synthesis of new molecules of the enzyme rather than an increase of its activity.

In conclusion, our data show that aldosterone is able to induce the synthesis of thymidine kinase in the kidney. This steroid possesses identical effects to those of estrogens and androgens in their target organs[1,10]. Thymidine kinase participates in the synthesis of DNA by the 'salvage pathway'. This seems to indicate that aldosterone participates in the development of the kidney, but at the present time, nothing indicates that the increase in thymidine kinase activity represents a fundamental process.

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Growth-hormone releasing factor does not antagonize somatostatin effects on pancreatic and gastric secretions

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Summary. The effect of human GRF 44 upon somatostatin-inhibited pancreatic and gastric secretions was studied in rats with chronic fistulas. GRF did not show any antagonist action of somatostatin on these gastro-intestinal organs. GRF alone had a small inhibitory effect on gastric acid output.

Key words. GRF; somatostatin; pancreatic secretion; gastric secretion.

Human growth hormone releasing factors (GRF's) have been isolated and characterized from human pancreatic tumors producing acromegaly[1-3] and from the hypothalamus[4], and the corresponding peptides synthesized. Since GRF is an antagonist of somatostatin on pituitary cells, the question arose whether antagonist effects might also exist on peripheral target organs. The aim of this study was to check this hypothesis on exocrine pancreatic and gastric secretions in rats.

Materials and methods. Male Wistar rats weighing 280–320 g were obtained from R. Janvier, 53 Le Genest, France.

1. Pancreatic secretion. Subacute pancreatic fistulas were prepared under light ketamine anesthesia as described in detail elsewhere[5]. Briefly, all of the pure pancreatic juice was collected from a catheter in the lowest common bile duct, while bile was recirculated to the duodenum through the choledoco-duodenal junction, using a Silastic shunt, and another Silastic catheter was inserted in the duodenum to allow infusions in the gut. The animals were kept in Bollman cages and used for experiments 24 h after surgery (surgery = day 0). Experiments were performed daily in conscious rats from the afternoon of day 1 until day 5. The central temperature of the animals was maintained at approximately 38 °C. Water was freely available, and food was given daily ad libitum between 5 p.m. and 8 a.m. The duodenal catheter was continuously infused with 2.5 ml/h of an equivolume mixture of 100 g/l glucose and Hartmann Ringer B21 electrolyte solution, containing 8000 IU/ml of porcine trypsin (Sigma I 0134). The final pH was 5.5.

2. Gastric secretion. Under light ketamine anesthesia, a Thomas cannula was inserted into the ruminal portion of the stomach and was kept closed between experiments. Rats were free to move normally and had free access to food between experiments. No more than two experiments a week were performed, in rats fasted for 18 h.

3. Experimental protocol. Secretions were collected in 20 min samples, starting with a basal period of 20 or 40 min, and followed by a 2 h venous infusion with one of the following: GRF (human pancreatic synthetic 1–44 GRF, a gift of Prof. R. Guillemin) 2.5 nmol/kg·h, somatostatin (14 aa, a gift of Clin-Midy Laboratories) 2.5 nmol/kg·h, somatostatin+GRF, each at 2.5 nmol/kg·h, saline alone. Gastric secretion was collected for 40 min, and pancreatic secretion for 2 h, after the infusion had finished. Eight to nine animals were used in each group, with usually one, and exceptionally two experiments done in each animal.

Pancreatic and gastric juice volumes were estimated by weighing the samples in tared vials. Total protein was determined by measuring the absorbance at 280 nm after appropriate dilution. Bicarbonate was determined with an Auto-Analyzer technique[6]. Gastric acid was titrated to pH 7 with 0.01 N NaOH. The significance levels given below were obtained using Student's t-test relative to the control groups.

Results. 1. Pancreatic secretion. Pancreatic secretion did not change significantly during the experiment in the control group. GRF infused alone did not change significantly the volume of pancreatic juice (−27 ± 11%). The output of bicarbonate was slightly (−39 ± 11%, fig. 1) but not significantly reduced during the second hour of infusion, while pancreatic protein output did not change (−16 ± 15%, fig. 2).

