Human calcitonin stimulates salivary amylase output in man¹

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SUMMARY Salivary amylase output in man increases after injection of synthetic human calcitonin. This effect is dose dependent.

All known effects of calcitonin in gastrointestinal glands are of an inhibitory nature. The present study describes a stimulatory effect of synthetic human calcitonin (hCT) on an exocrine gland. hCT increases amylase output from human salivary glands. *In vitro* experiments with isolated cells of rat salivary glands have confirmed this effect and suggest a direct action of hCT on the cellular level.

Methods

Six healthy normocalcaemic subjects (four males, two females) were studied. All tests were started at 8 a.m. Whole saliva was collected by a standardized method (Blum and Woodall, 1972; Makhoul and Blum, 1972). Every 15 minutes the subjects emptied their mouth by carefully swallowing saliva. For exactly five minutes they made chewing movements and voided saliva into a preweighted beaker by spitting four times per minute. The subjects rested during an interval of 10 minutes. Then they swallowed again and spat saliva for another five minute period into a new beaker. This procedure was repeated four times during a basal period of 55 minutes and another four times after injection of the test substance. Out of the four basal samples the first one was discarded.

Injection of one of the following test substances was done intravenously during three minutes:

1. Synthetic human calcitonin (hCT; 47 175-Ba; Ciba-Geigy) dissolved in 2 ml distilled water in concentrations of 0.16, 0.39, 0.63, 0.94 and 1.25 µg/kg.

2. Pancreozymin (PZ; Pancreozymin, Boots) dissolved in 2 ml 0.9% NaCl in concentrations of 0.5, 1.0, and 2.0 U/kg.

3. Two millilitres of physiological saline as placebo.

A butterfly needle was inserted in a cubital vein before the tests were started. Injections were performed in a single blind manner. Every 15 minutes the subjects recorded possible side effects on a pre-printed protocol-form.

In one subject the following additional experiments were performed: subcutaneous injections of bethanechol chloride (Urecholine chloride, Merck Sharp and Dohme) in doses of 8, 20, 40, 60, and 80 µg/kg.

The amount of saliva was determined by weighing. Bicarbonate was determined by a back titration method. Amylase was determined by an amyloclastic method (Phadebas, Pharmacia).

In every experiment with one of the test substances, stimulation of a salivary constituent is calculated by the equation

$$Q = \frac{H_s \cdot P_b}{H_b \cdot P_s}$$

H is the output in an experiment with hormone injection and

P the output in a placebo test

s represents the first hour after injection and

b represents the basal period before injection, respectively.

Statistical significance was determined by Wilcoxon's signed rank test.

Results

In the placebo experiments amylase output is 32.6 ± 7.6 U/h·kg (mean $\pm$ SEM) before injection of NaCl and 35.2 ± 8.4 U/h·kg after injection respectively ($P > 0.1$).

Injection of hCT leads to a significant increase of amylase output. This effect is dose dependent. The

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Figure Dose response curve with human calcitonin and pancreozymin. Stimulation (Q) is defined in the text. ●—● Amylase (mean $\pm$ SEM). ○—○ Volume (mean $\pm$ SEM). ■····■ Bicarbonate (mean $\pm$ SEM). * $P < 0.05$.

Table Human salivary gland: volume and amylase output (mean $\pm$ SEM)

	Calcitonin ($\mu\text{g/kg}$)					Pancreozymin (U/kg)				NaCl (%)
	0.16	0.39	0.63	0.94	1.25	0.5	1.0	2.0	0.9	
Amylase (U/h·kg)										
Basal	26.64 $\pm$ 9.66	26.22 $\pm$ 10.44	21.66 $\pm$ 8.67	26.46 $\pm$ 8.07	24.75 $\pm$ 8.07	35.94 $\pm$ 6.75	28.38 $\pm$ 6.84	29.07 $\pm$ 5.58	32.55 $\pm$ 7.62	
First hour	58.86 $\pm$ 31.08	44.13 $\pm$ 9.93	47.46 $\pm$ 14.82	57.30 $\pm$ 10.11	64.53 $\pm$ 18.03	49.11 $\pm$ 11.49	35.13 $\pm$ 9.27	44.22 $\pm$ 10.38	35.16 $\pm$ 8.37	
Volume ($\mu\text{l/h}\cdot\text{kg}$)										
Basal	576 $\pm$ 195	486 $\pm$ 126	531 $\pm$ 159	573 $\pm$ 132	543 $\pm$ 135	603 $\pm$ 93	558 $\pm$ 90	579 $\pm$ 84	675 $\pm$ 138	
First hour	564 $\pm$ 177	537 $\pm$ 114	570 $\pm$ 132	558 $\pm$ 123	597 $\pm$ 174	645 $\pm$ 96	585 $\pm$ 96	630 $\pm$ 105	525 $\pm$ 75	

dose-response curve is shown in the Figure. Amylase output rises from 26.5 ± 8.1 to 57.3 ± 10.1 U/h·kg with a hCT dose of $0.94 \mu\text{g/kg}$, and from 24.8 ± 8.1 to 64.5 ± 18.0 U/h·kg with a dose of $1.25 \mu\text{g/kg}$, respectively. With 0.94 and $1.25 \mu\text{g/kg}$ hCT Q_{amylase} is 3.0 ± 0.9 ($P < 0.05$). The mean volume output ranges from 486 to $675 \mu\text{l/h}\cdot\text{kg}$ and does not show any significant difference before and after injection. Bicarbonate output parallels volume output both before and after stimulation (Table).

