Effect of Atropine on Pigeon Pancreas
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It is well established that atropine administration results in decreased pancreatic secretion; however, little information has been available concerning the metabolic effects of this agent on the pancreas. Recently it was shown that atropine administration to pigeons was associated with changes in pancreatic enzyme secretion and synthesis (1). The present investigations were performed for two reasons: (a) to obtain additional specific information concerning the mechanism of action of atropine on pancreatic protein synthesis; and (b) to determine general mechanisms concerned with reduction of pancreatic protein synthesis.

These studies show that uridine-^{3}H incorporation into tissue RNA was decreased 2, 4, and 6 hr following atropine administration. However, no change was apparent in uridine-^{3}H incorporation into nuclear RNA 2 and 4 hr following atropine. The major decrease in uridine-^{3}H incorporation was into soluble RNA. Pancreatic microsomes and supernatant from atropine-treated birds incorporated fewer counts following *in vitro* incubation than microsomes and supernatant from saline-treated birds. These results suggest that the initial decrease in L-phenylalanine-^{14}C incorporation results primarily from changes involving the translational level of protein synthesis.

Methods. Fasted or fed white Carneau pigeons, age 6–8 weeks (wt 500 g) were used for all studies (2). All animals had free access to water.

Materials and methods used have been previously described (2–4).

Pigeons (control group, 3 birds; atropine group, 3 birds) were given atropine, 0.8 mg/kg, or saline in pectoral muscles according to the following schedules: (1- and 2-hr studies) atropine, 0.4 mg each 30 min, two or four times; (4- and 6-hr studies) atropine, 0.4 mg each hour, four or six times; saline, 1.0 ml was given to control birds at the same time. Atropine administration at these doses and periods of time resulted in no apparent change in the well being of the animals.

Studies of uridine-^{3}H incorporation into tissue RNA. After atropine or saline treatment pigeons were killed, pancreatic slices were prepared, and incubated in tissue culture media containing 1 μCi/ml of uridine-^{3}H for 30 min at 37°. The reaction was stopped by addition of 10% perchloric acid, slices were homogenized, precipitate was washed, RNA and DNA were separated, and measured as described (3).

Studies of uridine-^{3}H incorporation into nuclear RNA. Pancreatic slices (1.0 g/10 ml of media/20 μCi of uridine-^{3}H) were incubated for 30 min at 37° as described (3). At completion of incubation, supporting media were aspirated, the slices were washed three times with ice-cold media, and resuspended in 2.5% citric acid (1:10; w/v) (5). The slices were homogenized using a Teflon homogenizer (0.02 in. clearance; 25 passes). The homogenate was passed through three layers of gauze and centrifuged 600*g* for 10 min. The supernatant was aspirated, the pellet was resuspended in citric acid, 2.5%, and centrifuged two additional times. The pellet from the third centrifugation was resuspended by homogenization in 8 ml of 0.25 M sucrose containing 1.5% citric acid. The nuclear pellet was layered over 15 ml of 0.88 M sucrose–citric acid (1.5%) and centrifuged at 900*g* for 10 min. The supernatant was

TABLE I. Effects of *in Vivo* Atropine on Uridine-^{3}H Incorporation into Tissue RNA by Pancreatic Slices.[a]

Time of sacrifice (hr)	Fed or fasted	No. of expts.	Uridine-^{3}H (cpm in RNA/100 μg of DNA)		% Diff.	p value
			Control	Atropine		
1	Fed	3	160 ± 24	158 ± 50		NS
2	Fed	8	165 ± 60	116 ± 35	−30%	<0.01
2	Fasted	7	98 ± 22	81 ± 15	−17%	<0.02
4	Fed	7	132 ± 44	102 ± 22	−23%	<0.02
6	Fed	5	157 ± 45	102 ± 23	−35%	<0.02

[a] Values are means ± SD.

aspirated, the nuclear pellet was resuspended in sucrose–citric acid, and the step was repeated two times. At completion of centrifugation, the nuclear pellet was precipitated and washed with 0.7 *N* perchloric acid (3×), 90% EtOH–sodium acetate (2%) (1×), and with ether–ethanol (1:3) (2×). The nuclear pellet was analyzed for RNA, DNA, and radioactivity as described (2–4).

Studies of uridine-^{3}H incorporation into RNA isolated by phenol extraction. Tissue slices or nuclei were made up in 0.02 *M* sodium acetate (1:10; w/v), Macaloid (0.5%), sodium dodecyl sulfate (0.5%) and homogenized. Immediately following homogenization, an equal volume of buffered saturated-phenol (pH 5.0, sodium acetate) was added and the homogenate was shaken vigorously for 10 min. One "cold" (4°) and one "hot" (60°) extraction with phenol was performed. The aqueous layer was removed, made up to volume with sodium acetate, and a third phenol extraction was performed. The RNA was precipitated with sodium chloride (2 *M*) and 95% ethanol at −20° for 30 min. The RNA was dissolved in sucrose and placed on a 5–25% sucrose gradient as described by Steele and Bush (6). Separation of RNA was obtained by 18-hr centrifugation at 24,000 rpm using an SW-27 rotor (Spinco). Fractions were collected; RNA and radioactivity were determined.

