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Evidence of a Direct TRH Effect on the Rat Exocrine Pancreas

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In the present study, the effect of TRH on amylase secretion was determined both *in vivo*, by cannulating the pancreatic duct of rats, as well as *in vitro*, by using isolated lobules and dissociated acini. The results show that TRH inhibited both basal and stimulated *in vivo* amylase secretion. Nevertheless, the *in vitro* experiments failed to show a TRH-related inhibitory effect when TRH was used alone, although the hormone did blunt the secretion elicited by CCK_8 and bethanechol from isolated lobules and dissociated acini. Results suggest that TRH can inhibit stimulated amylase secretion in rats through a direct effect on acinar cells.

Key words: TRH, Amylase secretion, Exocrine pancreas.

The thyrotropin-releasing hormone (Pyroglutamyl - histidil - proline amide) (TRH) was initially isolated from the hypothalamus. However, its distribution was ubiquitous and it has been shown by LEPPÄLUOTO *et al.* (17) that TRH is found throughout the gastrointestinal tract of the rat.

KAWANO *et al.* (12) have demonstrated that TRH can be measured by specific radioimmunoassay in pancreas extracts from adult rats. The highest tissue concentration was found in newborn rats, progressively decreasing toward lower levels in adult animals (6, 16). There is compelling evidence that TRH is located in the islets of Langerhans and more specifically within the β cells producing insulin (1, 13). Its physiological role has not yet been ascertained, but DOLVA and STADDAS (5) and UBERTI *et al.* (22) have proposed that TRH could be involved in the regulation of the digestive function, including gastric secretion and motility.

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Recently, GLASBRENNER *et al.* (8) have shown that chronic TRH administration in rats induces pancreatic hyperplasia but decreases the pancreatic concentration of digestive enzymes, probably by interfering with enzyme synthesis. On the other hand, a study conducted by GULLO and LABO (10) in healthy human subjects, using intravenous TRH infusion, showed a marked inhibitory effect of the hormone on the enzymatic pancreatic secretion stimulated by secretin and cholecystokinin. Since TRH could influence exocrine pancreatic secretion through and extrapancreatic effect, the possibility of an indirect effect due to TRH on acinar cells cannot be excluded.

The present study aims at ascertaining if such an indirect mechanism does exist and to evaluate the action of TRH on *in vivo* and *in vitro* secretion.

Materials and Methods

In vivo *amylase secretion.* After an overnight fast, male Wistar rats, weighing 200-250 g, were intraperitoneally anesthetized with sodium pentobarbital (50 mg/kg). The abdominal wall was opened through a midline surgical incision, the duodenum identified and incised along the anteromesenteric border, and the pancreatic duct cannulated with vinyl tubing (Venen-Katheter 0.5 × 0.9 mm, B. Braun AG.). Each rat was intravenously infused with saline solution at a flow rate of 20 µl/min.

In the control group, after the collection of basal secretion every 10 minutes during half an hour, 100 µl of saline solution were infused intravenously as a bolus, over a period of one minute, followed by a resumption of saline infusion. Pancreatic secretion following the saline bolus was collected for one and a half hours as nine 10 min samples. The same procedure was performed infusing a 100 µl TRH bolus of 1.5 ng/kg, CCK_8 in dosis of 30 ng/kg and TRH + CCK_8 in the same doses. Pancreatic secretion was collected in the same way and the rat central temperature was maintained at 38 ± 0.5 °C. Amylase activity was determined in all 10 min samples of pancreatic juice.

