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Mechanisms Involved in the Control of Exocrine Pancreatic Secretion in the Interdigestive State in the Rabbit*

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The effect of rapid wash-out of the duodenum with phosphate buffered saline on exocrine pancreatic secretion and plasma levels of secretin, VIP, gastrin and CCK was studied. Furthermore, the possible nervous role in this effect was checked after atropine and hexamethonium treatment. Rapid wash-out significantly increased protein output (35.0 μg/min, in the control group without duodenal perfusion and 72.15 μg/min, in the perfused group) and the plasma levels of CCK (from 5.2 to 13.17 fmol/ml). Intravenous infusion of atropine significantly reduced the protein output (from 78.19 to 32.45 μg/min) and the plasma levels of CCK (from 10.1 to 5.55 fmol/ml), with no change in the remaining parameters in the intraduodenally perfused group. Intravenous administration of hexamethonium significantly stimulated hydroelectrolyte secretion (from 6.99 to 15.15 μl/min) and the plasma levels of VIP (from 4.8 to 7.3 fmol/ml) and reduced the protein output (from 61.47 to 30.75 μg/min) and the plasma levels of CCK (from 14.56 to 6.25 fmol/ml) in the intraduodenally perfused group. Our results suggest that, in the interdigestive state, the exocrine pancreatic secretion of the rabbit is tonically inhibited. This inhibition can be divided into two different mechanisms: on the one hand there is a decrease in enzyme secretion produced by a duodenal factor and mediated by CCK and muscarinic mechanisms and on the other, there is an inhibition of hydroelectrolyte secretion with no duodenal participation which is probably controlled by nervous non-muscarinic mechanisms and VIP involvement.

Key words: CCK, Cholinergic, Exocrine pancreas, Secretin and VIP.

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There is experimental evidence in animals to suggest that pancreatic enzymes in the duodenum exhibit a feedback control of pancreatic exocrine secretion (2, 5, 7, 11, 12). Moreover the discovery that gastrointestinal peptides were secreted intraluminally (5, 13, 22) suggested a regulated mechanism of exocrine pancreatic secretion from duodenum in the interdigestive state, without food implications. One approach to study the exocrine pancreatic secretion regulation from duodenum in the interdigestive state has been the washout of duodenum with phosphate buffered saline to remove regulatory factors present in intestinal secretion (15, 18).

Preliminary results obtained in our laboratory indicate that pancreatic enzyme secretion in rabbits which were perfused with phosphate buffered saline was higher than that in non perfused animals; these results are different from those found in rat (15, 18). Because of this, the effect of duodenum washout with phosphate buffered saline on exocrine pancreatic secretion in the interdigestive state in the anesthetized rabbit, has been studied. In order to gain a better characterization of the pancreatic regulation the plasma levels of CCK secretin, VIP and gastrin have been measured as well as by means of atropine and hexamethonium the nervous influence on the pancreatic response and release of these peptides during duodenum washout.

Materials and Methods

Materials. — Atropine sulfate, hexamethonium VIP, CCK and Gastrin were purchased from SIGMA Chemical Co Secretin was provided by Dr. Viktor Mutt (Karolinska Institutet, Stockholm, Sweden). Gastrin antibodies and CCK-Gastrin common pentapeptide antibodies were kindly provided by Dr. Stephen R. Bloom (Royal Postgraduate Medical School, London, U.K.). All the other reagents were of the highest commercially available grade.

Animals and surgical technique.— Male adult New Zealand rabbits weighing 2-2.5 kg were fasted for 24 h with free access to water. Coprophagy was prevented by a collar. Rabbits were anesthetized with sodium pentobarbital (30 mg/kg body weight) and tacheotomized. After a median laparotomy, the pancreatic duct was cannulated following ligation of the pylorus and cannulation of the bile duct to deviate bile flow to the exterior. Blood samples were taken from the portal vein, replacing the volume withdrawn by injecting the same volume of dextran saline (Bax-Dextran saline, Braum).

When necessary, intraduodenal perfusion, flow rate 3 ml/min, was carried out by means of two cannulae; one placed at the proximal end of the duodenum through the pylorus and the other three centimeters distal to the outlet of the pancreatic duct.

Experimental design. — In the control group (without duodenal perfusion, n = 24), an equilibration period of 30 min was followed by a 20 min basal period. During the basal period the pancreatic flow was collected and a sample of portal venous blood was taken.

In phosphate buffered saline (PBS) group (n = 24), the same experimental design was used. In this experimental group the duodenal lumen was perfused with phosphate buffered saline during both the equilibration and basal period.

