mean glucagon value again rose within one to two minutes to 390 pg./ml. (SEM ± 70) and insulin returned to low values within four to five minutes. The potency of glucose in suppressing release of glucagon is clearly seen in figure 1; when the glucose concentration was suddenly decreased from 25 to 0 mg./100 ml., glucagon output immediately increased to very high values.

Effect of acetyl choline during perfusions with glucose concentrations of 25 and 150 mg./100 ml. Infusion of acetyl choline at concentrations of 10 or 100 μM. for ten minutes stimulated release of glucagon both at low and at high concentrations of glucose in the perfusing medium. Figure 1 shows a representative experiment. At a glucose concentration of 25 mg./100 ml., glucagon rose rapidly within one to two minutes to a plateau about three- to fourfold that of the prestimulation value. The plateau slightly increased until termination of the infusion, when glucagon output leveled off to the prestimulation value over five to ten minutes. At a glucose concentration of 150 mg./100 ml., acetyl choline also stimulated release of glucagon. At this high glucose concentration, however, the glucagon rise was smaller than that obtained at the low glucose concentration and increased slightly until termination of the infusion, when glucagon reached the prestimulation value after five to six minutes of perfusion. Thus, the glucagon response to acetyl choline was monophasic, like

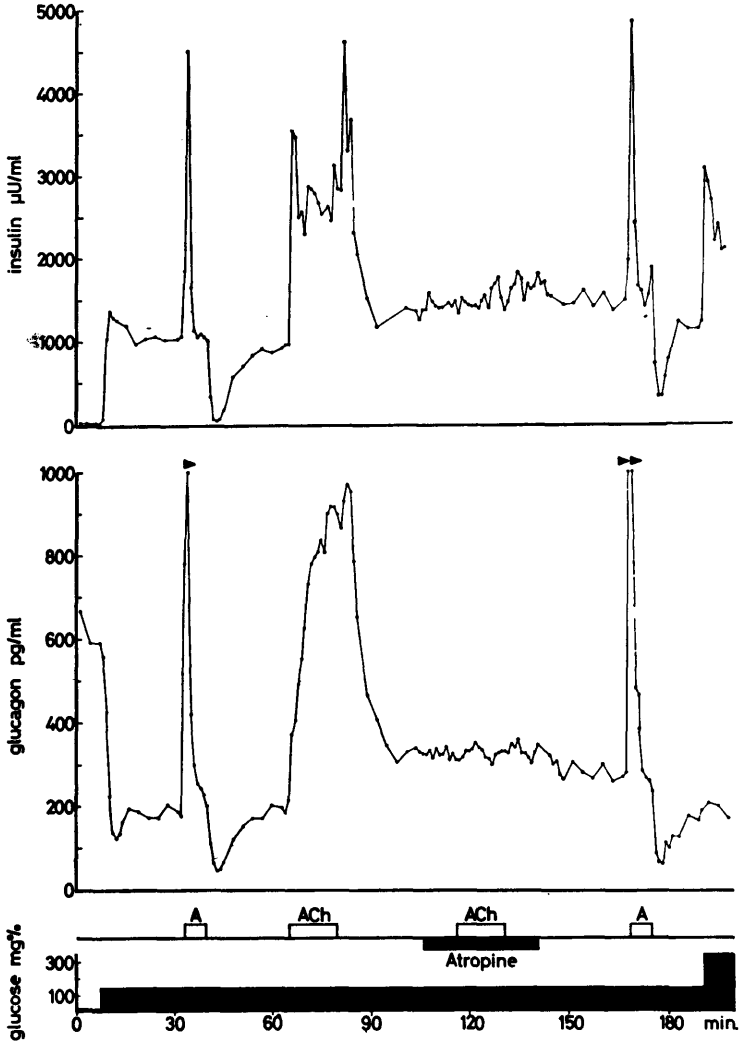

FIGURE 2

Effect of arginine on the secretion of pancreatic glucagon and insulin during perfusion with a glucose concentration of 150 mg./100 ml. before and after a single infusion of acetyl choline at a concentration of 100 μM. and before and after an infusion of acetyl choline at the same concentration preceded by atropine. ► peak value 1,105 pg. per milliliter ►► peak value 1,027 and 1,322 pg. per milliliter.

