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Effect of Tetracaine on Pancreatic Protein Synthesis[1] (38788)

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(Introduced by R. Lyman)

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The major interest regarding the pancreatic acinar cell function concerns the mechanism whereby processes of secretion and synthesis are integrated. We now have good evidence from in vivo experiments that feeding (1), cholinergic drugs and pancreozymin (2) are associated with increased pancreatic enzyme secretion as well as synthesis. Moreover, recent data obtained *in vivo* (3) and *in vitro* (4) suggest that changes in the rate of protein synthesis are related to the secretion of the digestive enzymes.

Experiments performed with atropine failed to demonstrate such a dissociation between the two processes; indeed, the anticholinergic drug was shown to initially reduce enzyme secretion in pigeons (5) and cats (6) and later, to decrease protein (5) and RNA synthesis in pigeons (7). In search for an inhibitor of the pancreozymin effect on pancreatic enzyme secretion, it was recently shown that tetracaine blocked secretion initiated by both the duodenal hormone and urecholine (8). The present investigation was therefore performed to determine if the local anesthetic also cause a reduction in protein synthesis and thus perhaps add another evidence for the control of pancreatic protein synthesis by some factors related to the secretory process.

Materials and Methods. Studies were performed using male albino rats of Sprague–Dawley strain (335–355 g wt). Before sacrifice, they were prepared under a definite schedule as previously described (4).

In vivo experiments. Control rats received intraperitoneally 0.5 cc of saline; treated rats received 14.7 mg/kg of tetracaine. All animals were sacrificed by decapitation 30 min after the injection and their pancreases were incubated as described in the *in vitro* experiments.

In vitro experiments and L-phenylalanine-^{14}C incorporation studies. Rats were sacrificed and their pancreas removed; fat and excess tissue were trimmed and pieces of pancreatic tissue (400 mg) were incubated in tissue culture media (5 ml) containing 1 μCi of L-phenylalanine-^{14}C (0.37 mCi/μmole) for 30 min at 37°. Pancreozymin was used at a dose of 2 IVY units/5 ml medium and urecholine 1×10^{-5} *M*. The 2 mM concentration for tetracaine was selected because of the 81% inhibition of amylase secretion observed following CCK-PZ stimulation (8). The reaction was stopped by the addition of 2.1 *N* perchloric (PCA) acid and the tissues were homogenized. The precipitates were washed twice with vigorous stirring in 5 ml of 0.7 *N* PCA. At completion of the washing procedure, the precipitate was dried out at 37° for 20 min. DNA was then extracted by the addition of 3 ml of 0.5 *N* PCA and heated at 90° for 20 min. The final precipitate was then dissolved in 3 ml of 0.3 *N* KOH and heated again at 90° for 20 min. Samples of this solution (0.5 ml) was placed in glass counting vials along with 10 ml of aquasol and radioactivity was determined in a Packard Tri-Carb liquid scintillation counter. The amount of free L-phenylalanine-^{14}C accumulated in pancreatic tissue was estimated as previously described (5). DNA was assayed according to Volkin and Cohn (17). Cholecystokinin-pancreozymin was obtained from the GIH Laboratories, Karolinska Institute, Stockholm, Sweden. The tissue culture medium was prepared by mixing 50 ml of NCTC-109, 10 ml of the nonessential amino acids mixture (MEM 100X), 21.2 mg of calcium chloride, and 1.9 g of glucose and 1 liter of 0.01 *M* Krebs–Ringer phosphate buffer, pH 7.4.

Results. The *in vivo* effect of tetracaine on pancreatic protein synthesis measured *in vitro* is demonstrated in Table I. The local

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TABLE I. EFFECT OF *in vivo* TETRACAINE ON *in vitro* L-PHENYLALANINE-^{14}C INCORPORATION INTO TOTAL PROTEINS AND ACCUMULATION INTO PANCREATIC TISSUE.

Groups	Incorporation[a] L-phenylalanine-^{14}C (DPM/μg DNA)	% Diff.[c]	Accumulation[b] L-phenylalanine-^{14}C (DPM/μg DNA)	% Diff.[c]
Control (8)[e]	233.8 ± 9.3		60.6 ± 7.3	
Tetracaine[d] (12)	179.7 ± 13.5*	−23.2	84.1 ± 6.4**	+41.0

[a] Pancreatic tissue (400 mg/5 ml medium) was incubated for 30 min at 37° and incorporation of L-phenylalanine-^{14}C (1 μCi) into PCA insoluble material was determined. Results are the mean ± SE.

[b] Amount of L-phenylalanine-^{14}C into cold PCA soluble material.

