Effect of Stimulation on Pancreatic Amylase Secretion and Nuclear RNA Synthesis (34369)

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It is well known that stimulation of the pancreas by feeding or cholinergic drugs results in zymogen secretion. Little information is available correlating the temporal relationship between pancreatic secretory and synthetic events. This paper reports studies of amylase secretion and uridine-^{3}H incorporation into nuclear RNA by pigeon pancreas after feeding or methacholine administration.

Methods and Materials. White Carneau pigeons, 6–8 weeks of age, weighing 450–500 g, were purchased from Palmetto Pigeon Farm, Sumter, South Carolina. "Fed" birds were fed *ad libitum*; "fasted" birds were denied food for 3 days prior to study. "Refed" birds were fasted 3 days, but allowed to eat for 2 hr prior to study. All birds had free access to water.

Sources of materials have been previously described (1).

Initial experiments were performed to identify differences in amounts of uridine-^{3}H incorporated into nuclear RNA by pancreatic slices prepared from groups of fasted, fed, or refed pigeons. Each experiment utilized pooled pancreatic tissue obtained from three pigeons.

Twenty-one experiments were performed to determine if methacholine administration was associated with enhancement of uridine-^{3}H incorporation into nuclear RNA. For each experiment, four birds were used; two were given 1.0 mg methacholine intramuscularly in 0.5 ml saline and two were given saline alone. The pigeons were killed 5, 15, 30, 60, or 120 min after injection.

Preparation of tissue slices. Pigeons were decapitated, the pancreas quickly removed, and slices prepared and incubated as previously described (1).

After incubation for 60 min at 37° the incubating media was removed, the slices washed once with cold media (4°) containing 1 m*M* uridine and once with cold media alone. The slices were suspended in sucrose solution homogenized with a Teflon Potter homogenizer (0.01-mm clearance, Kontes Glass Company), and homogenates filtered through four layers of cheesecloth to remove large particulate matter.

Isolation of nuclear and subcellular fractions. Nuclei were separated from pancreatic brei by two methods. The first was similar to that described by Siekevitz and Palade (2). The second method was similar to that described by Widnell and Tata (3).

Nuclear and other fractions were made up in sucrose solution, 1 vol of 10% perchloric acid added, and the precipitate treated as previously described (1). RNA and DNA were separated by methods described by Schmidt and Thannhauser (4). RNA was assayed by the orcinol method and DNA by the diphenylamine method (5, 6). Radioactivity was counted as previously described (1).

Secretory studies. The pigeons were anesthetized with 0.75 g of urethane given intramuscularly. They were placed on their backs, wings secured, and a horizontal incision made between the rib cage and pelvis. The duodenum was exteriorized and inflow and outflow cannulas inserted.

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At the beginning of each experiment the duodenum was washed with 150 ml of saline solution and then perfused by means of a constant-infusion pump at a rate of 1 ml/min. The first 10 ml of this wash solution was collected and identified as zero-time sample. After irrigation of the duodenum, four 10-min (10-ml) samples were collected; methacholine given, and fractions 5–15 collected. The second injection of methacholine was made after collection of sample 15 and samples 16–20 collected. Methacholine was given intramuscularly in doses of 0.1, 0.25, 0.50, 0.75, and 1.0 mg in 0.5 ml of saline.

Assay of amylase activity. Amylase activity was assayed by the Bernfeld method using litner starch as substrate. A unit of amylase activity represents that amount which catalyzes the formation of 1 mg of maltose in 3 min at 30° (7). Amylase activity represents amounts secreted per 10-min period of collection.

Results. The rate of amylase secretion into the duodenum after methacholine administration is graphed in Fig. 1. These data show the secretory response of the pigeon after intramuscular methacholine to occur within minutes, to reach maximal rates within 20–30 min, and to return to basal rates within 60–80 min. The zero time values show the wide spread in amounts of amylase present in the duodenum of pigeons fed *ad libitum*.

Table I shows the distribution of uridine-^{3}H-labeled RNA in pancreatic subcellular fractions isolated from tissue slices prepared from fasted or fed pigeons. After 60 min of *in vitro* incubation the label is found largely in nuclear and soluble RNA fractions. Attempts to obtain significant labeling of microsomal RNA with increasing periods of *in vitro* incubation were unsatisfactory.

Table II shows data obtained when nuclei were isolated by use of 0.88 *M* sucrose solution containing 1.5 m*M* calcium chloride (3). Greater amounts of uridine-^{3}H were incorporated into nuclear RNA by slices prepared from fed and 2-hr refed pigeons compared to fasted controls. The nuclear RNA/DNA ratios in Table II agree with results published by other workers for other tissues (8).