the response seen after epinephrine, norepinephrine, and isoproterenol, and contrasted with the biphasic release pattern seen after gastrin, pancreozymin, and arginine, as previously reported from our laboratory.[6]

Acetyl choline infusion stimulated release of insulin only slightly and inconsistently at the low glucose concentration. Immediately upon termination of the infusion, however, a consistent spike in insulin output was observed reaching a peak of about 80 to 100 μU. per milliliter. At the high glucose concentration, acetyl choline at the same concentration was a powerful stimulus for insulin release. Insulin output rose immediately, exhibiting a typical biphasic pattern of release. Within the first minute insulin reached a very high value of 3,000 μU./ml. After two to four minutes it declined rapidly to a value about fourfold that of the prestimulation value and remained almost constant to the end of the infusion. On cessation of the infusion, the 'off-effect' phenomenon was again observed: a transient high spike in insulin was followed by a decline of the release to prestimulation levels over a period of ten to twelve minutes.

The results from the individual experiments are recorded in table 1.

Effect of atropine on the acetyl choline stimulated release of glucagon and insulin. During perfusion with a glucose concentration of 150 mg./100 ml., the glucagon and insulin response to a ten minute infusion of acetyl choline (10 to 100 μM.) was compared with the responses obtained in the same experiment after a ten minute infusion of acetyl choline preceded by a ten to fifteen minute infusion of atropine at a concentration of 25 μM. Figure 1 shows a representative experiment. When atropine had been infused, the stimulatory effect of acetyl choline upon glucagon release was completely abolished and the insulin response was greatly and almost totally suppressed. The results from the individual experiments are depicted in table 2.

Infusion of atropine alone at a concentration of 25 μM. had no effect upon release of glucagon. A negligible stimulatory effect upon insulin release was observed in one of five experiments (see figure 1 and table 3). That

TABLE 1

Effect of acetyl choline on the secretion of PG* and I†

Exp.	Glucose‡ concentration		Prestimulation§	Concentrations during stimulation (Column headings indicate time in minutes) 1	2	3	4	5	6	7	8	9	10
111#	25	PG	500.8±11.0 (6)‖	628	—	1,725	1,635	1,600	1,850	2,280	2,327	2,375	2,500
		I	8.8± 1.0 (7)	9	11	15	22	23	—	18	16	—	23
	150	PG	359.5±10.8 (7)	425	1,085	1,320	1,360	1,390	1,540	2,225	1,970	2,270	2,350
		I	455.0±20.8 (6)	476	3,160	3,280	2,335	1,830	1,980	1,960	2,315	2,315	2,135
261**	25	PG	414.7± 6.6 (8)	501	1,048	892	873	766	773	801	832	904	900
		I	10.5± 0.5 (6)	9	10	12	13	17	17	22	21	23	25
	150	PG	255.4± 9.1 (10)	332	445	474	565	550	591	623	666	874	967
		I	732.5±36.5 (7)	993	1,770	1,762	1,550	1,435	1,424	1,380	1,354	1,439	1,520
1111**	25	PG	547.2±33.0 (10)	539	1,042	1,042	1,005	815	873	1,035	978	940	1,012
		I	5.6± 1.0 (5)	3	4	4	4	6	7	6	7	8	7
	150	PG	204.8± 6.8 (9)	220	310	395	537	550	633	675	780	748	722
		I	665.4±17.5 (7)	622	1,533	1,321	1,039	887	942	1,072	1,169	1,194	1,094
317**	25	PG	226.6±12.0 (3)	—	725	600	655	750	780	880	910	890	1,000
		I	2.6± 0.4 (5)	2	2	8	10	10	11	12	11	7	9
	150	PG	155.0± 6.3 (6)	267	540	544	615	858	855	965	1,020	1,000	1,040
		I	1,312.0±45.8 (5)	1,770	2,790	3,170	2,800	2,655	2,730	2,800	2,770	2,810	2,580
908**	25	PG	128.0±10.8 (4)	120	552	520	505	482	492	440	520	517	545
		I	3.4± 0.7 (5)	7	16	24	26	33	33	30	38	34	33
	150	PG	56.0± 2.8 (6)	90	116	124	115	137	155	185	180	180	210
		I	337.0±13.0(4)	362	917	1,000	903	705	597	650	608	720	775