[c] Percentage difference between control and tetracaine.

[d] Tetracaine was injected intraperitoneally at the dose of 14.7 mg/kg and the animals were sacrificed 30 min later.

[e] Number of animals in each group.

* $P < 0.01$.

** $P < 0.02$.

anesthetic at a dose of 14.7 mg/kg caused a significant decrease of 23.2% ($P < 0.01$) in total protein synthesis. However the amount of radioactive L-phenylalanine accumulated into pancreatic tissue was significantly increased by 41% ($P < 0.02$).

Table II shows the *in vitro* effect of tetracaine on pancreatic protein synthesis. When added alone to the incubation medium, tetracaine (2 m*M*) reduced significantly by 38% ($P < 0.001$) the incorporation of L-phenylalanine-^{14}C into total protein. This inhibitory effect of tetracaine does not differ significantly from that of 29% ($P < 0.001$) observed when pancreozymin was added to the medium. However, the reduction in total protein synthesis was further decreased to 60% ($P < 0.001$) of controls when tetracaine was combined to CCK-PZ. When compared to pancreozymin, the addition of tetracaine to the duodenal hormone caused a 44% ($P < 0.001$) decrease in protein synthesis. These inhibitory effects of pancreozymin and tetracaine on protein synthesis do not seem to depend on a reduced availability of the radioactive amino acids. Indeed, increases in free L-phenylalanine-^{14}C into pancreatic tissue were observed after pancreozymin, 25% ($P < 0.01$); pancreozymin plus tetracaine, 40% ($P < 0.001$) and tetracaine, 65% ($P < 0.001$) additions.

Table III shows the *in vitro* effect of tetracaine on pancreatic protein synthesis following *in vitro* urecholine stimulation. Urecholine (1×10^{-5} *M*) was associated with a decrease of 36% ($P < 0.001$) in total protein synthesis when compared to control. The addition of tetracaine to urecholine further reduced the synthesis to 60.5% ($P < 0.001$) when compared to control. However, when compared to the cholinergic drug, the addition of tetracaine to urecholine caused a 38% ($P < 0.001$) decrease in total protein synthesis. The fact that urecholine and urecholine plus tetracaine increased the free L-phenylalanine-^{14}C into pancreatic tissue by 17% ($P < 0.05$) and 41% ($P < 0.001$) cannot account for the decrease observed in protein synthesis.

Discussion. The question of whether secretion is a prerequisite for any increases in pancreatic protein synthesis is debated. It was believed that synthesis was a continuous process unaffected by changes in secretion (9, 10) while growing evidence suggests that synthesis can vary upon changes in the rates of secretion (3, 4, 11). Even if increases in secretion and synthesis can be dissociated in the early moments following the initiation of secretion (3), it remains that synthesis is always increased *in vivo* at least 30 min up to 4 hr after stimulation (2, 12). Moreover, when secretion was inhibited by atropine, a decrease in protein synthesis was also observed (5).

Data from Table I show that *in vivo* tetracaine reduced pancreatic protein synthesis as did atropine (5); however, this reduction appeared earlier than that observed after atropine: 30 min compared to 2 hr

TABLE II. EFFECT OF TETRACAINE ON L-PHENYLALANINE-^{14}C INCORPORATION INTO TOTAL PROTEINS AND ACCUMULATION INTO PANCREATIC TISSUE FOLLOWING *in vitro* PANCREOZYMIN STIMULATION.

Groups	Incorporation[a] L-phenylalanine-^{14}C (dpm/μg DNA)	% Diff.[c]	Accumulation[b] L-phenylalanine-^{14}C (dpm/μg DNA)	% Diff.[c]
Control (22)[e]	220.7 ± 11.4		74.9 ± 4.5	
Pancreozymin[d] (12)	156.6 ± 6.7**	−29.0	93.6 ± 5.6*	+25.0
Pancreozymin + Tetracaine (11)	87.7 ± 6.3**, xxx	−60.3	105.2 ± 5.9**	+40.5
Tetracaine[d] (11)	136.4 ± 14.8**	−38.2	123.5 ± 7.7**, xxxx	+65.0

[a] Pancreatic tissue (400 mg/5 ml medium) was incubated for 30 min at 37° and incorporation of L-phenylalanine-^{14}C (1 μCi) into PCA insoluble material was determined. Results are the mean ± SE.
[b] Amount of L-phenylalanine-^{14}C into cold PCA soluble material.
[c] Percentage difference between control and the other groups.
[d] Concentration of PZ (2 IVY units/5 ml) and tetracaine (2 *mM*).
[e] Number of animals in each group.
* $P < 0.01$ when compared to controls.
** $P < 0.001$ when compared to controls.
xxx $P < 0.001$ when compared to PZ.
xxxx $P < 0.01$ when compared to PZ.

