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# Effect of Secretin on Vagal Stimulation-Evoked Exocrine Pancreatic Secretion in the Rat

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The effect of secretin on nerve-mediated pancreatic juice secretion in the anaesthetized rat has been studied. Vagotomy caused a significant reduction in the rate of pancreatic juice flow, total protein output and amylase secretion being compared to control values prior to vagotomy. Both secretin (intravenous infusion $10^{-10}$ mol/kg body weight/h) and electrical stimulation of the vagus nerves (4 V, 2 ms, 20 Hz) caused marked increases in flow, total protein output and amylase output. Pretreatment of rats with atropine (0.1 mg/kg body weight) abolished the electrical stimulation-evoked secretion. However, simultaneous intravenous infusion of secretin and electrical stimulation did not yield either a clear additive response or a potentiation of secretory responses.

Key words: Exocrine pancreatic secretion, Secretin, Vagus.

The secretion of pancreatic juice is basically controlled by both the autonomic nervous system (12, 15, 19) and the two naturally occurring gut hormones, secretin and cholecystokinin (6). The parasympathetic nerves form the major stimulatory neural pathway in regulating exocrine pancreatic secretion (12) and the neurotransmitter released in response to vagal stimulation is mainly acetylcholine (15). There is also evidence that adrenergic (17) as well as non-cholinergic non-adrenergic (18) nerves can also influence exocrine pancreatic secretion to a lesser extent when compared to cholinergic stimulation.

Cholecystokinin and acetylcholine act by stimulating phosphatidyl-inositol 4,5-bisphosphate breakdown to produce dia-

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cylglycerol and inositol 1,4,5-trisphosphate, with subsequent activation of protein kinase C by diacylglycerol and $Ca^{2+}$ mobilization by inositol 1,4,5-trisphosphate (3). Secretin and vasoactive intestinal polypeptide activate adenylate cyclase to produce cAMP with subsequent activation of protein kinase A (13).

Over the past few years several studies, using both *in vivo* and *in vitro* preparations, have investigated the interactions between secretagogues in an attempt to ascertain precisely how they may act to elicit pancreatic juice secretion. Results have shown that two or more secretagogues acting via different stimulus-secretion coupling pathways can either potentiate or attenuate one another (10). The phorbol ester 12-0-tetradeconyl-phorbol-13-acetate can potentiate the responses to ACh, noradrenaline, the $Ca^{2+}$ ionophore A23187 and dibutyryl cyclic AMP (7, 9, 22) but attenuate pancreatic juice flow elicited by secretin (21) and enzyme and protein secretion induced by cholecystokinin (1, 8). Similarly, a combination of secretin with cholecystokinin can also result in marked reduction in secretory pancreatic parameters (20, 23).

Since the parasympathetic nerves and the gut hormone, secretin form two major neural and humoral pathways in the control of exocrine pancreatic secretion, it was decided to investigate how they may interact to elicit exocrine secretion.

## Materials and Methods

*Animals and surgical technique.* — Wistar rats of either sex weighing 250-300 g were fasted for 24 h with free access to water. The animals were anaesthetized with 0.75-1.0 g/kg i.p. urethane. Saline and drugs were administered via a cannula inserted in the jugular vein while systemic blood pressure was measured via a carotid artery cannula. Both cervical vagus nerves were dissected free from connective tissues but left unsevered. After laparotomy, the pylorus was ligated and the bile-pancreatic duct cannulated at its entrance to the duodenum for the collection of pancreatic juice. The hepatic end of the common bile-pancreatic duct was also cannulated and bile reinfused into the duodenum. The body temperature was maintained at 36-38 °C with heating lamps.

*Experimental design.* — Pancreatic juice was continuously collected on ice in preweighed capillary tubes for periods of either 20 or 30 min over a duration of 130 min. Following collection of basal sample, for 30 min, the right and left vagus nerves were cut in the neck followed by another 30 min collection period of secretion after vagotomy. Saline (0.9 % NaCl) or synthetic porcine secretin ($10^{-10}$ mol/kg b.w./h) (Sigma) were infused continuously via the jugular vein for 20 min followed by another 20 min of infusion plus electrical stimulation (4 V, 2 ms, 20 Hz) of the distal end of both vagus nerves and pancreatic juice collected separately during these two stimulatory periods. At the end of the stimulatory periods, pancreatic juice was collected for a final unstimulatory period of 30 min. In some experiments atropine (0.1 mg/kg b.w.) was i.v. injected prior to electrical stimulation of the vagus nerves.