In vitro L-*phenylalanine-^{14}C incorporation using microsomes.* Pancreatic tissue from atropine or saline treated animals was homogenized (10% w/v) in 0.44 *M* sucrose using a tight Teflon homogenizer (0.004 in. clearance; 8 passes; 500 rpm). Microsomal and cell sap fractions were obtained by centrifugation (2500 rpm for 10 min; 11,000 rpm for 15 min; 65,000 rpm for 30 min) (Spinco L2-65B and no. 65 rotor). The supernatant fractions obtained from the high speed centrifugation were used as a source for activating enzymes. The microsomal pellet was washed twice, resuspended in 0.25 *M* sucrose and incubated for 10 min with GTP and an energy generating system. These methods are similar to those used by Redman *et al.* (7). Radioactivity and protein were measured.

Results. Table I shows results obtained for uridine-^{3}H incorporation into whole tissue RNA by pancreatic slices prepared from pigeons given atropine or saline. There was no

TABLE II. Effects of Atropine on Uridine-^{3}H Incorporation into Nuclear-RNA by Pancreatic Slices (citric acid method).[a]

Time of sacrifice (hr)	Fed or fasted	No. of expts.	(cpm in RNA/100 μg of DNA)		(cpm in RNA/100 μg of RNA)		RNA (μg/100 μg of DNA)	
			Saline	Atropine	Saline	Atropine	Saline	Atropine
2	Fed	7	178 ± 80	185 ± 26	1633 ± 894	1536 ± 760	12 ± 5	14 ± 5
4	Fed	4	233 ± 86	239 ± 59	2458 ±1039	2990 ±1300	10 ± 4	10 ± 6

[a] Values are means ± SD.

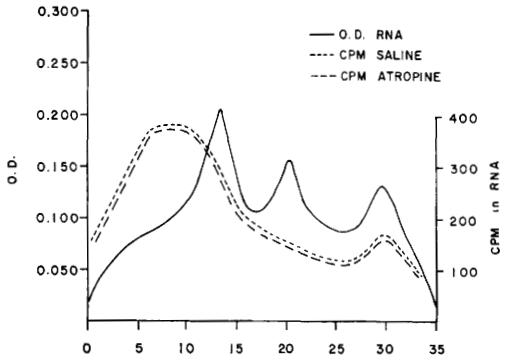

FIG. 1. Effects of *in vivo* atropine on uridine-^{3}H incorporation into RNA isolated from pancreatic nuclei (0, bottom; 35, top).

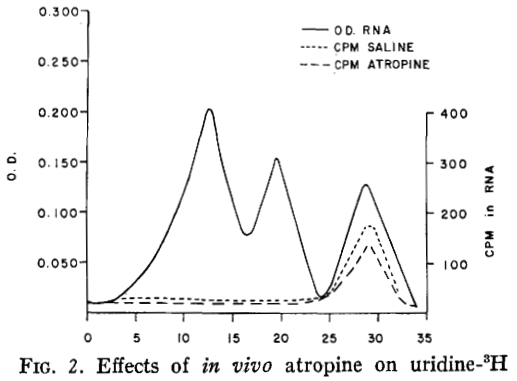

FIG. 2. Effects of *in vivo* atropine on uridine-^{3}H incorporation into whole tissue RNA by pancreatic slices (0, bottom; 35, top).

difference between control and atropine-treated groups of pigeons killed 1 hr after atropine; however, pigeons killed 2, 4, or 6 hr following atropine showed a 30, 23, or 35% decrease in amounts of uridine-^{3}H incorporated into RNA. The decrease in uridine-^{3}H incorporation into RNA was apparent whether the pigeons were fed or fasted (—30 and —17%).

Table II shows effects of *in vivo* atropine on uridine-^{3}H incorporated into nuclear RNA whether the data were expressed in terms of DNA or RNA. Moreover, RNA/DNA ratios for nuclei were about the same for both control and experimental animals. These observations suggest that the decreased incorporation of uridine-^{3}H obtained with the slice–whole tissue system (Table I) might largely be into soluble RNA. Whether or not atropine administration for longer periods of time or at greater doses might alter nuclear RNA synthesis was not determined.

Figure 1 shows effects of atropine on the pattern of uridine-^{3}H incorporation into nuclear RNA. The patterns of labeling as well as total specific activity of nuclear RNA isolated from saline or atropine-treated groups of birds (saline, 2420 cpm/mg of RNA; atropine, 2250 cpm/mg of RNA) were identical. The greatest labeling of RNA was obtained in fractions preceding the 28 s peak; such presumably reflects heavier types of RNA (*i.e.*, 45 s).

