EFFECT OF TETRAGASTRIN, CAERULEIN AND PANCREOZYMIN ON THE ENZYME SECRETION OF THE RABBIT PANCREAS IN VITRO AND IN VIVO

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It is known that pancreozymin and acetylcholine stimulate the pancreatic enzyme secretion. Experiments using pancreatic tissue slices in vitro showed that pancreozymin and acetylcholine both stimulated the enzyme secretion directly. In these experiments the effect of acetylcholine was inhibited by atropine, while that of pancreozymin was not (1). Stening and Grossman reported recently that caerulein, pancreozymin, gastrin I and gastrin II, each sharing the C-terminal pentapeptide had a stimulatory effect on the chronic pancreatic fistula of the dogs in vivo (2).

Thus it was decided to investigate, whether the effect of these related peptides on the pancreatic enzyme secretion is direct or indirect. Indirect action through the humoral or neural mechanism may be possible, as these peptides may release some mediator substances such as endogenous pancreozymin from the duodenum by their strong enterokinetic action, or acetylcholine from nerve endings by parasympathetic stimulation. In order to elucidate these problems, experiments were carried out in vitro and in vivo using New Zealand rabbit pancreas.

The tissue used in vitro was prepared according to Rothman's method (3), except that the duodenum attached to the pancreas was completely removed so that the endogenous pancreozymin or other humoral substances would be eliminated. The isolated organ was then bathed in 300 ml of a solution, the composition being in terms of m moles/l: NaCl 118.6, CaCl₂ 2.5, KH₂PO₄ 1.2, NaHCO₃ 24.9 and MgCl₂ 1.2. The bath solution was bubbled continuously with 95% O₂ + 5% CO₂ and maintained at 30°C. The main pancreatic duct was cannulated by the polyethylene tube (PE 50) and the secreted fluid was collected at 20 min intervals for approx. 8 hr. Two or three hr after mounting, when the secretion became constant in flow rate, the test substance was added in the bath solution. After a 20 min collection, the organ was washed in fresh bath solution free of the test substances and the secreted fluid was collected again for 40 to 60 min. Substances tested were as follows: tetragastrin (Nippon Kayaku Co. Ltd. Japan, synthetized): Amyloxy carbonyl-Try-Met-Asp-Phe-NH₂, caerulein (Farmitalia, Italy, synthetized): Pyr-Glu-Asp-Tyr(SO₃H)-Thr-Gly-Try-Met-Asp-Phe-NH₂, pancreozymin (Boots Pure Drug Co. Ltd, England, extracted from porcine duodenum): Lys-(Ala-Gly-Pro-Ser)-Arg-Val-(Ile-Met-Ser)-Lys-Asp-
(Asp-Glu-His-Leu2-Pro-Ser2) Arg-Ile-(Asp-Ser)-Arg-Asp-Tyr (SO₃H)-Met-Gly-Try-Met-Asp-Phe-NH₂. Volume of the obtained sample was determined by weighing, and protein concentration was measured by Lowry’s method. As for enzyme activity, amylase and

Fig. 1. Dose response curves of the effect of tetragastrin, caerulein and pancreozymin on the pancreatic enzyme secretion.

(A) In vitro experiments; Dose is expressed as μg or unit per one ml of bath solution. Enzyme secretion is expressed as the increase of protein output in the pancreatic juice for 40 min after stimulation. Points connected by dashed lines represent measurements in the same isolated pancreas.

(B) In vivo experiments; Dose is expressed as μg or unit per kg body weight. Enzyme secretion is expressed as protein output in the pancreatic juice for one hr after stimulation. Solid bar is the mean value.
Trypsinogen were assayed by the method of Caraway (4) and Hummel (5). It was found that in pancreatic juice, enzyme activity was strictly proportional to protein concentration. In studying the pancreatic enzyme secretion, therefore, it was reasonable to observe the change of protein output in the secreted fluid, instead of measuring each enzyme activity.

Dose responses of the protein output to these three peptides in vitro are summarized in Fig. 1-A. Similar experiments were carried out in vivo and the results are shown in Fig. 1-B. It is obvious that tetragastrin, caerulein and pancreozymin showed a stimulatory effect on the pancreatic enzyme secretion in vitro, the order of their potency being caerulein, pancreozymin and tetragastrin. The order was similar to that in vivo. It is interesting that the response to tetragastrin in vitro was less apparent than that in vivo. Acetylcholine stimulated the pancreatic enzyme secretion both in vivo and in vitro, its minimal effective concentration was 50 μg infused intravenously for 15 min in vivo and 0.01 μg per one ml of bath solution in vitro. When atropine was added to the bath, 40 μg per one ml of bath solution, the effect of acetylcholine, 20 μg per one ml of bath solution, was completely inhibited but the effect of tetragastrin, caerulein and pancreozymin, 1 μg, 0.01 μg and 0.1 Unit per one ml of bath solution respectively, was not affected. In order to investigate protein composition, the collected pancreatic juice was analysed by polyacrylamide gel electrophoresis. Pancreatic juice obtained after stimulation by tetragastrin, caerulein, pancreozymin and acetylcholine showed a similar change in the electrophoretic pattern of protein. Furthermore, there was no difference between results obtained in vitro and in vivo.

It is concluded that tetragastrin, caerulein and pancreozymin, each sharing C-terminal tetrapeptide, have a direct effect on pancreatic acinar cells. Modes of these peptides' action are assumed to be identical, because their dose response curves showed similar changes between in vivo and in vitro, and similar electrophoretic pattern of proteins was revealed in the secreted juices obtained after stimulation. Modes of action may be, however, different from that of acetylcholine as their effects were not inhibited by atropine both in vivo and in vitro.

REFERENCES