* Pancreatic glucagon pg./ml.
† Insulin μU./ml.
‡ Glucose concentration in the perfusate mg./100 ml.
§ The prestimulation period is defined as the ten to fifteen minutes preceding infusion of the stimulatory substance.
‖ Number of determinations in the prestimulation period.
Acetyl choline concentration 100 μM.
** Acetyl choline concentration 10 μM.

TABLE 2

Effect of atropine on the acetyl choline stimulated secretion of PG* and I† at a glucose concentration of 150 mg./100 ml.

Exp.			Prestimulation‡	Concentrations during stimulation (Column headings indicate time in minutes) 1	2	3	4	5	6	7	8	9	10
31‖	ACh − Atrop.	PG	255.4± 9.1 (10)§	332	445	474	565	550	591	623	666	874	967
		I	732.5±36.4 (7)	993	1,770	1,762	1,550	1,435	1,424	1,380	1,354	1,439	1,520
	ACh + Atrop.	PG	393.8± 5.8 (5)	329	292	292	316	305	314	319	310	328	285
		I	1,109.4±51.3 (5)	1,002	996	946	917	938	963	904	874	843	897
1#	ACh − Atrop.	PG	359.5±10.9 (7)	425	1,085	1,320	1,360	1,390	1,540	2,225	1,970	2,270	2,350
		I	455.0±20.8 (6)	476	3,160	3,280	2,335	1,830	1,980	1,960	2,315	2,315	2,135
	ACh + Atrop.	PG	431.6±27.4 (3)	427	395	387	392	412	400	412	410	400	392
		I	417.6±22.6 (6)	590	550	590	625	525	505	525	566	578	490
7‖	ACh − Atrop.	PG	155.0± 6.3 (6)	267	540	544	615	858	855	965	1,020	1,000	1,040
		I	1,312.0±45.9 (5)	1,770	2,790	3,170	2,800	2,655	2,730	2,800	2,770	2,810	2,580
	ACh + Atrop.	PG	175.0±15.3 (3)	178	175	135	137	132	123	130	128	145	135
		I	857.3±33.7 (3)	808	587	725	732	780	826	660	730	655	595
2#	ACh − Atrop.	PG	178.1± 7.6 (6)	214	372	405	491	552	695	732	780	793	807
		I	890.2±24.2 (5)	958	3,532	3,458	2,499	2,554	2,293	2,859	2,830	2,764	2,660
	ACh + Atrop.	PG	330.8± 6.9 (10)	311	309	317	332	332	336	351	339	336	319
		I	1,330.6±40.7 (3)	1,410	1,325	1,502	1,464	1,425	1,415	1,407	1,388	1,476	1,540
8‖	ACh − Atrop.	PG	56.0± 2.8 (6)	90	116	124	115	137	155	185	180	180	210
		I	337.0±13.0 (4)	362	917	1,000	903	705	597	650	608	720	775
	ACh + Atrop.	PG	66.8± 5.4 (5)	55	55	30	32	55	53	65	69	55	65
		I	277.0± 7.3 (7)	287	223	208	258	243	245	216	228	231	212

* Pancreatic glucagon pg./ml.
† Insulin μU./ml.
‡ The prestimulation period is defined as the ten to fifteen minutes preceding infusion of the stimulatory substance.
§ Number of determinations in the prestimulation period.
‖ Acetyl choline concentration 100 μM.
Acetyl choline concentration 10 μM.

the islets could respond normally to a physiological stimulus after the atropine infusion can be seen in figure 2: L-arginine, at a concentration of 1 mM., was infused over a period of seven minutes before and after a single infusion of acetyl choline and before and after an infusion of acetyl choline preceded by atropine. A normal biphasic response of both hormones can be seen, which are essentially identical before and after the acetyl choline-atropine infusions, proving intact responsiveness of the islets throughout the experiment.

