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Hormonal Control of Exocrine Pancreatic Secretion in the Isolated Intact Rat Pancreas

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This study employs the isolated perfused rat pancreas to investigate the actions of the two gut hormones, cholecystokinin-octapeptide (CCK-8) and secretin on pancreatic juice flow and total protein output. Perfusion of the pancreas with either CCK-8 (100 pM) or secretin (100 pM) resulted in marked both pancreatic juice flow and total protein output. The effect of CCK-8 was three-fold larger compared to the responses obtained with secretin alone. Simultaneous application of CCK-8 and secretin (all 100 pM) failed to elicit either a potentiation or an additive effect in both pancreatic juice flow and total protein output. The net increases in both secretory parameters were reduced during combined application of CCK-8 and secretin compared to the responses obtained with CCK-8 alone. The results indicate that optimal concentrations of either CCK-8 or secretin can display marked secretagogue effects on the exocrine pancreas but when administered simultaneously they failed to elicit either an additive response or a potentiation in pancreatic juice secretion.

Key words: Pancreas, Secretin, Cholecystokinin.

The two naturally occurring gut hormones, cholecystokinin (CCK) and secretin, play important physiological roles in the control of pancreatic juice secretion. These hormones are released from mucosal cells into the circulation following the arrival of acidic chyme from the stomach. The hormones are then transported to the pancreas where they regulate the secretion of pancreatic juice. CCK stimulates mainly pancreatic acinar cells to release a juice which is rich in digestive enzymes, whereas secretin stimulates mainly ductal cells to secrete bicarbonate (5). The action of CCK on acinar cells is associated with the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) resulting in the

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formation of inositol 1,4,5-trisphosphate (IP_3), inositol 1, 3, 4, 5-tetrakisphosphate (IP_4) and diacylglycerol (DG) (1, 3, 16). Both IP_3 and IP_4, are now kown to mobilize cellular calcium (Ca^{2+}) which in turn mediates secretion (1, 3, 12, 16). DG activates protein kinase C which is also associated with enzyme secretion (3, 4, 6, 11). On the other hand, the action of secretin involves the metabolism of adenosine 3,5-cyclic monophosphate (cAMP) which in turn mediates the secretion of bicarbonate from ductal cells and enzymes from acinar cells (3, 8, 16).

Several studies have now demonstrated marked interactions between secretagogues resulting in either inhibition or potentiation of pancreatic secretion (4, 6, 7, 13-15). However, the interaction between secretin and CCK is still unclear. It was the increasing conviction that since the two gut hormones are acting via different stimulus-secretion coupling mechanisms, involving cellular Ca^{2+} mobilization and cAMP metabolism, then they should potentiate one another (7, 13, 16). Recent studies have shown differential secretory effects of CCK and secretin in the in vivo rat pancreas (13) and an attenuation in enzyme secretion in isolated pancreatic segments (4, 15). In this study we have decided to employ the isolated perfused intact rat pancreas (8-10) to investigate further the interaction between CCK and secretin. This preparation is advantageous because it is devoid of the autonomic nervous system (ANS), sensitive to physiological doses of secretin and CCK and furthermore, both the secretion of pancreatic enzymes and juice flow can be measured simultaneously.