*Analytical methods.* — Secretory rates were determined by reweighing the tared capillary tubes, assuming a density of secretion equal to water. Total protein concentrations were estimated by the method of BRADFORD (4), and amylase concentrations were determined by the method of BERNFELD (2) adopting arbitrary units defined by HICKSON (11).

*Statistical analysis.* — Data were compared by Student's test and only values with $P < 0.05$ were accepted as significant.

## Results

In this series of experiments the mean ± SEM resting rate of pancreatic juice flow, total protein output and amylase output in the anaesthetized rat prior to vagotomy were 0.37 ± 0.09 µl/min (n = 18), 6.34 ± 0.87 µg/min (n = 18) and 30.03 ± 3.32 mU/min (n = 18), respectively. After vagotomy the rate of pancreatic juice flow, total protein output and amylase output were 0.27 ± 0.07 µl/min (n = 18), 4.61 ± 0.84 µg/min (n = 18) and 23.62 ± 3.05 mU/min (n = 18), respectively. These results show that vagotomy caused a significant ($P < 0.05$) reduction in all secretory parameters compared to values obtained prior to vagotomy.

Figure 1 shows that intravenous infusion of saline caused a small increase in flow. However, when secretin was infused continuously there was a 233 % increase in flow compared to vagotomy values. Electrical stimulation of the vagus in the presence of saline resulted in a 213 % increase in pancreatic juice flow. This value was significantly ($P < 0.001$) reduced by the cholinergic muscarinic antagonist,

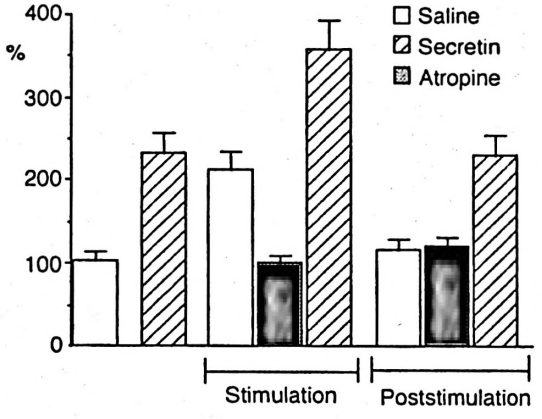


Fig. 1. *Rate of pancreatic juice flow expressed as a percentage of unstimulated flow after vagotomy.* Each bar represents mean ± SEM of six experiments.

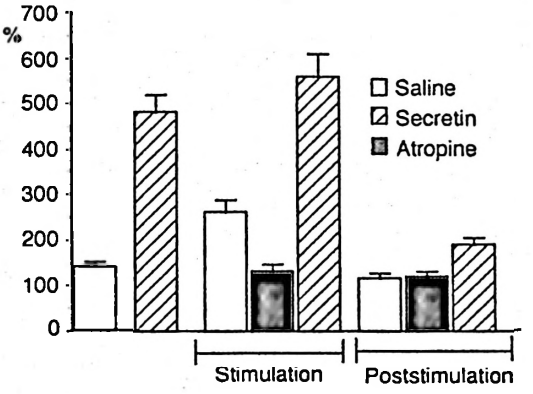


Fig. 2. *Total protein output expressed as a percentage of unstimulated protein output after vagotomy.* Each bar represents mean ± SEM of six experiments.

atropine. In contrast, when the vagus nerves were stimulated in the continuous presence of infused secretin the flow rate increased by about 357 % indicating that the gut hormone is not potentiating the response evoked by vagal stimulation. During the post-electrical stimulation period pancreatic juice flow decreased to the unstimulated levels following vagotomy in those experiments in which saline was infused, but remained partially elevated in these experiments in which electrical stimulation was combined with secretin infusion.