DISCUSSION

In the present investigation a stimulatory effect of acetyl choline upon glucagon and insulin release has been reported. Furthermore it has been amply demonstrated that this effect could be blocked by atropine, demonstrating that acetyl choline was the chemical mediator of the effect.

The stimulatory effect of acetyl choline upon glucagon release has not been previously shown either in vitro or in vivo. Vance et al.[7] briefly reported that acetyl choline was unable to release glucagon in the isolated islet system. However, they also reported failure of gastrointestinal hormones[8] and adrenergic substances[7] to influence release of glucagon and gastrointestinal hormones to release insulin, which may reflect an altered responsiveness of this in vitro system during the isolation procedure.

The finding that acetyl choline is able to release glucagon may explain the rise in blood glucose, in place of the expected hypoglycemia, with vagal-stimulated insulin release reported by Frohman et al.[9] Likewise, it may explain the absence of hypoglycemia when a visual or olfactory stimulus is associated with a rise in serum insulin, as recently reported,[10] and the normoglycemia during insulin secretion secondary to glucose administration in the awake dog with intestinal fistula.[11]

The present finding of a stimulatory effect of acetyl choline upon insulin secretion and its inhibition by atropine is consistent with results obtained in the isolated islet system.[12] Reports on the stimulation of the vagal nerve regarding an effect of the vagus nerves on

TABLE 3

Effect of atropine on the secretion of PG* and I† at a glucose concentration of 150 mg./100 ml.

Exp.		Prestimulation‡	Concentrations during stimulation (Column headings indicate time in minutes) 1	2	3	4	5	6	7	8	9	10
261	PG	393.8± 5.8 (5)§	332	360	346	380	360	412	376	326	305	300
	I	1,109.4±51.3 (5)	1,086	1,065	1,047	1,133	1,078	1,106	1,013	1,026	1,037	1,000
111	PG	431.6±27.4 (3)	372	367	390	400	415	395	355	400	430	402
	I	417.6±22.5 (6)	379	463	471	—	517	543	463	471	531	—
317	PG	175.0±15.3 (3)	202	190	155	125	140	130	127	177	175	180
	I	857.3±33.6 (3)	846	867	920	817	900	806	873	870	759	845
222	PG	330.8± 6.9 (10)	324	324	332	316	337	321	322	341	310	325
	I	1,330.6±40.7 (3)	1,363	1,374	1,567	1,465	1,403	1,389	1,399	1,595	1,445	—
908	PG	66.8± 5.4 (5)	60	55	44	48	58	59	58	50	62	71
	I	277.0± 7.2 (7)	288	250	240	263	294	237	240	272	251	234

* Pancreatic glucagon pg./ml.
† Insulin μU./ml.
‡ The prestimulation period is defined as the ten to fifteen minutes preceding infusion of the stimulatory substance.
§ Number of determinations in the prestimulation period.

pancreatic insulin release have been conflicting. Daniel et al.[13] found that stimulation of the vagal nerve subdiaphragmatically in baboons produced a slight increase in the insulin content measured in the splenic vein. Nelson et al.[14] reported failure to influence insulin release in dogs by stimulation of the vagal nerve in the neck, as well as supra- and subdiaphragmatically. Finally, Frohman et al.[9] reported a fall in portal vein insulin content following cervical vagotomy and an increase in insulin after stimulation of the distal end of the transected nerve. This effect could be blocked by an infusion of atropine.