Materials and Methods

Animals and surgical preparation. — All experiments were performed on the isolated pancreas of overnight fasted adult Wistar rats (200-300 g b. w.) of either sex. The animals were anaesthetized with 1.5 g kg^{-1} intraperitoneal urethane, and the pancreas was isolated and perfused following the technique described by KANNO *et al.* (8-10). Briefly, after ligating and sectioning the vascular connections, stomach, spleen and the intestinal tract (but duodenum) were separated from the pancreas. The bile duct and the hepatic artery were ligated and sectioned, and the pancreatic duct cannulated. The celiac artery and the mesenteric artery, as inlets, and the portal vein, as an outlet, were cannulated, and perfusion was started with the modified Krebs-Henseleit solution at a rate of 2 ml min^{-1} using a Watson-Marlowe peristaltic pump, with a Krebs-Henseleit (KH) of the following composition (mM): NaCl 131, KCl 5.6, $CaCl_2$ 2.5, $MgCl_2$ 1, $NaHCO_3$ 25, NaH_2PO_4 1 and glucose 2.5. Dextran T-70 and bovine serum albumine (BSA) were added to a final concentration of 5 % and 0.25 %, respectively, the former to maintain osmotic pressure and BSA to prevent adhesion of the peptides to internal surfaces of the perfusion tubes. The mesentery with its embedded pancreas and duodenum was then removed and transferred to an organ bath containing 50 ml of the KH solution without Dextran. The KH was maintained at 37 °C, gassed with 5 % CO_2 and 95 % O_2 and the pH was 7.3-7.4.

Experimental design. — Following a stabilization period of 30 min pancreatic juice was collected in pre-weighed capillary tubes attached to pancreatic cannula. The samples were taken at 10 min intervals during 70 min. Pancreatic juice collected the first 10 min during perfusion with KH solution was taken as basal value. This was followed by continuous infusion for 40 min with either normal KH solution, 100 pM secretin, 100 pM CCK-8 or a combination of secretin and CCK-8 (all 100 pM). The test solutions were subsequently replaced with control KH solution and the pancreas perfused for a further 20 min period.

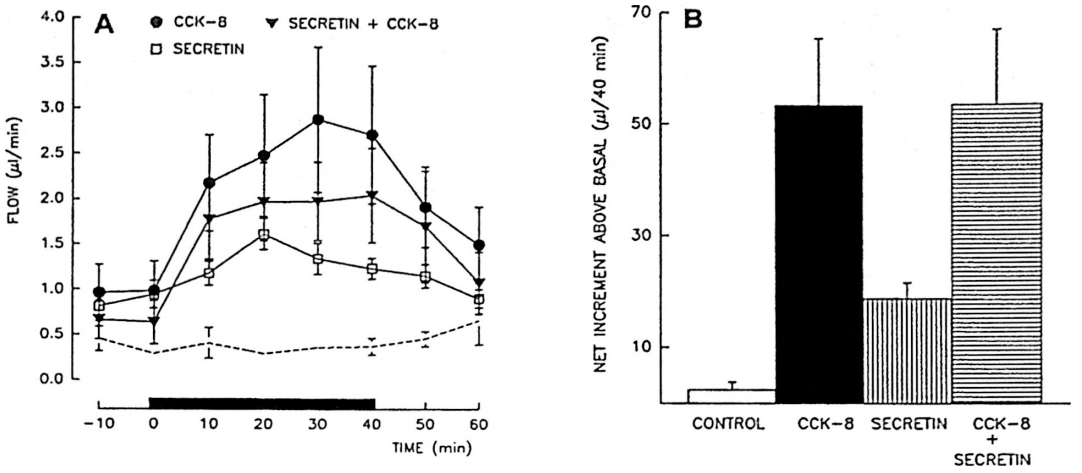

Fig. 1. *Effect of CCK-8 (100 pM, n=6), secretin (100 pM, n=6) or both (n=5) on pancreatic juice flow from the isolated perfused rat pancreas (A) and on mean net increases above basal in pancreatic juice flow during the infusion period (B).*
The saline control (broken line, n=9) is shown for comparison. The solid horizontal bar indicates the duration of infusion of secretagogues. Each point is mean ± SEM. Each column represents mean ± SEM.

Estimation of pancreatic juice flow and total protein. — Following collection, the pancreatic juice was frozen immediately. Secretory rate was determined by re-weighing the capillary tubes (assuming the density of secretion was equal to water), and total protein concentrations were estimated by the method of BRADFORD (2) using BSA as standard.