Amylase secretion from pancreatic lobules. After an overnight fast, male rats weighing 200-250 g were sacrificed and isolated lobules were prepared by the method described by SCHEELE and PALADE (19). The lobules were incubated for sixty minutes in 5 ml Krebs Henseleit bicarbonate buffer in 25 Erlenmeyer flasks, placed in a shaking water bath (60 cycles/min) at 37 °C, and bubbled with 95 % O_2, 5 % CO_2. Different experiments were performed with 10^{-11} to 10^{-6} M CCK_8, 10^{-7} to 10^{-4} M TRH and 10^{-8} to 10^{-3} M bethanechol. In other experiments, lobules were incubated with 10^{-5} M TRH in combination with 10^{-11} to 10^{-6} M CCK_8 or in combination with 10^{-8} to 10^{-3} M bethanechol, and 10^{-5} M bethanechol in combination with 10^{-10} to 10^{-4} M TRH. After an hour of incubation, the medium was removed and the lobules were homogenized in 5 ml phosphate buffer, using a ground-glass homogenizer. The amylase content in the medium and homogenates was determined.

Amylase secretion from dissociated acini. Dissociated acini were prepared by previously described methods (20). After preincubation for 30 min in Krebs Henseleit bicarbonate buffer (Ca^{++} 1.25 mM), the samples were poured into graduated centrifuge tubes, spun for 5 min at 500 rpm and resuspended. After a thorough mixing of the sample with the solution, aliquots of dissociated acini were distributed in incubation flasks. The effects of bethanechol (10^{-4} M), bethanechol (10^{-4} M) in combination with TRH (10^{-5} M), and TRH (10^{-5} M) alone were studied. At the beginning of each incubation, 0.5 ml samples were centrifuged at 11.000 rpm

for 1 min. The amylase activity in the supernatant was subtracted from the values obtained following incubation with peptides to determine the enzyme released during the 30 min incubation period. The cell pellets were washed once with normal saline, and an aliquot of distilled water was added to each cell pellet. The pellets were sonicated and amylase activity was determined in the samples.

Amylase and protein determination. — Amylase in all samples was assayed by the methods described by BERNFELD (2), using Litner's starch as substrate. One unit of amylase is the amount of starch hydrolized to 1 mg maltose in 3 min at 37 °C. Amylase released into the medium was expressed as the percentage of the total content of enzyme present in lobules or cell pellets at the beginning of the incubation. The protein was assayed by using the BRADFORD's method (3).

TRH radioimmunoassay. — After dissection, the pancreas was weighed and TRH was extracted according to a technique described by FAIVRE-BAUMAN *et al.* (7) for hypothalamic tissues. Samples were assayed immediately or stored in methanol at −20 °C. They were then evaporated to dryness and taken up in PBS just before assay.

Tissue levels of TRH were measured using a highly specific antiserum prepared by VARA and TAMARIT-RODRÍGUEZ (23). TRH was radioionidinated with $Na^{125}I$ by the procedure of chloramine-T (18) and purified by cation-exchange chromatography on Sephadex SP-C25 (9). Radioimmunoassay was performed as follows: 50 µl of diluted antiserum, 50 µl of labelled TRH, 50 µl of sample or standard TRH solutions and 200 µl of buffer (0.02 mol/l NaH_2PO_4 and 0.14 mol/l NaCl, pH = 7, containing 1 % bovine serum albumin) were incubated at 4 °C for 48 h. Separation of free from bound hormone fractions was achieved with a 0.25 % (w/v) charcoal suspension in radioimmunoassay buffer (2 ml/tube). The sensitivity of the assay was 0.47 ± 0.1 pg/tube (N = 5). The intra-assay variation ranged from 10 % (lower part of standard curve) to 6 % (middle) and 11 % (upper) and the inter-assay variation oscillated between 6.2 % and 9.8 %.

TRH Prem was from Frumtost S.A. (Barcelona, Spain); bethanechol chloride from Merck, Sharp and Dome (West Point, Penn.); cholecystokinin (fragment 26-33), collagenase (*Clostridium histolyticum*, 614 units/mg), α-chymotrypsin (bovine pancreas, 64 units/mg) and hyaluronidase (bovine testes) were from Sigma and Sephadex SP-C25 was from Pharmacia Fine Chemicals. All other chemicals were of analytical grade from Merck.

Analysis of data. — The results obtained are expressed as the mean ± SEM of several observations. Data were analyzed by two-way analysis of variance with replication and the Newman Keuls test. Values of $p < 0.05$ were considered to be statistically significant (4). In *in vivo* experiments, values for each 10 min period after the administration of peptide bolus were expressed as the percentage of the mean of 30 min basal values.