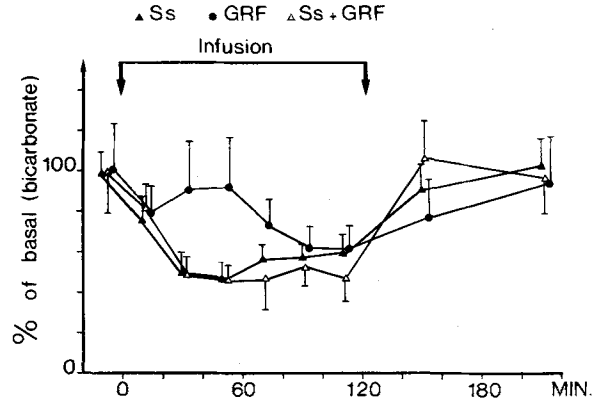

Figure 1. Bicarbonate output in pancreatic juice of rats receiving somatostatin and/or GRF (2.5 nmol/kg·h, i.v.). Average output during the basal period (20 min) was 29.2 ± 4.9 μmol/h (M ± SEM).

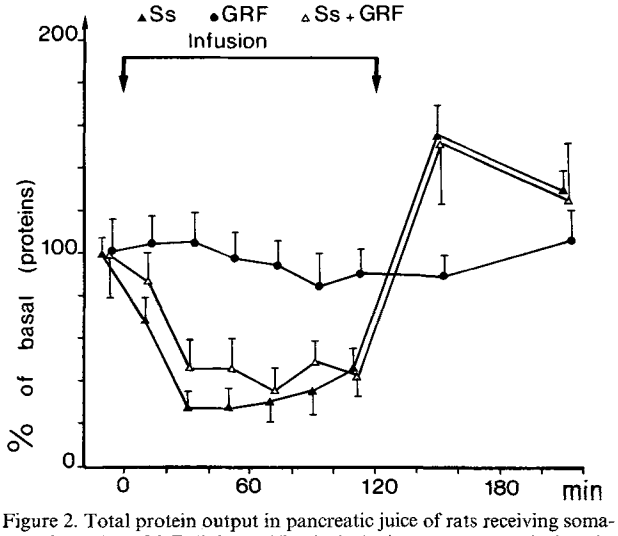

Figure 2. Total protein output in pancreatic juice of rats receiving somatostatin and/or GRF (2.5 nmol/kg·h, i.v.). Average output during the basal period (20 min) was 17.4 ± 2.3 mg/h (M ± SEM).

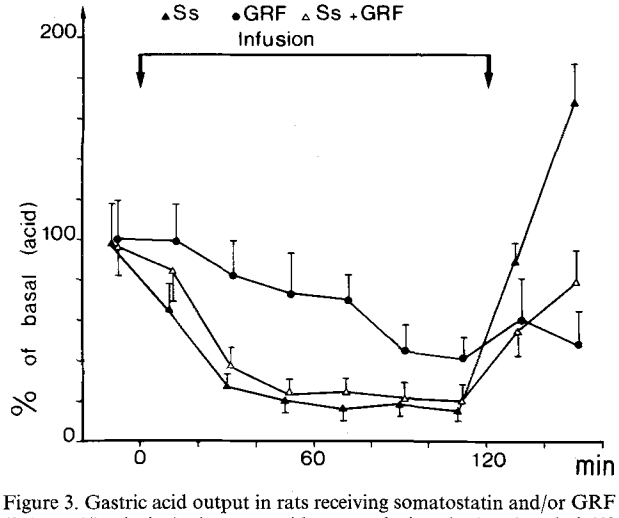

Figure 3. Gastric acid output in rats receiving somatostatin and/or GRF (2.5 nmol/kg·h, i.v.). Average acid output during the basal period (40 min) was 30.0 ± 3.1 μmol/h (M ± SEM).

