N.J.) was freshly dissolved in 0.3*M* sodium acetate solution and injected intraperitoneally at a dose of 13 mg/kg, 1 to 2 hours before the challenging dose of arachidonic acid.

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Selective Pancreatic Enzyme Secretion due to a New Peptide Called Chymodenin

Abstract. *Chymodenin, a peptide newly isolated from porcine duodenum, elicited a rapid threefold increase in secretion of chymotrypsinogen while increasing secretion of total protein by only about 40 percent; lipase secretion was unaffected. The pancreas appears able, under chymodenin stimulation, to rapidly alter the transport of an individual enzyme as opposed to producing en masse secretion of enzyme.*

Digestion in higher organisms is the result of enzymatic hydrolysis of a wide variety of chemical linkages present in the ingested meal, including selected peptide bonds, glycosidic linkages, and esters of fatty acids. The major source of digestive enzymes, the exocrine pancreas, apparently exerts control over specific enzymes over the long term in response to specific diets (*1*, *2*). It is not clear, however, whether the pancreas may in the short term (that is, within the digestive period of a single meal) alter the rate of secretion of individual enzymes selectively, reflecting intestinal digestive demands. Rapid selective secretion of protein by pancreatic tissue was proposed nearly a century ago (*3*) and appears to occur in other tissues, such as the pituitary gland. Recent work from this laboratory demonstrated that the pancreas responds to a specific stimulus—the presence of lysine in the intestine—by rapidly altering the relative amounts of enzymes transported into the secreted fluid (*4*). We have suspected that if short-term selective secretion of enzyme from the pancreas were to play a role in the regulation of digestive catalysis, then the regulation would in all likelihood involve changes in the transport pattern for specific enzymes (expressed within minutes) followed by longer term compensatory alterations in enzyme synthesis.

We examined the effect of intravenous administration of a newly isolated peptide, purified from porcine duodenum, upon the secretion of enzyme from the pancreas of the anesthetized rabbit in situ. We report here that administration of the new peptide caused dramatically increased secretion of chymotrypsinogen (ChTg) within 15 minutes, whereas lipase output remained unchanged and only a modest increase in protein output was observed. We have named this peptide chymodenin, after the enzyme which secretion it stimulates and its tissue of origin.

The starting material for the chymodenin purification was a peptide-containing extract of porcine duodenum that had been separated from cholecystokinin-pancreozymin (CCK-PZ) and secretin after extraction with acidic methanol followed by precipitation with KOH as described by Mutt (*5*). This fraction of the extract had previously been shown to contain peptides which in low concentrations were capable of releasing enzymes from isolated rat pancreatic zymogen granules in vitro (*6*). The fraction was further purified to yield an apparently homogeneous peptide by sequential ion exchange and gel-filtration chromatography on SP-Sephadex C-25, eluted in an ammonium bicarbonate gradient; on carboxymethyl cellulose in an ammonium bicarbonate gradient; by equilibrium chromatography on QAE-Sephadex A-25 in sodium pyrophosphate; and finally by gel filtration on Sephadex G-75. Following this purification, the peptide was homogeneous as judged by polyacrylamide disc gel electrophoresis in both acidic and sodium dodecyl sulfate gel systems. It differed in amino acid composition and electrophoretic mobility from any of the known gastrointestinal hormones and had an apparent molecular weight of about 5000 determined both by minimal amino acid composition and gel-filtration chromatography. It showed no secretin or CCK-PZ activity in the cat even in milligram doses. Details of the purification and chemistry will be presented elsewhere (*7*).

Male albino New Zealand rabbits (weighing 1.7 to 2.5 kg) were anesthetized with Dial with urethane [0.8 ml per kilogram of body weight (Ciba Pharmaceutical Co.)]. After cannulation of the pancreatic duct and an initial 1-hour stabilization period, secretion was collected for sequential 20-minute periods, and all collected samples were analyzed for protein, ChTg, lipase, and fluid output. Chymodenin was administered intravenously at a dose of 10 μg in 1.0 ml of 0.9 percent NaCl per animal; controls received an equal volume of 0.9 percent saline. Injections of either chymodenin or saline were made in each animal at 2 hours and again at 4 hours after cannulation. Animals were treated exclusively with either chymodenin (nine animals) or saline (nine animals). Samples taken during the three periods in the hour immediately following each injection were used as the treated samples. Protein, ChTg, and lipase were measured as described elsewhere (*8*), and fluid output was measured by weight.

