Effects of atropine on pigeon pancreas

JEAN MORISSET AND PAUL D. WEBSTER

Section of Gastroenterology, Veterans Administration Hospital, Augusta 30904, and
Medical College of Georgia, Augusta, Georgia 30902

MATERIALS AND METHODS

White Carneau pigeons (6-8 weeks of age, weighing 450-500 g) obtained from Palmetto Pigeon Farm, Sumter, S. C. were used. Atropine sulfate, 0.4 mg/ml, was purchased from Eli Lilly Co., Indianapolis, Ind. L-Phenylalanine-\(^{14}\)C (0.37 mc/\(\mu\)mole) uniformly labeled (UL), l-leucine-\(^{14}\)C (UL) (0.35 mc/\(\mu\)mole), glucose-\(^{14}\)C (UL) (14.4 \(\mu\)c/\(\mu\)mole), and palmitate-\(^{14}\)C (UL) (5.0 mc/\(\mu\)mole) were purchased from New England Nuclear Corporation, Boston, Mass. Preparation and materials for tissue culture media have been described (19). Dithylyminocetyl cellulose (DE 52) was purchased from Scientific Division, Reeve Angel, Clifton, N. J. Sephadex G-75 was purchased from Pharmacia, Uppsala, Sweden.

Birds referred to as fed had food in their cages at all times; fasted birds were denied food for three days prior to time of study. Desirability of a 3-day fast for pigeons has been described (23). All birds had free access to water. All studies were done on paired groups of pigeons (three control and three experimental) in an attempt to minimize day-to-day variability in metabolic activity of pancreatic tissue. We have observed that similar groups of birds studied on the same day yield almost identical values for measurements of amino acid-\(^{14}\)C incorporation, whereas similar groups of birds studied on different days may show as much as a 50% difference. This variability has been commented on by others (7, 24).

Atropine dose schedule. For all experiments, atropine was given according to one of the following schedules: 1- and 2-hr studies, atropine 0.4 mg in 1 ml saline each 30 min 2 or 4 times; 4- and 6-hr studies, atropine 0.4 mg in 1 ml of saline each hour 4 or 6 times. Saline, 1.0 ml, was given to control birds at the same time. All injections were into the pectoral muscle. Atropine administration at these dose levels and time periods resulted in no apparent change in the well being of the animals.

Studies of L-phenylalanine-\(^{14}\)C and l-leucine-\(^{14}\)C incorporation into tissue protein. At indicated time periods following atropine or saline, pigeons were killed, pancreas removed, tissue slices prepared and incubated in vitro for 60 min at 37 C under 95% \(O_2\) 5% \(CO_2\) (23). When rates of L-phenylalanine-\(^{14}\)C incorporation were studied, slices were incubated for 0, 20, 40, and 60 min.

Studies of L-phenylalanine-\(^{14}\)C incorporation into microsomal proteins. Experiments were performed to determine amounts of L-phenylalanine-\(^{14}\)C incorporation into deoxycholate (DOC)-soluble and -insoluble fractions of microsomal
proteins by tissue slices from atropine or saline treated birds. Atropine, 0.4 mg, was given each 30 min for 2 hr. Tissue slices were prepared, L-phenylalanine-^{14}C 1 μc/500 mg tissue added, and incubated for 60 min. Upon completion of in vitro incubation, the supporting media were aspirated, the slices washed 2 times with ice-cold media containing unlabeled phenylalanine, and homogenized in 0.44 M sucrose containing 0.01 M Tris buffer (pH 7.2) (homogenization performed by 15 passes, 250 rpm, using a glass Teflon (Kontes) homogenizer with a clearance of 0.004 inches). The homogenate was centrifuged 2 times at 15,000 g for 15 min and the upper four-fifths of the supernatant removed and centrifuged at 105,000 g for 60 min in a Spinco model L2 65B preparative ultracentrifuge using a no. 65 rotor. The microsomal pellet was resuspended by three passes with a Teflon homogenizer in 3.5 ml of sodium phosphate-buffered saline (pH 7.2) and sufficient deoxycholic acid buffered with Tris (pH 7.2) added to make 0.5%. Particulate material was precipitated by centrifugation at 245,000 g for 30 min. This pellet representing the DOC-insoluble portion of microsomal fraction was washed 2 times with buffer; protein content and trichloroacetic acid (TCA)-precipitable radioactivity determined. The supernatant representing DOC-soluble material was analyzed for protein content and TCA-precipitable radioactivity.