Results

Pancreatic TRH content was 0.66 ± 0.4 pg/mg of tissue or 0.02 ± 0.01 pg/mg of protein. TRH concentration in the acini preparations was not detectable.

In vivo *studies.* — Figure 1-A shows the effect of TRH bolus infusion on basal amylase secretion in pancreatic juice. The peptide induced a statistically significant decrease in enzyme secretion and the inhibition persisted for ninety minutes after the TRH dose (1.5 ng/kg) had been administered.

Figure 1-B shows the effect of bolus in-

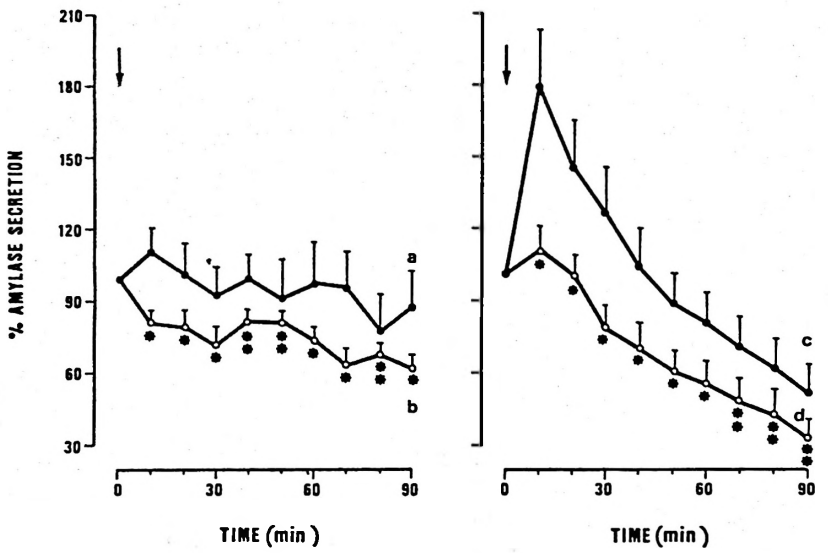

Fig. 1. *Kinetics of amylase secretion in pancreatic juice* in vivo, *following bolus injection (→) with saline solution (a) and TRH (1.5 ng/kg) (b) and with CCK_8 (30 ng/kg) (c) and CCK_8 plus TRH (d).* The results are expressed as a percentage of secretion, considering 100 % the mean of the amylase secretion for three initial 10 min periods before the bolus is injected (0 time). Each time point represents the mean ± SEM of seven experiments. * $p < 0.01$; ** $p < 0.05$.

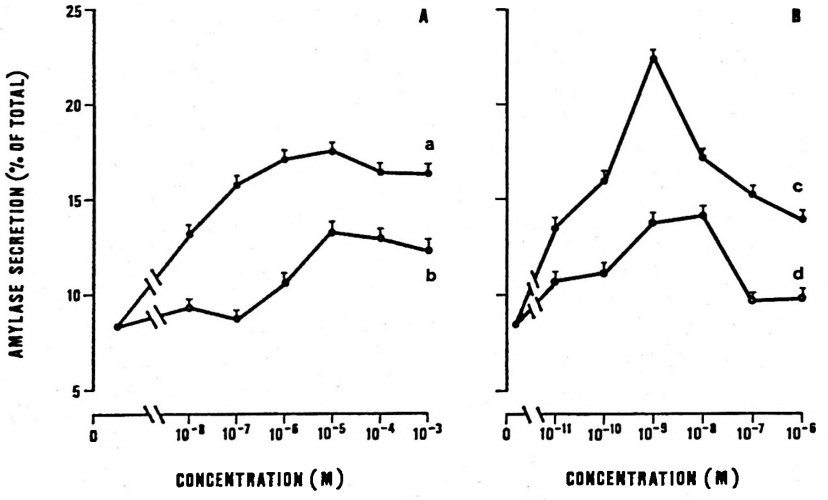

Fig. 2. *Effects of different concentrations of two secretagogues alone and concomitant with TRH (10^{-5} M) on amylase secretion from isolated pancreatic lobules incubated during 60 minutes.* A) The lobules were incubated with bethanechol (a) and bethanechol plus TRH (b). B) the lobules were incubated with CCK_8 alone (c) and concomitant with TRH (d). In each figure the result is the mean ± SEM for nine experiments. * $p < 0.01$; ** $p < 0.05$.