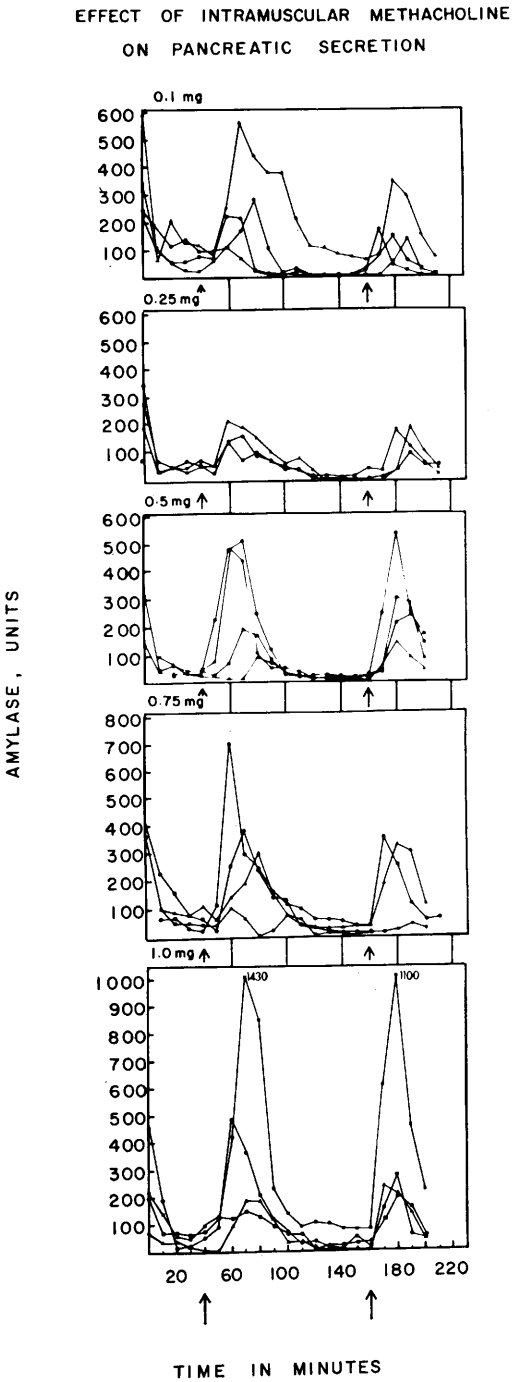

FIG. 1. Rate of amylase secretion into pigeon duodenum after 0.1, 0.25, 0.5, 0.75, and 1.0 mg intramuscular methacholine. Ordinate represents amylase units; abscissa represents time in 10-min intervals.

TABLE I. Effects of Fasting and Feeding on Uridine-^{3}H Incorporation into Subcellular Fractions by Pancreatic Slices.[a]

No. of expt.	Fasted or fed	Fraction	Count/min[b]		μg RNA[b]
			100 μg DNA	100 μg RNA	100 μg DNA
7	Fasted	Total	380 ± 85	550 ± 123	53 ± 16
		Nuclear	248 ± 43	243 ± 109	26 ± 8
		Microsomal		79 ± 32	
		Postmicrosomal		440 ± 206	
7	Fed	Total	924 ± 305	623 ± 133	146 ± 16
		Nuclear	821 ± 470	1870 ± 880	34 ± 18
		Microsomal		144 ± 75	
		Postmicrosomal		955 ± 321	

[a] Tissue slices were prepared and incubated in tissue culture media for 60 min at 37°. The slices were homogenized in 0.88 *M* sucrose solution and subcellular fractions prepared by differential centrifugation.

[b] Mean values ± SD. RNA expressed in terms of RNA-ribose.

Figure 2 shows greater rates of uridine-^{3}H incorporation into nuclear RNA by pancreatic slices prepared from fed compared with fasted controls.

Table III shows results obtained when effects of methacholine administration on uridine-^{3}H incorporation into nuclear RNA were studied. As shown, slices prepared from similar groups of birds given saline and killed immediately (zero time) incorporated approximately equal amounts of uridine-^{3}H into nuclear RNA. However, when one group of birds was given saline and a similar group methacholine and both groups killed 5, 15, 30, 60, or 120 min later, the slices prepared from methacholine pretreated pigeons incorporated greater amounts of uridine-^{3}H into nuclear RNA than did the saline controls.