The infusion of somatostatin decreased maximally the volume of juice by 51% ($p < 0.01$), the output of bicarbonate by 54% ($p < 0.001$, fig. 1) and the output of protein by 73% ($p < 0.001$, fig. 2). This effect began immediately after the onset of the infusion and lasted for the whole somatostatin infusion period. A rebound effect on the output of protein was observed just after the end of the infusion period (+50% with respect to the basal level, $p < 0.01$, fig. 2).

When GRF was associated with somatostatin infusion in equimolar amount, the inhibition of volume, bicarbonate output and protein output induced by somatostatin remained unchanged and no significant difference was observed between the animals receiving somatostatin alone or GRF+somatostatin (figs 1 and 2). A rebound effect occurring just after the end of the infusion was observed on protein output, and was not significantly different between somatostatin and GRF+somatostatin.

2. Gastric secretion. Gastric acid output decreased slightly and steadily by 9% per hour in the control group.

GRF infused alone slightly and progressively decreased gastric acid output, by 59% ($p < 0.01$, fig. 3) after 2 h of infusion.

Somatostatin alone decreased maximally gastric acid output by 84% ($p < 0.001$, fig. 3). The maximal inhibition was reached after about half an hour of infusion, and lasted for all the time of somatostatin infusion. Immediately after the end of the infusion, a rebound effect amounting to +70% with respect to the basal level was observed (fig. 3).

The association of an equimolar amount of GRF to the infusion of somatostatin did not change the amplitude and duration of the somatostatin-induced inhibition. However, no rebound effect was observed 40 min after the mixed infusion had ceased (fig. 3).

Discussion. Somatostatin is a potent inhibitor of exocrine pancreatic secretion in the rat, and it has been shown to inhibit primarily CCK- and vagally-induced secretion, but not secretin-induced secretion[7,8]. In previous work, using similar experimental conditions, we found that 2.5 nmol/kg·h inhibited pancreatic secretion by about 50%[7]. In the present study, the same range of inhibition was achieved with somatostatin.

Somatostatin also inhibits gastric acid secretion in the rat and in other species[9] and the dose used here has been shown to reduce by 50% pentagastrin-stimulated secretion in the rat stomach[10]. Although GRF efficiently antagonizes the effect of somatostatin on pituitary cells[11], no similar antagonism occurred on either gastric or pancreatic secretion under the conditions of this study. This suggests either that no specific receptors of GRF are actually present on parietal or acinar cells, or that the intracellular mechanisms involved in the somatostatin effect on these cells[12,13] differ to some extent from those activated in pituitary cells.

It has been recently shown, however, that GRF was able to interact with surface receptors present on the membrane of guinea pig pancreatic acinar cells[14], but experimental evidence accumulated thus far suggests that this interaction occurs with VIP receptors, due to the partial structural identities between GRF and VIP[15]. No stimulation of basal pancreatic secretion has been observed with GRF alone, but this is not unexpected, since in the conditions of this study, pancreatic secretion is already basally stimulated by neuro-humoral endogenous factors[16,17], a situation which makes it difficult to demonstrate small stimulatory effects, and also since very high doses of VIP (and hence still more of GRF) are necessary to stimulate rat pancreatic secretion in vivo[18]. Gastric secretion, however, was slightly decreased by GRF alone, and we suggest that this effect may be related to the VIP-like properties of the GRF molecule.

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Action of TSH on nuclear ADP-ribosylation in dog thyroid slices

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Summary. Treatment of dog thyroid slices with thyrotropin (TSH) results in an increase in ADP-ribosylation in nuclei isolated thereafter. This increase is time-dependent and is observed with concentrations of TSH eliciting physiological responses. The technique described here does not involve permeabilization of cell membranes, thereby avoiding artefacts which could arise from hypotonic shock. Cyclic AMP mimicked the stimulatory action of TSH.
Key words. ADP-ribosylation; thyroid; thyrotropin; cyclic AMP.