Preliminary experimental results indicated that unstimulated output amounts of lipase, ChTg, and protein were highly variable from time to time and from animal to animal. This variability, common in studies of this nature and apparently caused by endogenous unspecified influences on pancreatic output, frequently introduces difficulty in statistically distinguishing effects due to treatment. Thus data collected from glands in a potentially "responsive" state (unstimulated) are not usually separated from data derived from the same glands during periods of endogenously stimulated secretion. In the present study, preliminary observations indicated that lipase secretion, although variable as a function of time and animal, was unaffected by the peptide, and therefore a high lipase output in any sample collection period was taken to indicate substantial endogenous stimulation of enzyme secretion during that period. To identify periods of active endogenous secretory stimulation, we calculated the mean lipase outputs for the control group and the group treated

with chymodenin during the hour following the first and, separately, the second injections. Individual 20-minute samples that exhibited a lipase output above the mean of the group were evaluated for ChTg and protein outputs independently of samples below the mean for that group; all samples were placed in either one group or the other. In this way, either one, two, or three observations after each injection were placed in one of the two groups (high or low lipase). The means were determined for the values after the injections, which gives $N = 1$ for each independent injection (Table 1). This analysis, a simple method for separating "basally" from "actively" secreting intervals, while useful in permitting the inclusion of all samples in the analysis, in all likelihood underestimates the true responsiveness of basal glandular states to a given stimulus.

Chymotrypsinogen output was increased approximately threefold following chymodenin administration in the "basal" lipase output range (Table 1). Even in endogenously stimulated glands, the relative proportion of ChTg in the secreted protein mixture was seen to increase in some experiments (Fig. 1; the mean lipase output was high in this animal, 34.2 μmole substrate hydrolyzed per minute by enzyme secreted per 15-minute collection period). Within the first 15-minute period, ChTg specific activity (substrate hydrolyzed per minute per 15-minute collection period per milligram of protein for same period) increased above the preceding preinjection period and returned to or below preinjection levels by 45 to 60 minutes (Fig. 1). The response was clearly visible at the lowest dose employed, 100 ng of chymodenin per kilogram of body weight. As expected, pancreatic lipase output in the "basal" range was not augmented

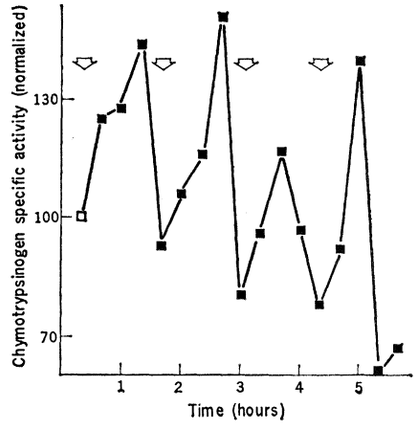

Fig. 1. Specific activity of chymotrypsinogen after repetitive hourly injections of chymodenin in a single animal. The observed specific activities were normalized so that 100 equals the specific activity in the period immediately prior to the first injection. Increasing doses of peptide were given at the times indicated by the arrows. The sequence was 100, 250, and 500 ng, and 2.5 μg (per kilogram of body weight).

by injection of the peptide (Table 1). Injection of chymodenin also increased protein output by about 40 percent in this range of low lipase output (Table 1). In contrast, in "endogenously stimulated" samples, indicated by lipase outputs above the group mean, no enhancement of ChTg or protein was observed following peptide injection (Table 1). Mean lipase output was lower in the "endogenously stimulated" range, but not significantly lower (Table 1).