Figure 2 shows a profile of RNA isolated from whole tissue slices. Under these conditions, there was greater labeling of soluble RNA. Moreover, the specific activity of sRNA isolated from saline groups was greater than that from atropine groups. Thus, the difference shown in Table I must result largely from differences in tRNA labeling.

Table III shows effects of *in vivo* atropine on *in vitro* L-phenylalanine-^{14}C incorporation into protein by pancreatic microsomes. Microsomes and supernatant from control animals incorporated more counts per minute than microsomes and supernatant from atropine-treated animals (6769 vs 4908 cpm). Control and atropine microsomes incubated with atropine supernatant incorporated approximately the same number of counts (5574 vs 4908 cpm). The fact that microsomes from control and atropine-treated groups incorporated L-phenylalanine-^{14}C at similar rates suggest they were primarily unaffected by atropine. Moreover, when control microsomes were incubated with supernatant from control and atropine-treated groups of birds, the decrease observed seemed to be

TABLE III. Effects of *in Vivo* Atropine on *in Vitro* L-Phenylalanine-^{14}C Incorporation into Protein by Pancreatic Microsomes.[a]

Microsomes	Supernatant	L-phenylalanine-^{14}C (cpm/mg of protein microsome)	p value
Control	Control	6769 ± 102	<0.05
	Atropine	5574 ± 55	
Atropine	Atropine	4908 ± 963	<0.05
	Control	5964 ± 603	

[a] Values are means ± SD.

a function of the atropine supernatant (6769 vs 5574 cpm). Atropine microsomes incubated with supernatant from atropine and control animals showed the greatest incorporation in the control supernatant group (4908 vs 5964 cpm).

These studies suggest that the major decrease in amino acid-^{14}C incorporation following atropine resulted from changes involving factors found or isolated in the supernatant fraction.

Discussion. Little information has been available concerning effects of atropine on pancreatic metabolism. Recently it was shown that atropine administration to pigeons was associated with the following changes in pancreatic enzyme secretion and synthesis: (a) an increase in amylase content; (b) a decrease in incorporation of L-phenylalanine-^{14}C into proteins as well as amylase; (c) a decrease in oxygen uptake; (d) a decrease in oxidation of glucose-^{14}C and palmitate-^{14}C to $^{14}CO_2$. The increase in amylase content was apparent within 1 hr following atropine; the decrease in incorporation of L-phenylalanine-^{14}C into protein and amylase and decrease in oxidation of substrates were not apparent until 2 hr or more following atropine (1).

The studies show that *in vivo* atropine was associated with decreased uridine-^{3}H incorporation into whole tissue RNA at 2, 4, and 6 hr (30, 23, and 35%, respectively). No change was apparent when pigeons were killed 1 hr following atropine. The decrease in uridine-^{3}H incorporation was apparent whether fasted or fed animals were studied. Atropine administration was associated with decreased uridine-^{3}H incorporation into soluble-RNA but not nuclear-RNA.

A number of factors have been identified in either cell sap or microsomes which influence or are necessary for polypeptide synthesis on ribosomes. Under the dose and time conditions established in this study, these data indicate that atropine decreases *in vitro* amino acid-^{14}C incorporation by altering soluble factors. This suggests control of protein synthesis at the translational level.

It has been shown that administration of cholinergic drugs to pigeons (urecholine and methacholine) was associated with increased pancreatic protein secretion and synthesis. Synthesis was initiated within 5 min, peaked within 30 min, and returned to basal levels within 60 to 80 min. Enhancement of amino acid-^{14}C incorporation into protein, palmitate-^{14}C oxidation to $^{14}CO_2$, and uridine-^{3}H incorporation into soluble and nuclear RNA were also observed (8). It is likely that the effect of atropine may be mediated directly by blocking the stimulatory effect of cholinergic drugs on cell membrane receptor sites. If such is the case, cholinergic drugs must effect an increase of an intracellular substance which enhances synthesis at the ribosomal level. Atropine, in turn, must block release of this substance, thus effecting a decrease in synthesis. Since a decrease in synthesis was only observed 2 hr after atropine administration, it would seem that the half-life of this substance or effects produced thereof must be about 2 hr.

It is of interest to point out a further difference in effect between drugs causing stimulation and those causing inhibition of protein synthesis. Urecholine and methacholine administration were associated with increased uridine-^{3}H incorporation into nuclear RNA. Atropine administration, on the other hand, did not have an effect on nuclear RNA synthesis even after 2 or 4 hr. It seems the pancreatic acinar cell possesses the capability to reduce rates of protein synthesis within short periods of time and that these changes must be effected at the ribosomal level.

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