Discussion. Methacholine administration to

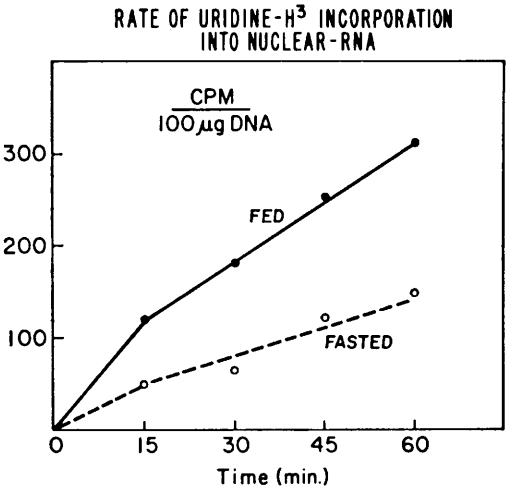

FIG. 2. Rate of uridine-3H incorporation into nuclear RNA by pancreatic slices from fasted or fed pigeons.

TABLE II. Effects of Fasting and Feeding on Uridine-^{3}H Incorporation into Nuclear-RNA by Pancreatic Slices.[a]

No. of expt.	Fasted or fed	Fraction	Count/min[b]		μg RNA[b]
			100 μg DNA	100 μg RNA	100 μg DNA
3	Fasted	Nuclear	120 ± 36	274 ± 161	26 ± 12
3	Fed	Nuclear	512 ± 243	1032 ± 484	24 ± 7
5	Refed	Nuclear	493 ± 163	1500 ± 542	35 ± 3

[a] Tissue slices were prepared and incubated in tissue culture media for 60 min at 37°. The slices were homogenized in 0.88 *M* sucrose solution containing 1.5 m*M* calcium chloride and nuclear fractions isolated by centrifugation.

[b] Mean values ± SD. RNA expressed in terms of RNA-ribose.

TABLE III. Effect of *in Vivo* Methacholine on Uridine-^{3}H Incorporation into Nuclear RNA by Pigeon Pancreatic Slices.[a]

Time before sacrifice (min)	No. of expts.	*In vivo* treatment	Count/min[b] 100 μg DNA	% Incorp.	Count/min[b] 100 μg RNA	% Incorp.
0	3	Sal.	335 ± 159		690 ± 449	
		Sal.	370 ± 192	9%	690 ± 259	0%
5	3	Sal.	374 ± 246		485 ± 128	
		Meth.	574 ± 418	53%	790 ± 92	63%
15	4	Sal.	410 ± 54		493 ± 182	
		Meth.	800 ± 227	95%	1400 ± 691	183%
30	5	Sal.	390 ± 207		775 ± 373	
		Meth.	500 ± 258	28%[c]	1460 ± 400	72%[c]
60	3	Sal.	303 ± 140		567 ± 261	
		Meth.	419 ± 102	38%	866 ± 292	52%
120	3	Sal.	260 ± 90		369 ± 158	
		Meth.	410 ± 142	57%	473 ± 149	28%

[a] Paired groups of pigeons were given either saline 0.5 ml or 1.0 mg methacholine in 0.5-ml saline im. After 0, 5, 15, 30, 60, 120 min the birds were killed, slices prepared, and uridine-^{3}H incorporation into nuclear RNA measured.

[b] Mean values ± SD.

[c] Significance between means of paired groups of pigeons; $p < 0.05$.

pigeons was associated with increased amylase secretion into the duodenum within 5 min, maximal secretion within 20–30 min, and a return to basal secretion within 60–80 min. In these experiments the effects of methacholine on processes involving changes in rates of secretion and synthesis closely parallel one another.

These studies suggest that feeding most likely attended with vagal stimulation results in acetylcholine release, which, in turn, initiates biochemical processes eventuating in secretion and synthesis of pancreatic digestive enzymes.

Many people have suggested that methacholine has its effect on some receptor site located on the external cell membrane. If such is the case, the present experiments indicate at least three possible sites for metabolic events in the cell; (1) attachment of methacholine molecule to a receptor site on the external cell membrane; (2) enhancement of nuclear RNA synthesis; (3) biochemical process resulting in extrusion of zymogen granules. The mechanisms whereby these effects are accomplished remains unknown.

Summary. These studies were designed to obtain information concerning the temporal correlation of synthetic and secretory events in the pigeon pancreas after stimulation.

After methacholine administration secretion of amylase into the duodenum was apparent within 5 min, approached maximal rates within 20–30 min, and returned to basal levels by 60–80 min.

Feeding and methacholine administration were associated with enhanced uridine-^{3}H incorporation into nuclear RNA by pancreatic tissue slices. The increased incorporation of uridine-^{3}H into nuclear RNA after methacholine was apparent within 5 min, approached maximal rates within 20–30 min, and returned toward basal levels within 60 min. Methacholine activates processes resulting in secretion as well as synthesis. In these particular experiments the two processes were closely related in time.

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