Studies of L-phenylalanine-^{14}C incorporation into amylase. Pigeons were treated with atropine for 2 hr. Pancreatic slices (2 g) were incubated in tissue culture media containing 5 μc phenylalanine-^{14}C for 60 min at 37°C. On completion of in vitro incubation, the supporting media were aspirated, the tissue slices washed 2 times with ice-cold media and 1 time with 0.02 M potassium phosphate buffer (pH 8.0). The slices were then suspended in 0.02 M potassium phosphate buffer (pH 8.0) and homogenized using a ground-glass tissue grinder (25% w/v). The homogenate was centrifuged 2 times at 15,000 g for 15 min and 1 time at 269,000 g for 30 min. The supernatant was dialyzed for 24 hr against 0.02 M potassium phosphate buffer (pH 8.0). The dialysate was centrifuged 15,000 g for 15 min. The supernatant was placed on a Sephadex G-75 column and proteins eluted with 0.02 M potassium phosphate (pH 8.0) buffer. The eluent containing maximal amylase activity was then dialyzed against 0.005 M potassium phosphate (pH 8.0) buffer and placed on DEAE cellulose column with proteins eluted using a gradient of 0.005 to 0.4 M potassium phosphate buffer (pH 8.0). Ten milliliter samples were collected. Amylase activity, protein content, and radioactivity were determined for each sample. The methods employed in the separation of amylase using DEAE are similar to those used by Keller and Cohen (9) and later by Marchis-Mouren et al. (11) except that lyophilization was not used to concentrate proteins.

Studies of L-phenylalanine-^{14}C accumulation. Rates of accumulation of L-phenylalanine-^{14}C by pancreatic slices prepared from fasted or fed pigeons treated with atropine or saline for 2 hr were investigated. Slices were prepared and incubated in tissue culture media (100 mg tissue per 1 ml media) for 5, 10, 20, 40, and 60 min. On completion of incubation, the supernatant was aspirated; slices washed 2 times with ice-cold media, and homogenized in 5% TCA (1 ml/100 mg tissue wet wt). Radioactivity in a sample of the TCA-soluble fraction was determined after ether extraction. Paper chromatographic separation of amino acids in the TCA-soluble fraction was accomplished using butanol, acetic acid, and water as described by Smith (15).

Studies of amylase content. Tissue from atropine- or saline-treated pigeons was homogenized in sodium phosphate buffer (pH 6.9) and amylase activity of the homogenate determined by the method of Bernfeld (1) using limmer starch as substrate. Amylase activity is expressed in units per milligram tissue wet weight. Amylase activity is expressed in terms of milligrams of maltose liberated in 3 min at 37°C.

Assay of protein content. Protein content was determined using methods described by Gornall et al. (5) or Lowry et al. (10).

Assay of DNA content. DNA content was measured using diphenylamine (14). Calf thymus DNA was used as standard.

Studies of oxygen uptake. Pigeons were treated with atropine or saline for 2 hr. Pancreases were removed, slices prepared, and oxygen uptake determined using a Warburg respirometer as described by Umbreit and co-workers (17).