Post-translational modification of proteins include a varitey of reactions, such as methylation of arginine residues[1], phosphorylation[10] and ADP-ribosylation[6,12]. In this last reaction, acceptor proteins are mono- or poly-ADP-ribosylated from NAD and this biochemical event has been proposed to be linked to DNA replication, cell proliferation and refractoriness to hormones. An enzyme that catalyzes ADP-ribosylation has been found in different subcellular fractions both in eukaryotes and in prokaryotes[6,12]. ADP-ribosylation has been shown to be under hormonal control, for example variations in the amounts of monomeric and polymeric ADP-ribose residues have been demonstrated in the mouse kidney after castration and testosterone treatment[5], and in the mouse liver after thyroidectomy and thyroxine treatment[9]. As far as the thyroid is concerned, TSH has been reported to increase total cell ADP-ribosylation in permeabilized cells[4] and in membranes of bovine and rat tissue[2,14]. The aim of the present study was to investigate the action of TSH on ADP-ribosylation in purified dog thyroid nuclei.

Materials and methods. Dogs were pretreated with thyroid powder for one day, as already described[13], and the thyroid glands were obtained by surgery under pentobarbital anesthesia. Slices were obtained with a Stadie-Riggs microtome (A. Thomas) and preincubated under 95% O_2/5% CO_2 for 30 min at 37 °C in Krebs-Ringer bicarbonate buffer (KRB) pH 7.4 containing 8 mM glucose and 1 mg/ml of bovine serum albumin. At the end of this period the slices were transfered to flasks containing fresh buffer, with or without TSH, and incubated for different lenghts of time. Approximately 1 g of slices was incubated in 7–10 ml KRB. The slices were then homogenized in 0.32 M sucrose, 30 mM Tris-HCl pH 7.8, 1 mM $MgCl_2$, 1 mM dithiothreitol (DTT) with a Potter Elvehjem tissue homogenizer with a teflon pestle, at 4 °C. The homogenate was filtered through gauze and centrifuged at 800 × g for 10 min. The resulting pellet was resuspended in 3 ml 2.1 M sucrose in the same buffer, and centrifuged at 50,000 rpm for 60 min in a SW_{56} rotor[7] to provide a nuclear pellet. Each pellet was resuspended in 200 µl of 30 mM Tris buffer pH 7.8 containing 25% (v/v) glycerol, 1 mM DTT and 1 mM $MgCl_2$. To this suspension 550 µl of 30 mM Tris-HCl buffer pH 7.8, 80 mM KCl, 10 mM $MgCl_2$, 1 mM DTT, 1 mM ATP and 0.1 mM NAD were added. Aliquots of 150 µl of the resuspended nuclei were utilized for the assay. DNA content was determined fluorometrically with ethidium bromide[11], after RNAse treatment.

ADP-ribosylation was measured following the method of Kostka and Schweiger[8], by incubating the purified nuclei in triplicate with a tracer amount (usually around 100,000 cpm) of ^{3}H-NAD (2,8 ^{3}H-NAD, New England Nuclear; 3.4 Ci/mmole sp. act.). After 6 min at 25 °C the reaction was stopped by the addition of 0.5 ml of cold 20% (w/v) TCA. The precipitates were filtered and washed with 10% TCA on GF/C Whatman glass fiber filters on a Millipore 1225 apparatus. The filters were dried,

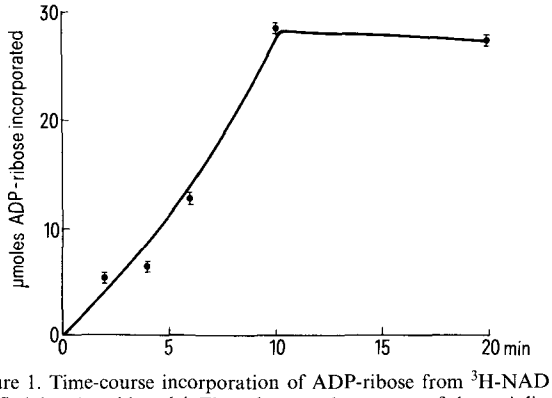

Figure 1. Time-course incorporation of ADP-ribose from ^{3}H-NAD by purified dog thyroid nuclei. The values are the average of close triplicates from a typical experiment. Each incubation tube contained purified nuclei corresponding to 65 mg of fresh thyroid.