TABLE III. EFFECT OF TETRACAINE ON L-PHENYLALANINE-^{14}C INCORPORATION INTO TOTAL PROTEINS AND ACCUMULATION INTO PANCREATIC TISSUE FOLLOWING *in vitro* URECHOLINE STIMULATION.

Groups	Incorporation[a] L-phenylalanine-^{14}C (dpm/μg DNA)	% Diff.[c]	Accumulation[b] L-phenylalanine-^{14}C (dpm/μg DNA)	% Diff.[c]
Control (22)[e]	220.7 ± 11.4		74.9 ± 4.5	
Urecholine[d] (12)	141.1 ± 2.1*	−36.1	87.8 ± 2.9**	+17.0
Urecholine + Tetracaine[d] (12)	87.3 ± 4.8*, xxx	−60.5	107.8 ± 3.2x, xxx	+41.3

[a] Pancreatic tissue (400 mg/5 ml medium) was incubated for 30 min at 37° and incorporation of L-phenylalanine-^{14}C (1 μCi) into PCA insoluble material was determined. Results are the mean ± SE.
[b] Amount of L-phenylalanine-^{14}C into cold PCA soluble material.
[c] Percentage difference between control and the other groups.
[d] Concentration of urecholine (1×10^{-5} *M*) and tetracaine (2 m*M*).
[e] Number of animals in each group.
* $P < 0.001$ when compared to controls.
** $P < 0.05$ when compared to controls.
xxx $P < 0.001$ when compared to urecholine.

(5). Moreover, the decrease in protein synthesis following tetracaine happened before any changes in enzyme content can be seen (data not included) while with atropine, increases in pancreatic amylase content was observed one hour before any detectable effect on protein synthesis (5).

The inhibition of pancreatic protein synthesis following *in vitro* pancreozymin (29%, Table II) and urecholine (36%, Table III) confirms what has been previously shown in similar conditions (2). This decrease in incorporation of amino acids into protein could be ascribed to a regulated distribution of the energy supply available within the cell; indeed, as previously suggested, the energy would be used in the early moments following stimulation for enzyme secretion (3) and RNA synthesis (13).

One possible explanation for the effect of tetracaine on protein synthesis is the following: the drug might prevent the ATP translocation from the mitochondria to the cytoplasm as suggested by Spencer and Bygrave

in rat liver (14). The local anesthetic however does not inhibit oxygen consumption by pancreatic tissue in our conditions (8). These data thus suggest that tetracaine would not interfere with the ATP generating system but would impair the availability of ATP into the cytoplasm.

Webster *et al.* (15) have shown that pancreozymin and methacholine injections to pigeons were associated with increases in rates of palmitate-^{14}C oxidation; they suggested that this increment in fatty acid oxidation may in part fulfill the energy requirements necessitated for protein synthesis and possibly enzyme secretion. Siddle and Hales (16) indicated that tetracaine was a potent inhibitor of lipolysis in isolated rat fat-cells stimulated by adrenaline. This inhibitory effect of tetracaine on fatty acid oxidation might be another alternative accounting for the decrease in protein synthesis seen after tetracaine alone or in combination with pancreozymin and urecholine by reducing part of the energy necessary for this process.

These two possibilities might explain the reduction in protein synthesis if one agrees that tetracaine gets into the cell which has not yet been demonstrated. Another explanation for the decrease in protein synthesis might be related to an effect of tetracaine at the plasma membrane level. Case *et al.* (6) have shown that acetylcholine and CCK-PZ were associated with two distinct peaks in pancreatic cyclic AMP content; one happening within one minute and the other starting between 15 and 20 min. The second cyclic AMP peaks has been timed with increases in protein synthesis. Previously, we have demonstrated (8) that tetracaine was a good inhibitor of pancreatic adenylate cyclase and in doing so it will abolish the formation of the cyclic nucleotide that might be necessary for phosphorylation of protein kinases directly involved in critical steps of protein synthesis.

Because tetracaine had been shown to be a potent inhibitor of CCK-PZ and urecholine stimulated amylase secretion (8), we thought that the drug might be a good tool to study and determine if the gastrointestinal hormone and cholinergic drug can increase protein synthesis while secretion has been inhibited. The present data show that both secretagogues cannot overcome the inhibitory effect of tetracaine on protein synthesis and hence add another evidence that pancreatic protein synthesis might be controlled by factors related to the secretory process.

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