Inactivation of Gastrin and Pentagastrin by Tissue Slices[1] (37278)

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The physiologic actions of exogenously administered gastrin appear to be short lived (1). Direct measurement by radioimmunoassay of the rate of disappearance of exogenous gastrin from the circulation indicated a very rapid disappearance of gastrin; the hormone was found to have a half-life of less than 10 min (2). The mechanisms of such rapid catabolism have yet to be accurately determined but they would appear to involve alteration or destruction of the active C-terminal tetrapeptide amide of gastrin. The sites of catabolism are not fully known but are probably widespread in distribution or located in organs receiving large volumes of blood. Amidase activity capable of catalyzing the degradation of hormonally active gastrin has been demonstrated in homogenates of several rat tissues (3). *In vivo* studies in dogs have shown that gastrin is partially inactivated by the liver and kidney (4–6), while there was no loss of immunochemical potency on passage through the hind limb (7). We have studied the capacity of slices of several mammalian tissues to inactivate synthetic human gastrin I and its analog, pentagastrin (provided by Ayerst Laboratories).

Materials and Methods. The tissues studied were obtained from fasting adult mongrel dogs that weighed between 15 and 18 kg. The dogs were stunned with a captive-bolt pistol and samples of kidney, liver, lung, gastric fundic mucosa and skeletal muscle were quickly obtained prior to cessation of circulation. The tissues were washed 3 times in Krebs–Ringer bicarbonate (KRB) buffer at 0° and then stored in a similar solution at 0° prior to testing. Slices of each tissue sample, approximately 0.5 cm^2 and 10 μm thick, were prepared with a commercial tissue chopper (Brinkman Instruments, Westbury, NY).

Slices of each of the tissues sampled were added to flasks containing 100 ml of KRB and either synthetic human gastrin I (1.3 μg/ml) or pentagastrin (5 μg/ml). Two sets of control flasks were prepared, one containing identical ingredients but no secretagogue while the other contained identical amounts of each secretagogue but no tissue slices. Before incubation at 37° for 2 hr in a metabolic shaking incubator, 0.5 ml of a solution containing a mixture of 100,000 units of penicillin and 0.125 g streptomycin was added to each flask. A mixture of 95% O_2 and 5% CO_2 was bubbled through each flask during the entire incubation period. Immediately afterwards, the incubates were centrifuged and the supernatant was stored at —20° prior to assay.

Gastrin and pentagastrin were measured by radioimmunoassay using a double antibody technique (8). All samples were assayed in duplicate; the reproducibility of the assay was within ±10%. The average of the duplicates was taken as the residual concentration in each of the incubates. The amount of secretagogue measured in each test solution was expressed as a percentage of that recovered from the control solutions which were incubated without tissue slices.

Bioassays of the samples were performed on dogs with denervated Heidenhain pouches (HP) of the gastric fundus. The solutions were diluted with normal saline solution and infused via a constant-rate infusion pump into a peripheral vein for 1 hr. The rate of infusion was calculated to deliver a dose that would result in a ½ maximal secretory response if no secretagogue had been destroyed.

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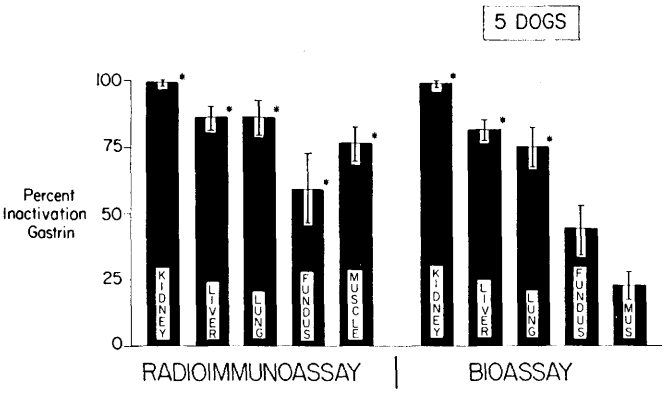

FIG. 1. Inactivation of synthetic human gastrin I (%) during incubation for 2 hr with slices of various tissues. Asterisks identify statistically significant differences from control incubations ($p < 0.05$). Narrow vertical bars denote ± 1 SEM.

This rate was 1.25 μg/kg/hr for gastrin and 5 μg/kg/hr for pentagastrin. Each dog was in a basal secretory state prior to testing and collections of HP secretion were made every 30 min during infusion. The average 60-min secretory response in the 5 studies of each tissue was compared to the average secretory response to infusion of the control solutions which contained no tissue. This allowed an expression of the percent of each secretagogue inactivated.

Students' *t* test was used to determine the significance of the percentage inactivation produced by each of the tissues.

Results. No significant amounts of secretagogue activity were found (as determined by either radioimmunoassay or bioassay) in any of the control solutions containing tissue slices but no gastrin or pentagastrin. These values were not considered further in the calculation of the percentage inactivation produced by the various tissue slices.

All of the tissues sampled produced a significant inactivation of gastrin as determined by radioimmunoassay (Fig. 1). Homogenates of kidney, liver and lung also produced significant inactivation as determined by bioassay.

Similarly, incubation of the various tissue samples with pentagastrin produced a significant loss of immunochemical potency, and all but fundic mucosa caused statistically significant inactivation as measured by bioassay (Fig. 2).

Discussion. These *in vitro* studies demonstrated that there is a potential for the in-

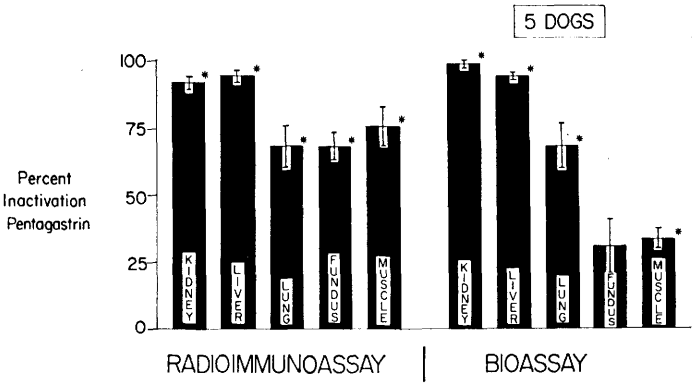

FIG. 2. Inactivation of pentagastrin (%) during incubation for 2 hr with slices of various tissues. Asterisks identify statistically significant differences from control incubations ($p < 0.05$). Narrow vertical bars denote ± 1 SEM.

activation of gastrin in many tissues. *In vivo* studies to date have failed to confirm a widespread potential for the catabolism of gastrin. A single transit of the kidney was found to inactivate approximately 30% of endogenous gastrin (5) and 40% of exogenous gastrin (6). Hepatic transit was found to reduce the serum concentration of exogenous gastrin (4), but to have no effect on levels of circulating endogenous gastrin (9). These previous studies suggest that there are different catabolic mechanisms for endogenous and exogenous heptadecapeptide gastrin. It is important to note that the *in vitro* capacity to degrade a hormone should not be extrapolated to *in vivo* states.

Summary. We have studied the capacity of several mammalian tissues to inactivate synthetic human gastrin I and its synthetic analog, pentagastrin. Slices of each tissue studied were incubated with either gastrin or pentagastrin and the degree of inactivation was determined by both radioimmunoassay and bioassay. The results demonstrate that there is an *in vitro* potential for the inactivation of gastrin in all the tissues tested. There was a close correlation between the results obtained with radioimmunoassay and those obtained with bioassay.

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