Since the response was observed within 15 minutes, the secretion of newly synthesized ChTg is unlikely to account for the enhanced secretion of this enzyme, at least in the early period, and the response is probably the result of increased transport of this enzyme by the pancreas. Estimates of the ChTg content of the pancreatic fluid in mammals including dog, beef, rat, and pig are between 10 to 20 percent of total digestive enzyme protein (*9*). Unless the rabbit value is less than 10 percent, at least half of the increased protein output can be accounted for by the increase in ChTg; a value of 20 percent would require that increased ChTg secretion completely account for the increase in protein output. This should be susceptible to direct experimental verification for individual treated samples.

In vitro studies on the effect of chymodenin on enzyme output by the rabbit pancreas (*10*) show a response similar to that reported here for in situ studies: a marked increase in ChTg output, no change in lipase secretion, and a moderate protein response. This response differs from the in vitro response to CCK-PZ (*4, 11*) or methacholine chloride (*10*). Cholecystokinin-pancreozymin produced a large increase in protein output accompanied by little or no increase in ChTg secretion; methacholine treatment in vitro elicited a massive increase in protein output accompanied by increased output of lipase and ChTg. The response to chymodenin thus appears unique and specific.

The observation that a peptide, chymodenin, purified from duodenal extracts elicits a specific increase in ChTg secretion from the exocrine pancreas suggests that the regulation of digestive enzyme secretion may be enzyme-specific and raises the possibility that other peptides with analogous functions may exist. In addition, these results are difficult to reconcile with the current commonly held view that enzyme secretion by the pancreas, and by other glands as well, occurs only by the parallel discharge en masse of all molecules stored within the secretory granule (*12*). Since evidence suggests that the different species of enzymes

Table 1. Effect of chymodenin on chymotrypsinogen, lipase, and protein output by the rabbit pancreas in situ. The data include the mean (± standard error of the mean) after treatment of all 20-minute sample collection periods in the hour following injection, in which the lipase output was in the "basal" or the "endogenously stimulated" output ranges as described in the text. Enzyme activity is expressed in micromoles of substrate hydrolyzed per minute by enzyme secreted per 20-minute collection period. Protein is expressed as micrograms secreted per 20-minute collection period. The numbers in parentheses are the numbers of injections of either saline or chymodenin administered (one or two per animal). Nine control animals and nine animals injected with chymodenin were used. The dose of chymodenin was 10 μg per injection; injections were administered 2 hours apart.

Chymotrypsinogen		Lipase		Protein	
Basal	Endogenously stimulated	Basal	Endogenously stimulated	Basal	Endogenously stimulated
		Saline			
3.1 ± 0.4 (9)	12.9 ± 3.9 (8)	3.9 ± 0.8 (11)	17.1 ± 1.78 (8)	556 ± 45 (10)	1013 ± 128 (7)
		Chymodenin			
9.1 ± 2.0 (16) $P < .01$*	12.8 ± 2.8 (11)	4.2 ± 0.6 (16)	13.2 ± 1.35 (9)	798 ± 71 (15) $P < .025$	1060 ± 126 (11)

* Calculated by using Cochran's (*16*) modification of the Behrens-Fisher test for populations of unequal variance.

are packaged together in the secretion granules (*13*), the enhanced ChTg secretion observed in these experiments did not result from secretion of the entire contents of the zymogen granule. Rather, the present observations fit with other examples of nonparallel and enzyme-selective transport reported during the past several years (*2, 11, 14*) and provide a potential physiological link between a natural stimulant and the enzyme-specific secretory response (*15*).

JOEL W. ADELSON
S. S. ROTHMAN
Department of Physiology, University of California, San Francisco 94143

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Radioreceptor Assay for 1α,25-Dihydroxyvitamin D_3

Abstract. *A competitive protein binding assay with a sensitivity of 80 picograms has been developed for 1α,25-dihydroxyvitamin D_3, the hormonal form of vitamin D_3. 1α,25-Dihydroxyvitamin D_3 displaced tritiated hormone from a cytosol-chromatin receptor preparation isolated from chick small intestine, providing a simple assay for the hormone. The concentration of 1α,25-dihydroxyvitamin D_3 in human plasma, as determined by this assay, is approximately 6 nanograms per 100 milliliters; in patients with renal disease the concentration of this kidney-produced hormone is significantly lower.*

Vitamin D_3 is metabolized to 25-hydroxyvitamin D_3 (25-OH-D_3) (*1*) and subsequently to 1α,25-dihydroxyvitamin D_3 [1α,25-$(OH)_2$-D_3] (*2, 3*). The latter conversion occurs exclusively in the kidney (*4*) and produces what is considered to be the hormonal form of the vitamin. 1α,25-$(OH)_2$-D_3 is the most active and fastest acting metabolite of vitamin D in stimulating both intestinal calcium transport and bone mineral mobilization (*5*).