The inconsistent results of these in vivo studies are not easily accounted for. One explanation for the negative results could be that stress-induced elevation of the catecholamines inhibited insulin release during vagal stimulation. Another explanation could be that epinephrine release from the adrenal medulla induced by acetyl choline suppressed insulin release. Stimulation of aberrant sympathetic nerve fibers located in the vagal trunk could also cause inhibition of insulin release. It is also possible that a direct effect of vagal stimulation on pancreatic blood flow could account for the negative findings in some of these studies, as pancreatic blood flow was not measured.

In the present study it should be emphasized that perfusion flow and perfusion pressure were uninfluenced by infusion of acetyl choline in the concentrations used. Likewise infusion of atropine alone did not affect release of glucagon or insulin, demonstrating the lack of nervous tone in the isolated organ. The concentrations of acetyl choline used may be regarded as high. However, there is reason to anticipate that acetyl choline released from postganglionic nerve fibers in direct contact with the alpha cells and the beta cells, would occur in much higher concentrations locally, thus justifying the concentrations chosen.

Acetyl choline, like most other stimuli for insulin release, was effective only when glucose concentration in the perfusing medium was elevated. An explanation for the rebound increase seen both at low and at high glucose concentrations in the medium at termination of the acetyl choline infusions cannot be given at the moment. Tentatively one might hypothesize that repolarization of the beta cell membrane and the associated ionic flux by the sudden deprivation of the receptors for acetyl choline might induce a transient release of insulin.

We have recently shown that release of glucagon is also stimulated by the adrenergic substances, epinephrine, norepinephrine, and isoproterenol, and that this effect could be blocked by propanolol. Furthermore, we have confirmed previous studies of the effect of adrenergic substances on insulin release. The islets of Langerhans in the pancreas are supplied with nerve fibers from both the sympathetic and the parasympathetic systems.[15,16] Evidence is thus accumulating that both parts of the autonomic nervous system may be intimately involved in the mechanisms, which control release of both pancreatic glucagon and insulin in the normal subject.

The physiologic role of the parasympathetic nervous system in the release of glucagon and insulin from the

islets of Langerhans is still unknown. The parasympathetic nervous system is stimulated before and during food intake, inducing the so-called cephalic phase of gastric and salivary secretion. As parasympathetic nerve fibers surround the islet cells, it may be inferred that stimulation of the vagal nerve during food ingestion also results in release of acetyl choline in the islets with a concomitant rise in both glucagon and insulin. A rise in insulin would contribute to the more marked rise in serum insulin following peroral glucose administration as compared to parenteral administration. A concomitant rise in glucagon output would appear expedient to prevent insulin-induced hypoglycemia when an olfactory or visual stimulus is not followed by food intake. When food intake possibly evokes an overshooting of insulin release it would serve to prevent hypoglycemia by replacing from the liver that glucose which accompanies the amino acids into tissues, as suggested by Unger et al.[17] We have previously shown that pancreatic glucagon increases after stimuli associated with protein intake, such as pancreozymin, gastrin, and amino acids, even at a glucose concentration which in itself suppresses glucagon.[6] Therefore, perhaps additional glucagon is released following acetyl choline principally in order to act in the metabolism of amino acids from ingested protein.

It is not known whether acetyl choline induces a hormone rise by acting directly or indirectly on a specific receptor. Stimulation of the vagal nerve results in a release of gastrin from the antral mucosa.[18,19] As gastrin is a potent stimulus for both glucagon and insulin release, it cannot be excluded that gastrin in the intact organism plays a role for the vagal-stimulated hormone release from the pancreas. It is also possible that acetyl choline releases gastrin in the pancreatic islets, which in turn could stimulate secretion of both glucagon and insulin. This suggestion is supported by the facts that gastrin-producing cells are found in the pancreas from the dog and that gastrin is found in the effluent from the perfused canine pancreas preparation.[20] Whether such a mechanism exists requires further investigation.