Statistical analysis. — The secretory rate was expressed as µl min^{-1}, and the total protein output as µg min^{-1}. All data are expressed as means ± standard errors of the mean (SEM). Data were compared by Student's *t* test, and only values with $p<0.05$ were accepted as significant. Net increases over basal value in flow rate and protein output were calculated as: 40 min total flow or protein output - (basal rate x 40 min).

Results

The basal pancreatic juice flow and total protein output in this series of experiments were 0.684 ± 0.079 µl min^{-1} (n=26) and 4.306 ± 0.679 (n=26) µg min^{-1}, respectively. Figure 1 shows the effect of the infusion of either saline, 100 pM CCK-8 alone, 100 pM secretin alone or in combination with CCK-8 on the time-course of pancreatic juice flow from the isolated intact rat pancreas, and on the net increases in pancreatic juice secretion above basal values in the isolated pancreas. The results demonstrate that saline alone had no significant effect on pancreatic juice flow. However, both CCK and secretin can elicit marked increases when infused separately. The response to CCK was three fold larger compared to the effect of secretin. On the other hand, when the two gut hormones were administered simultaneously they failed to evoke either a potentiation or an additive effect in juice flow.

Figure 2 shows the influence of the infusion or either saline, 100 pM CCK-8, 100 pM secretin or a combination of secretin and CCK-8 (all 100 pM) on the time-course increases in total protein output from the isolated rat pancreas and on

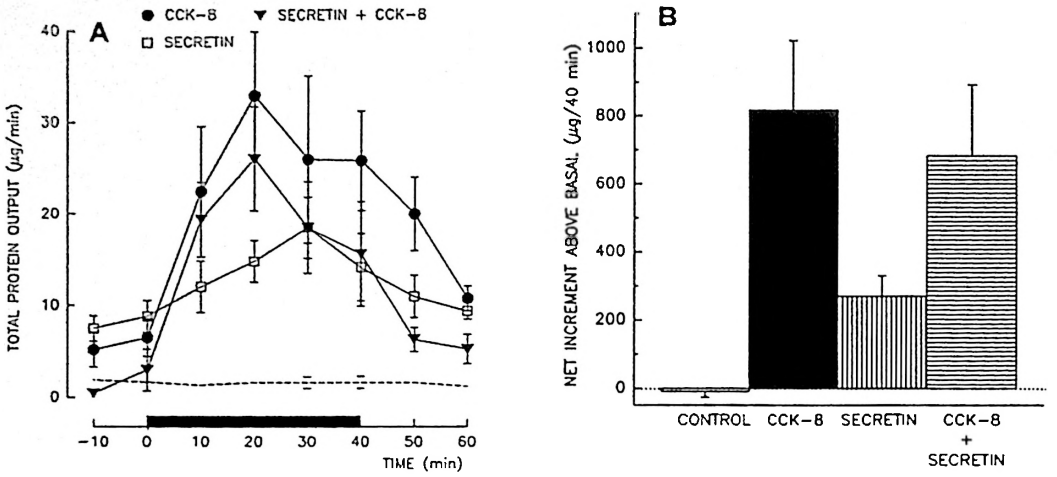

Fig. 2. *Effect of CCK-8 (100 pM, n=6), secretin (100 pM, n=6) or both (n=5) on total protein output from the isolated perfused rat pancreas (A) and on mean net increases in protein output during the infusion period (B).*

the net increases in total protein output above basal values. The results indicate that saline infusion caused a small no significant reduction on protein secretion whereas either CCK or secretin infusion resulted in marked increases in protein output. Again, CCK-8 evoked a three fold increase in protein output compared to the response obtained with secretin. When secretin was combined with CCK-8 there was a small decrease in protein output compared to the effect of CCK-8 infusion alone. Once more the results demonstrate that a combination of secretin and CCK-8 failed to evoke either a potentiation or an additive effect in protein secretion.