Studies of glucose-^{14}C and palmitate-^{14}C oxidation. Pancreatic tissue from pigeons given atropine for 9 or 4 hr was homogenized in freshly oxygenated Krebs-Ringer phosphate buffer (pH 7.4). One milliliter of the homogenate was incubated in 25 ml Erlenmeyer flasks containing glucose-^{14}C or palmitate-^{14}C. These methods are identical to those previously used (21).

Preparation of tissue slices. For all studies the pigeons were killed by decapitation, the pancreas quickly removed, connective tissue and fat excised, and the tissue chilled in ice-cold Krebs-Ringer phosphate buffer (pH 7.4). Techniques used in preparing slices have been previously described (19).

Preparation of proteins and radioactivity assay. Protein precipitates were washed 4 times in 5% TCA, 1 time with ethanol 95%, and 1 time with ether-ethanol (1:3). The washed precipitate was dissolved in 0.5 N potassium hydroxide. Radioactivity of protein was determined as previously described (23). When only small amounts of protein were available, the protein was coprecipitated with bovine serum albumin in addition of TCA; the precipitate was placed on a Millipore filter and washed with TCA (19).

RESULTS

Table 1 shows effects of atropine administration on L-phenylalanine-^{14}C incorporation into whole tissue proteins by pancreatic slices prepared from pigeons given atropine for 1, 2, 4, or 6 hr. During the 1st hr following atropine administration, no difference was observed between control and atropine-treated groups in amounts of L-phenylalanine-^{14}C incorporated into protein. However, atropine administration for 2 hr resulted in a 24% decrease in L-phenylalanine-^{14}C incorporation. Fasted pigeons incorporated lesser amounts of L-phenylalanine-^{14}C compared with fed controls, and atropine administration for 2 hr resulted in an even further reduction in amounts of L-phenylalanine-^{14}C incorporation. Pigeons given atropine (0.4 mg...
For each experiment six birds were used; three were given saline and three were given atropine. Values are means ± SD. *Time interval following injection of intramuscular saline or atropine before sacrifice. Dose schedule as follows: 1- and 2-hr studies, saline or atropine 0.4 mg each 30 min; 4- and 6-hr studies, saline or atropine 0.4 mg each 60 min. § Pigeons fed ad libitum or fasted 3 days prior to time of study. ¶ TriPLICATE batches of slices were prepared from pooled pancreatic tissue obtained from three birds. Slices were incubated for 60 min at 37°C in Erlenmeyer flasks containing 500 mg tissue, 5.0 ml of tissue culture media, 1.0 µc of L-phenylalanine-14C, and aerated with 95% O2-5% CO2.

Table 4 shows data derived when amylase was isolated by column chromatography from tissue slices obtained from atropine or saline treated pigeons. Atropine treatment for 2 hr resulted in a 35% decrease in specific radioactivity of protein.

For each experiment six birds were used; three were given saline and three were given atropine 0.4 mg each 30 min for 2 hr. Values are means ± SD. * TriPLICATE batches of slices were prepared from pooled pancreatic tissue obtained from three birds. Slices were incubated for 60 min at 37°C in Erlenmeyer flasks containing 500 mg tissue, 5.0 ml of tissue culture media, 1.0 µc of L-phenylalanine-14C, and aerated with 95% O2-5% CO2. $ Significance between means of paired groups of pigeons. † Percent difference between control and atropine groups.

Table 3 shows effects of atropine on L-phenylalanine-14C incorporation into deoxycholate-soluble and -insoluble fractions of microsomal proteins by pancreatic slices.