Similarly, saline infusion caused only a small increase in total protein output (fig. 2). However, continuous infusion of secretin resulted in a large and significant ($P < 0.001$) increase in protein output. Electrical stimulation of the vagus nerves caused a significant ($P < 0.001$) increase in protein output above vagotomy level but this value was less than that obtained with secretin infusion alone. Pretreatment of rats with atropine abolished the electrical stimulation-evoked protein output. In the continuous presence of secretin in-

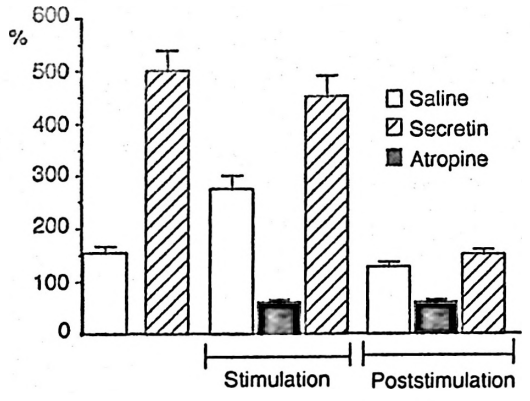


Fig. 3. *Amylase output expressed as a percentage of unstimulated amylase output after vagotomy.* Each bar represents mean ± SEM of six experiments.

fusion, electrical stimulation caused a large increase in total protein output which was not significantly different from the value obtained during secretin stimulation alone. During the post-electrical stimulation period total protein output fell to values close to the basal level.

Finally, figure 3 shows that intravenous saline infusion caused a small increase in amylase secretion compared to a large and significant ($P < 0.001$) release of the digestive enzyme in the presence of secretin infusion. Electrical stimulation of the vagus nerves also evoked a significant ($P < 0.001$) increase in amylase output but this value was less than that obtained with secretin stimulation. Pretreatment of rats with atropine abolished the electrical stimulation-induced amylase release. When electrical stimulation was applied in the continuous presence of secretin, amylase output was significantly elevated compared to vagotomy value. Again, this value, like protein output, was not significantly different when compared to the amylase released during secretin infusion alone. During the post-electrical stimulation period amylase secretion fell to almost the control vagotomy level.

## Discussion

In agreement with others (5) this study has demonstrated that stimulation of the vagus nerves cause an increase in pancreatic exocrine secretion that is reduced by atropine; moreover, vagotomy cause a significant reduction in exocrine pancreatic juice secretion in the anaesthetized rat. This suggests that vagus nerves can influence both basal as well as stimulated exocrine pancreatic secretion. The pattern of exocrine secretion during saline infusion in this study is similar to values obtained in other experiments using anaesthetized rats (8, 21, 23).

On the other hand, secretin is a known potent stimulant for pancreatic exocrine secretion by activating ductal and acinar adenylate cyclase (13). In fact, intravenous administration of secretin resulted in increased pancreatic juice flow and protein and amylase output. Although it is accepted that pancreatic intact cells exhibit synergistic stimulation by diacylglycerol-, $Ca^{2+}$- and cyclic AMP-mediated pathways (16), when secretin was infused during electrical stimulation the gut hormone did not potentiate the rate of pancreatic juice secretion elicited by the vagus nerves. These results do not agree with the view that the interaction of the distinct pathways that secretagogues employ should lead to potentiated secretory responses (1, 9, 13, 22). KATSUSHIMA *et al.* (14) showed that cholecystokinin octapeptide can induce down regulation of secretin receptors associated with diminished acinar response to secretin-induced cAMP and amylase release and that this inhibits the secretory responses compared to the effect of each secretagogue alone. If ACh, a calcium-dependent secretagogue like CCK, is capable of this secretin down regulation then this is a possible explanation for the secretion found with combining vagal stimulation and secretin in the present study. Indeed, carbachol, an analogue of ACh, has been shown to induce

this down regulation of secretin receptors but to a lesser extent than cholecystokinin (14).

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## Resumen

Se estudia el efecto de la secretina sobre la secreción de jugo pancreático inducida por estimulación nerviosa en rata anestesiada. La vagotomía causa una significativa reducción en el flujo de secreción de jugo pancreático, en la producción de proteína total y en la secreción de amilasa, respecto de los valores control previos a la vagotomía. Tanto la secretina (infusión intravenosa de $10^{-10}$ mol/kg peso corporal/h) como la estimulación eléctrica de los nervios vago (4 v, 2 ms, 20 Hz) causan incrementos marcados del flujo, de la producción de proteína total y de amilasa. El tratamiento previo de las ratas con atropina (0,1 mg/kg peso corporal) abole la secreción provocada por estimulación eléctrica. La aplicación simultánea de infusión intravenosa de secretina y la estimulación eléctrica no induce una respuesta aditiva clara ni una potenciación de las respuestas secretoras individuales.

Palabras clave: Secreción pancreática exocrina, Secretina, Vago.

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