Discussion

Perfusion of the isolated pancreas with saline resulted in a very small increase in net protein output and a small decrease in pancreatic juice secretion. The small increase in flow or the decrease in total protein output was not significantly different when compared to the basal secretory parameters prior to infusion of saline. The pattern of basal secretion in this study employing the isolated perfused rat pancreas is similar to that obtained with the use of the anaesthetized rat (6, 14, 15). These results indicate that basal secretion from the pancreas is unaffected when removed from the animal and placed in an *in vitro* situation.

In this study, application of optimal doses of either secretin or CCK-8 to the isolated intact perfused rat pancreas resulted in marked increases in both pancreatic juice flow and total protein output. Moreover, the effect of CCK-8 in both secretory parameters was three-fold larger compared to the responses obtained with secretin alone. Interestingly, similar results were obtained in a previous study where optimal physiological doses of the two gut hormones in the isolated rat pancreas were used (9, 10). In contrast, in the anaesthetized rat it was shown that infusion of animals with secretin resulted in a much larger increase in pancreatic juice flow compared to CCK-8 alone (15). The results of the present study have indicated that the ability of secretin to stimulate pancreatic juice flow in the isolated intact rat pancreas decreases when compared to

the anaesthetized animal. This interesting observation clearly suggests that the secretory effects of both CCK-8 and secretin may be influenced by the autonomic nervous system (ANS) *in vivo*. It is possible to postulate from the present study that the ANS is playing a functional role *in vivo* to modulate the secretory responses evoked by the gut hormones.

Simultaneous application of optimal concentrations of both CCK-8 and secretin to the isolated perfused rat pancreas failed to elicit either a potentiation or an additive response in both pancreatic juice flow and total protein output. In fact, both secretrory parameters were reduced when compared to the responses obtained with CCK-8 alone. Similar findings were obtained when similar concentrations of the two gut hormones were infused simultaneously to the anaesthetized rat (15) or when a submaximal dose of secretin was administered in the presence of a wide range of CCK-8 concentrations in superfused pancreatic lobules (4). In contrast, when different peptides or concentrations of the hormones were employed a potentiation in secretory parameters was observed (7, 10, 13). This observation suggests that the interaction between the gut hormones to elicit either a potentiation or an attenuation in secretory responses may depend critically on the kind and concentrations of the hormones employed in the study.

In conclusion, the results of this study have demonstrated that optimal doses of CCK-8 and secretin can stimulate juice flow and total protein output in the isolated pancreas when applied individually. However, when combined, they failed to induce either a potentiation or an additive response.

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P. J. CAMELLO, D. M. WISDOM, J. SINGH y G. M. SALIDO. *Control hormonal de la secreción exocrina pancreática en páncreas perfundido de rata.* Rev. esp. Fisiol. (J. Physiol. Biochem.), 50 (1), 35-40, 1994.

En páncreas de rata aislado y perfundido se investigan los efectos de las dos hormonas gastrointestinales, colecistoquinina octapéptido (CCK-8) y secretina, sobre el flujo de jugo pancreático y la producción total de proteínas. La perfusión del páncreas con CCK-8 (100 pM) o secretina (100 pM) da como resultado un marcado incremento en el flujo de jugo pancreático y en la producción de proteína total. El efecto de la CCK-8 es 3 veces mayor que las respuestas obtenidas con secretina sola. La aplicación simultánea de CCK-8 y secretina (ambas 100 pM) no provoca potenciación o efecto aditivo en el flujo de jugo pancreático ni en la producción total de proteínas. Los aumentos netos de ambos parámetros secretores incluso se reducen ligeramente durante la aplicación combinada de CCK-8 y secretina comparados con las respuestas obtenidas con CCK-8 sola. Los resultados indican que las concentraciones óptimas tanto de CCK-8 como de secretina pueden tener un marcado efecto secretagogo en el páncreas exocrino, aunque cuando se administran simultáneamente no provocan respuesta aditiva o potenciación en la secreción de jugo pancreático.

Palabras clave: Páncreas, Secretina, Colecistoquinina.

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