With three different doses of PZ, amylase, volume and bicarbonate output do not rise significantly.

Bethanechol chloride produces a dose dependent stimulation of amylase output. With a dose of $8 \mu\text{g/kg}$ Q_{amylase} is 2. Volume output and bicarbonate output are stimulated by the same factor. With 60 – $80 \mu\text{g/kg}$ Q_{amylase} is 20 and Q_{volume} is 8, respectively.

Thus, in contrast with hCT, cholinergic agents also stimulate volume and bicarbonate output and achieve higher rates of amylase output.

Two subjects complained of slight nausea after the injection of saline. In all hCT experiments, subjects complained of slight nausea. In PZ experiments, nausea was more marked. Serum calcium did not change in any of the tests. Bethanechol chloride produced epigastric pain and profuse sweating.

Discussion

In these experiments hCT has a stimulatory effect on salivary amylase output in man. As this effect is dose dependent, it might serve as a simple bioassay for hCT.

The stimulatory effect of hCT on salivary amylase

output is of interest because hCT inhibits amylase output from the pancreas (Schmidt *et al.*, 1971; Hotz *et al.*, 1973). This observation, therefore, is an example of the fundamental functional difference between pancreas and salivary glands despite their close histological resemblance. Other differences have previously been reported. For example, in mice alpha adrenergic stimulation leads to hyperpolarization of the cell membrane in parotis (Petersen and Pedersen, 1974), but has no effect on pancreatic acinar cells (Matthews and Petersen, 1973). Cholinergic stimulation leads to hyperpolarization of parotis (Petersen and Pedersen, 1974) and depolarization of pancreas (Matthews and Petersen, 1973; Matthews *et al.*, 1973). In rats cholinergic stimulation produces potassium and water secretion in slices of parotid tissue (Schramm and Selinger, 1974), while it leads to amylase output from slices of pancreatic tissue (Matthews *et al.*, 1973). Ca^{++} ionophore A 23 187 leads to potassium release in parotis tissue and to amylase secretion in pancreas tissue (Selinger *et al.*, 1974). As shown by the present study PZ leads to only very minor amylase secretion from the salivary gland while it produces maximal amylase output from the pancreas. Secretin, which leads to maximal stimulation of fluid and bicarbonate secretion from the pancreas, has virtually no effect on the salivary glands (Drack, unpublished observations).

Our results with secretin and PZ are in apparent contrast with the conclusions of Mulcahy and his colleagues who in 1972 reported a 50% stimulation of salivary amylase concentration after combined secretin—PZ stimulation. However, these authors do not report amylase output and placebo experiments. In our own series amylase output increased by 26% one hour after injection of NaCl. This effect is probably due to diurnal variations of amylase output. Therefore, secretin and PZ have no physiological effect on salivary secretion, as Bayliss and Starling (1902) have already pointed out.

The mechanism by which hCT stimulates amylase output from the salivary glands is unknown.

In vitro experiments using isolated cells of rat salivary glands suggest that stimulation is due to a direct action of calcitonin at the cellular level. Calcitonin stimulates amylase release of isolated cells. In contrast with adrenaline this effect is not accompanied by increased cellular uptake of calcium (Kondo *et al.*, 1976).

Many other effects of calcitonin on the gastrointestinal tract have been demonstrated in previous studies. For example, CT inhibits pentagastrin stimulated lower oesophageal sphincter pressure (Waldeck *et al.*, 1973); oral and parenteral CT inhibit gastric secretion (Hesch *et al.*, 1971; Hesch *et al.*, 1972; Becker *et al.*, 1974; Ziegler *et al.*, 1974); hCT

depresses fasting serum gastrin in patients with medullary carcinoma of the thyroid and those with peptic ulcer (Go *et al.*, 1972; Becker *et al.*, 1974); it inhibits contraction of the gallbladder (Winckler *et al.*, 1973); it stimulates small bowel motility (Gray *et al.*, 1973); and it inhibits glucose stimulated release of insulin from the endocrine pancreas (Ziegler *et al.*, 1972). All these effects including those on the exocrine pancreas (Schmidt *et al.*, 1971; Hotz *et al.*, 1973) and our own observations on salivary glands have been achieved with doses of hCT which produce serum concentrations about 100 times higher than those measured in normal man. Therefore, it is unlikely that the gastrointestinal effects of hCT are of major physiological importance.

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