Table 4 shows effects of atropine on L-phenylalanine-14C incorporation into amylase by pancreatic slices.
Table 5. Effects of atropine on pancreatic amylase content

<table>
<thead>
<tr>
<th>Time Before Sacrifice, hr</th>
<th>Fed or Fasted</th>
<th>No. of Exp</th>
<th>Amylase Units, mg Tissue</th>
<th>P Value</th>
<th>% Diff</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Saline</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Atropine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Fed</td>
<td>10</td>
<td>6.6 ± 3.0</td>
<td>&lt;0.05</td>
<td>+42</td>
</tr>
<tr>
<td>2</td>
<td>Fed</td>
<td>11</td>
<td>5.4 ± 1.0</td>
<td>&lt;0.01</td>
<td>+83</td>
</tr>
<tr>
<td>2</td>
<td>Fasted</td>
<td>7</td>
<td>5.3 ± 1.1</td>
<td>&lt;0.01</td>
<td>+66</td>
</tr>
<tr>
<td>4</td>
<td>Fed</td>
<td>9</td>
<td>7.2 ± 3.8</td>
<td>&lt;0.05</td>
<td>+82</td>
</tr>
<tr>
<td>6</td>
<td>Fed</td>
<td>3</td>
<td>10.0 ± 3.4</td>
<td>&lt;0.05</td>
<td>+70</td>
</tr>
</tbody>
</table>

For each experiment six birds were used; three were given saline and three were given atropine. Values are means ± SD.

Table 5 shows effects of atropine administration on amylase content of pancreatic tissue. Following atropine administration, there was a 45% increase in amylase content within 1 hr, an 83% increase within 2 hr, an 82% increase within 4 hr, and a 70% increase within 6 hr. The increase in amylase content following atropine was less when fasted pigeons were used (66 vs. 83%).

Figure 2 shows that oxygen uptake by pancreatic slices obtained from fed pigeons given atropine for 2 hr was less than saline-treated controls. The decreased rates of in vitro respiration were apparent within 60 min but became greater in magnitude after 180 min incubation.

Figure 3 shows rates of oxidation of glucose-14C and palmitate-14C to 14CO2 by pancreatic homogenates. Groups of three pigeons given saline or atropine 0.4 mg each 30 min for 2 hr. For glucose studies, μmole of glucose-14C oxidized to 14CO2 represented on ordinate, for palmitate-14C studies, μmole of palmitate-14C oxidized to 14CO2 represented on ordinate. Time in minutes on abscissa.

Table 6. Effects of atropine on glucose-14C and palmitate-14C oxidation to 14CO2 by pancreatic homogenates

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Time Before Sacrifice, hr</th>
<th>No. of Exp</th>
<th>Amount of Substrate Oxidized to 14CO2, μmol/mg Protein</th>
<th>P Value</th>
<th>% Diff</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose-14C</td>
<td>2</td>
<td>6</td>
<td>6.2±1.3</td>
<td>&lt;0.05</td>
<td>-21</td>
</tr>
<tr>
<td>Palmitate-14C</td>
<td>2</td>
<td>3</td>
<td>3.8±0.7</td>
<td>&lt;0.01</td>
<td>-10</td>
</tr>
<tr>
<td>Palmitate-14C</td>
<td>4</td>
<td>5</td>
<td>5.6±3.2</td>
<td>&lt;0.05</td>
<td>-48</td>
</tr>
</tbody>
</table>

For each experiment six birds were used; three were given saline and three were given atropine. Values are means ± SD. a Type of 14C-labeled substrate used. b Time interval following injection of intramuscular saline or atropine before sacrifice. c Significance between means of paired groups of pigeons. d Percent difference between control and experimental groups.
Paper chromatographic separation of amino acid from the TCA-soluble pool demonstrated the 14C-label migrated with the same RF as known phenylalanine-14C. The rate of incorporation of L-phenylalanine-14C into protein was linear when data were expressed in terms of milligram tissue wet weight.