Previous detection of 1α,25-$(OH)_2$-D_3 has been carried out only in experimental animals, and there is little information available on the occurrence of this hormone in humans. Defects in the production of this hormone may explain abnormal calcium metabolism in diseases such as chronic renal failure. There is also evidence that parathyroid hormone is involved in the regulation of the renal synthesis of 1α,25-$(OH)_2$-D_3 (*6*), suggesting that abnormal production of parathyroid hormone may affect calcium homeostasis by altering the circulating level of 1α,25-$(OH)_2$-D_3. In order to study the role of 1α,25-$(OH)_2$-D_3 in calcium metabolism in normal humans and to study its possible relation to metabolic bone disease, we have developed a sensitive, competitive protein binding assay capable of detecting circulating amounts of the hormone.

The assay depends upon the binding of 1α,25-$(OH)_2$-D_3 to its chromatin receptor in the small intestine (*7*). This receptor is saturable and binds 1α,25-$(OH)_2$-D_3 with high affinity ($K_d \approx 10^{-9}M$). The receptor is specific for 1α,25-$(OH)_2$-D_3, and the radioactive hormone is displaced from its binding site only by much higher concentrations of 25-hydroxyvitamin D_3 (150-fold excess) and vitamin D_3 (> 20,000-fold excess) (*8*). Moreover, 1α,25-$(OH)_2$-D_3 binds initially to a cytosol receptor protein, and this sterol receptor complex migrates into the nucleus, via a temperature-dependent process, where it associates with the chromatin (*8*). In practice the receptor system is prepared by isolating Triton X-100–washed chromatin from intestines of rachitic chicks (*7*) and reconstituting it with the 100,000*g* supernatant from a centrifuged homogenate of the same intestine. A 200-μl portion of the receptor system (containing 100 μg of DNA) was added to a small tube containing nitrogen-dried sterol or

Table 1. Concentration of 1α,25$(OH)_2$-D_3 in plasma of normal individuals and in patients with disorders in calcium metabolism; S.D., standard deviation.

Group	Patient (treatment)	Calcium (mg/100 ml)	1α,25-$(OH)_2$-D_3* (ng/100 ml ± S.D.)	Average of 1α,25-$(OH)_2$-D_3 (ng/100 ml ± S.D.)
Normal	20 (none)	10.5–11.7†	4.1–8.5†	6.4 ± 1.2
Renal disease	1 (no dialysis)	6.5	3.1 ± 0.4	
	2 (dialysis 1.3 yr)	12.2	2.9 ± 0.4	
	3 (dialysis 3.5 yr)	8.5	2.3 ± 0.6	2.6 ± 0.5‡
	4 (dialysis 1.7 yr)	11.8	1.9 ± 0.3	
	5 (dialysis 0.7 yr)	11.0	2.6 ± 0.2	
Postsurgical hypoparathyroid§	1 (surgery 1970)	9.5	3.3 ± 0.5	
	2 (surgery 6/1973)	8.6	6.7 ± 0.4	4.7 ± 1.8‖
	3 (surgery 1968)	10.1	4.1 ± 0.2	
Presumed primary hyperparathyroid	1 (no surgery)	12.6	12.3 ± 0.6	
	2 (surgically confirmed adenoma)	15.5	11.4 ± 0.7	
Rachitic chicken		5.3	1.8 ± 0.6¶	
Normal chicken		11.4	10.1 ± 1.2¶	

* Represents the average of four assays; corrected for losses occurring in purification sequence. † Range for 20 normal volunteers. ‡ Significantly different from normal, $P < .005$. § All patients received 100,000 international units of vitamin D_2 daily. ‖ Significantly different from normal, $P < .10$. ¶ Represents two separate assays on pooled plasma from 14 chickens each.