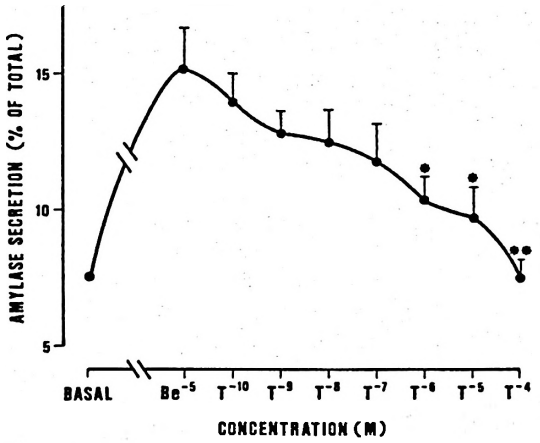

Fig. 3. *Amylase secretion from pancreatic lobules under the effects of 10^{-5} M bethanechol (Be) and 10^{-5} M bethanechol plus TRH (10^{-10} to 10^{-4} M) (T).*
Results are expressed as mean ± SEM of 6 experiments. * $p < 0.05$, ** $p < 0.01$.

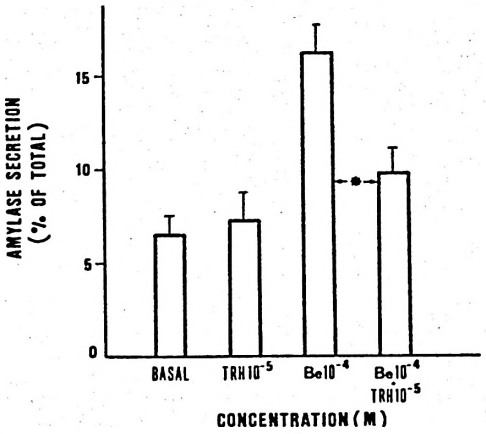

Fig. 4. *Amylase secretion from dissociated acini under basal conditions, and under the effects of 10^{-4} M bethanechol, 10^{-2} M TRH and bethanechol plus TRH.*
The results are expressed as mean ± SEM of 7 experiments. * $p < 0.01$.

fusion on pancreatic amylase secretion using CCK_8 (30 ng/kg) alone or in combination with TRH. The CCK_8 bolus caused significant stimulation of amylase secretion for the first three 10 min periods and a decline in the last 60 min. The administration of CCK_8 with TRH halted the increase in amylase secretion observed with CCK_8. The characteristic response to CCK_8 was thus blunted while the amylase output proved significantly lower in the TRH group.

In vitro *studies.* — TRH did not significantly influence basal amylase secretion (7.6 ± 1.0 to 8.0 ± 0.4 %) when different concentrations were used in the incubating medium (10^{-7} to 10^{-4} M). To test whether TRH modified the stimulated secretion, the dose responses for both bethanechol and CCK_8 were studied. A significant inhibition ($p < 0.01$) was found when the lobules were incubated with bethanechol (fig. 2-A) and CCK_8 (fig. 2-B) in the presence of TRH (10^{-5} M). The peak of stimulation is seen to be reduced by almost 50 % for both the CCK_8 and bethanechol dose-response curves. When the lobules were incubated with different concentrations of TRH (10^{-10} to 10^{-4} M) plus 10^{-5} M bethanechol (fig. 3), the increase in amylase secretion observed with bethanechol decreased with each concentration of TRH used, but only 10^{-6} M ($p < 0.05$), 10^{-5} M ($p < 0.05$) and 10^{-4} M ($p < 0.01$) TRH reduced the amylase secretion significantly.