**DISCUSSION**

Although the effects of anticholinergic drugs on pancreatic secretion have been extensively studied, there is little information concerning their effect on protein synthesis. Davies, Harper, and MacKay (2) reported that atropine completely prevented the increase in respiration observed following addition of acetylcholine to a cat pancreas slice system. Deutsch and Raper (3) confirmed the observations that atropine in vitro inhibited the enhancement of respiration observed following addition of acetylcholine to a cat pancreas slice system. In addition, they observed that atropine in vitro did not alter resting rates of oxygen uptake. Ilkin and Ilkin (6), using a pigeon slice system, found that atropine abolished the stimulatory effect of carbamylcholine on both enzyme secretion and incorporation of 35P into phospholipids. They also found that addition of atropine to an in vitro slice system did not alter resting rates of respiration (7). Dickman and Morrill (4) observed that atropine in vitro would inhibit the cholinergic-mediated secretion of ribonuclease and enhancement of respiration.

Two views prevail concerning the relationship of protein synthesis to secretion: 1) that synthesis is a continuous process unaffected by changes in secretion (7, 12); and 2) that synthesis is a variable process so that changes in synthesis fluctuate with changes in rates of secretion (18, 20). Our present understanding of control and integration of secretion and synthesis would lead one to conclude that atropine administration might be associated with either a decrease or no change whatever in protein synthesis. Our purpose in studying the effects of atropine on the pigeon pancreas was threefold: 1) to determine whether a decrease in secretion was associated with a decrease in synthesis; 2) to determine the temporal relationship of such a change, if found; and 3) to determine mechanisms involved with decreases in protein synthesis.

It has been shown that in vivo administration of methacholine results within 5-10 min in increased pancreatic amylase secretion, increased L-phenylalanine-14C incorporation into amylase, and increased uridine-3H incorporation into pancreatic nuclear RNA (20).

Pigeons killed 2, 4, and 6 hr following atropine administration were found to have decreased incorporation of L-phenylalanine-14C into tissue proteins (Table 1). However, during the 1st hr, there was no change in L-phenylalanine-14C incorporation, although there was increased amylase content (Table 5). Under these experimental conditions, atropine initially affects processes of secretion as reflected by increases in amylase content and later affects processes of synthesis as reflected by decreases in rates of amino acid-14C incorporation by tissue slice system. Redman et al. (13) have shown that newly synthesized amylase, first found on ribosomes attached to the endoplasmic reticulum, is transported through the endoplasmic reticulum into a deoxycholate soluble subfraction corresponding to contents of microsomal vesicles. The studies in Table 3 indicate decreased synthesis on ribosomes as well as decreased partition of nascent proteins into solubilized membranes and intracisternal content.

It is well recognized in such animals as dogs, cats, rats, and man that pancreatic exocrine secretion as determined by cannulation of the pancreatic duct is decelerated following atropine administration. Similar secretory studies are not available for pigeons. While secretion was not directly measured by intraembolic or pancreatic ductal cannulation, the increased amylase content as shown in Table 5 could only be obtained by decreased rates of secretion or increased rates of synthesis. Data presented in Table 1 indicate that increased rates of synthesis were not likely. Based on the data presented in Tables 1 and 5, it seems plausible to suggest that macromolecular synthesis and migration from ribosomes to the exterior of the cell (secretion) is delayed.

Studies of accumulation of L-phenylalanine-14C were undertaken to examine the possibility that effects of atropine might, in part, be on membrane transport systems conveying amino acids or other nutrients into the cell (Fig. 4). These studies showed no differences in accumulation of L-phenylalanine-14C by pancreatic slices obtained from saline- or atropine-treated animals.

When atropine was administered in vivo and oxygen uptake or oxidation of substrate studied in vitro, atropine administration was associated with decreased oxygen uptake, decreased oxidation of glucose-14C and palmitate-14C to 14CO2. Energy production was one point whereby protein synthesis could be immediately controlled without changes involving translational or transcriptive levels of protein synthesis. From the studies provided, one cannot determine whether decreases in oxidative metabolism were secondary to or preceded decreases in rates of protein syn-
thesis. Additional studies are underway to examine in detail these relationships.

This study was supported by the Veterans Administration and Grant AM13131-02 from the National Institutes of Health.

This paper was presented in part at the Meetings of the Federation

REFERENCES