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REFERENCES

[1] Iversen, J.: Adrenergic receptors for the secretion of glucagon and insulin from the isolated, perfused canine pancreas. J. Clin. Invest. 1973. In press.

[2] Iversen, J., and Miles, D. W.: Evidence for a feedback inhibition of insulin on insulin secretion in the isolated, perfused canine pancreas. Diabetes *20*:1, 1971.

[3] Thomsen, H. G.: Pancreatic glucagon and 'glucagon-like material' in the blood. Diabetologia *5*:430, 1969.

[4] Orskov, H.: Wick-chromatography for the immunoassay of insulin. Scand. J. Clin. Lab. Invest. *20*:297, 1967.

[5] Orskov, H., Yde, H., and Thomsen, H. G.: Wick-chromatography for the rapid and reliable immunoassay of insulin, glucagon and growth hormone. Nature (London) *219*:193, 1968.

[6] Iversen, J.: Secretion of glucagon and insulin from the isolated, perfused canine pancreas. J. Clin. Invest. *50*:2123, 1971.

[7] Vance, J. E., Buchanan, K. D., and Williams, R. H.: Glucagon and insulin release: influence of drugs affecting the autonomic nervous system. Diabetes *20*:78, 1971.

[8] Buchanan, K. D., Vance, J. E., and Williams, R. H.: Insulin and glucagon release from isolated islets of Langerhans. Diabetes *18*:381, 1969.

[9] Frohman, L. A., Ezdinli, E. Z., and Rouhollah, J.: Effect of vagotomy and vagal stimulation on insulin secretion. Diabetes *16*:443, 1967.

[10] Parra-Covarrubias, A., Riviera-Rodriguez, I., and Almaraz-Ugalde, A.: Cephalic phase of insulin secretion in obese adolescents. Diabetes *20*:800, 1971.

[11] Hommel, H., Knöfler, H., Retzlaff, K., and Fischer, U.: Insulin secretion secondary to glucose administration in the awake dog with intestinal fistulae. Diabetologia *6*:632, 1970.

[12] Malaisse, W., Malaisse-Lagae, F., Wright, P. H., and Ashmore, J.: Effects of adrenergic and cholinergic agents upon secretion of insulin in vitro. Endocrinology *80*:975, 1967.

[13] Daniel, P. M., Henderson, J. R.: The effect of vagal stimulation on plasma insulin and plasma glucose levels in the baboon. J. Physiol. (Lond.) *192*:317, 1967.

[14] Nelson, C. N., Blackard, W. G., Cocchiara, J. C., and Labat, J.: Influence of the vagus nerves on pancreatic insulin secretion. Diabetes *16*:852, 1967.

[15] Honjin, R.: The innervation of the pancreas of the mouse, with special reference to the structure of the peripheral extension of the vegetative nervous system. J. Comp. Neurol. *104*:331, 1956.

[16] Coupland, R. E.: The innervation of pancreas of the rat, cat and rabbit as revealed by the cholinesterase technique. J. Anat. *92*:143, 1958.

[17] Unger, R. H., Ohneda, A., Aguilar-Parada, E., and Eisentraut, A. M.: The role of the aminogenic glucagon secretion in blood glucose homeostasis. J. Clin. Invest. *48*:810, 1969.

[18] Olbe, L.: Vagal release of gastrin. *In* Gastrin, Grossman, M. J., editor, UCLA, Forum of Medical Sciences No. 5. University of California Press, Berkeley, 1966.

[19] McGuigan, J. E., and Trudeau, W. L.: Serum gastrin levels before and after vagotomy before pyloroplasty or vagotomy and antrectomy. N. Engl. J. Med. *286*:184, 1972.

[20] Rehfeld, J. F., and Iversen, J.: Evidence for gastrin release from the pancreas of the dog. (Abstract.) Proc. 8th Congr. I.D.F., Brussels. Excerpta Med. Found., I.C.S., 1973. In press.