Finally, figure 4 shows the effect of TRH on amylase secretion from dissociated acini. TRH (10^{-5} M) alone did not alter basal amylase secretion but it produced a marked inhibitory effect on the amylase secretion stimulated by bethanechol ($p < 0.01$).

Discussion

The present paper investigates an inhibitory TRH effect on basal and stimulated

pancreatic amylase secretion in anesthetized rats. TRH blunted the pancreatic secretion elicited by CCK_8 and bethanechol in isolated lobules and dissociated acini by acting directly on acinar cells.

It seems that TRH exerts an inhibitory effect on both basal and stimulated pancreatic secretion in humans (10, 14) and dogs (25). Furthermore, long term treatment with TRH in rats induces a decrease in digestive enzyme concentration and reduces amylase discharge from isolated pancreatic lobules (8). In this sense, our results are in accord with these findings since TRH decreases amylase pancreatic secretion both *in vivo* and *in vitro*.

However, the mechanism of action by which TRH influences pancreatic secretion is still unclear. GULLO and LABO (10) suggest a direct effect due to the rapid onset of action on the exocrine pancreatic function, when the hormone was administered by venous infusion. Furthermore, the experiments of KOMIYA *et al.* (14) with pancreatic perfusion in dogs, and their studies on humans with primary hypothyroidism and Graves disease, suggest that TRH probably operates directly on the exocrine pancreas. In this way, our *in vitro* experiments are conclusive. Although the dose response curve did not show a TRH effect on *in vitro* basal pancreatic secretion from lobules, the hormone exhibited an unquestionable inhibition on CCK_8 and bethanechol-stimulated secretion. The CCK_8 dose response curve displayed about fifty per cent inhibition in the presence of TRH and the maximal effect was also delayed. Moreover, the bethanechol dose response curve also changed in the same way, but in this case the maximum stimulation was reached at the same bethanechol concentration as without TRH. Thus, the inhibition of stimulated amylase secretion can be considered as a direct effect of TRH on the pancreatic lobules.

Since pancreatic lobules contain the islets of Langerhans, these are "contaminated" by endogenous TRH. Furthermore, other peptides such as somatostatin, glucagon and insulin are present in the tissue samples and could influence the pancreatic amylase secretion in the experiments with lobules (15, 21). In order to eliminate this possibility, the experiments were designed with dissociated acini. As described in the experiments with lobules, TRH did not alter basal amylase secretion from dissociated acini. Nevertheless, the increase in amylase secretion observed with bethanechol was inhibited by TRH. These findings suggest that TRH decreases amylase pancreatic secretion through a direct effect on acinar cells. Recently, TRH has been reported to be located in the ß cells of pancreatic Langerhans' islets (1, 13). TRH could be able to exert its inhibitory effect via the islet acinar portal vascular system, through an endocrine mechanism, or acting directly on the periinsular acini, through a paracrine mechanism (24).

At present, it is difficult to assess the physiological significance of the inhibitory TRH effect. The fact that TRH is able to directly influence the secretory function raises the possibility that the peptide may be involved in the control of pancreatic enzyme secretion.

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Resumen

Se estudia el efecto de la TRH sobre la secreción de amilasa *in vivo*, cateterizando el conducto pancreático de ratas, e *in vitro*, utilizando lóbulos pancreáticos aislados y acinos disociados. Los resultados muestran que la

TRH inhibe la secreción basal y estimulada de amilasa *in vivo*, que *in vitro* no tiene efecto inhibidor cuando es usada sola, pero bloquea la secreción estimulada por CCK_8 y betanecol en los modelos de lóbulos aislados y acinos disociados. Los resultados sugieren que la TRH puede inhibir la secreción estimulada de amilasa en ratas a través de un efecto directo sobre la célula acinar.

Palabras clave: TRH, Secreción de amilasa, Páncreas exocrino.

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