The effects of atropine (n = 8) and hexamethonium (n = 8) were studied in animals perfused with PBS. An intravenous bolus of atropine (1 mg/kg b.w.) or hexamethonium (25 mg/kg b.w.) was injected after the basal period. The pancreatic flow was then collected during two periods of 20 min and a blood sample was taken in the middle of each period. At no time did any of the animals show signs of atropine or hexamethonium toxicity.

Analytical methods. — The output of pancreatic juice was determined by weighing the 20 min aliquots on an electronic balance (Type H-35, Mettler, Switzerland), assuming the density of the juice to be 1.0. Total protein concentration was measured by the Coomassie blue binding method (3).

Secretin and VIP plasma levels were measured by RIA methods developed in our laboratory (9, 10). CCK and gastrin plasma levels were measured by RIA following the method of ADRIAN and BACARESE-HAMILTON (1).

Statistical Treatment. — Values are expressed as mean ± standard error of the mean. Data were compared by Student's *t* test for unpaired data or by ANOVA followed by Scheffé test where suitable. Differences were considered significant if $p < 0.05$.

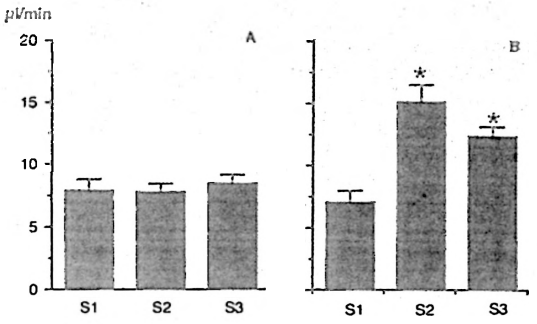

Fig. 1. *Flow rate (µl/min) in animals with an intravenous infusion of atropine (A) or hexamethonium (B).*
S1 is the basal 20 min sample, and S2 and S3 are the two 20 min. samples after the injection of atropine or hexamethonium. Values are means ± SEM of 8 animals. * Significant differences ($p < 0.05$) by ANOVA followed by Scheffé test from basal sample.

Results

Effect of intraduodenal perfusion of PBS. — Intraduodenal perfusion of PBS significantly increased the protein output and CCK plasma levels; the other parameters remained unchanged (table I).

Effect of atropine in perfused animals.— Atropine injection had no effect on pancreatic flow rate (fig. 1A) and portal plasma secretin and VIP levels. On the other hand, atropine significantly reduced the protein output (fig. 2A) and the plasma levels of CCK (fig. 3A). Furthermore, gastrin plasma levels were also significantly (fig. 3A) diminished by atropine.

Effect of hexamethonium in perfused animals. — Intravenous injection of hexamethonium significantly stimulated pancreatic flow rate (fig. 1B). Moreover, the hexamethonium also significantly in-

Table I. *Flow rate, protein output, bicarbonate output and hormonal plasma in the control group (without duodenal perfusion) and in the phosphate buffered saline (PBS) group after a 20 min basal period.*
Values are mean ± SEM, in parentheses number of animals.

	CONTROL Group	PBS Group
Flow (µl/min)	6.39 ± 0.33 (24)	6.61 ± 0.26 (24)
Bicarbonate output (mmol/min)	0.37 ± 0.02 (24)	0.40 ± 0.02 (24)
Protein output (µg/min)	35.0 ± 2.16 (24)	72.15 ± 4.21* (24)
CCK (fmol/ml)	5.20 ± 0.83 (24)	13.17 ± 1.27* (24)
Secretin (fmol/ml)	4.37 ± 0.58 (20)	4.10 ± 0.55 (20)
Gastrin (fmol/ml)	6.20 ± 0.83 (24)	6.02 ± 0.52 (24)

* significant differences ($p < 0.05$) by Student's *t* test from control group.

creased VIP release to portal plasma (figure 4). However, secretin plasma levels remained unchanged after hexamethonium injection.

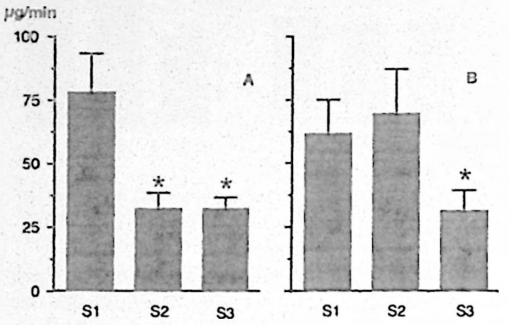

Fig. 2. *Protein output (µg/min) in animals with an intravenous infusion of atropine (A) or hexamethonium (B).*
Legend as in figure 1.

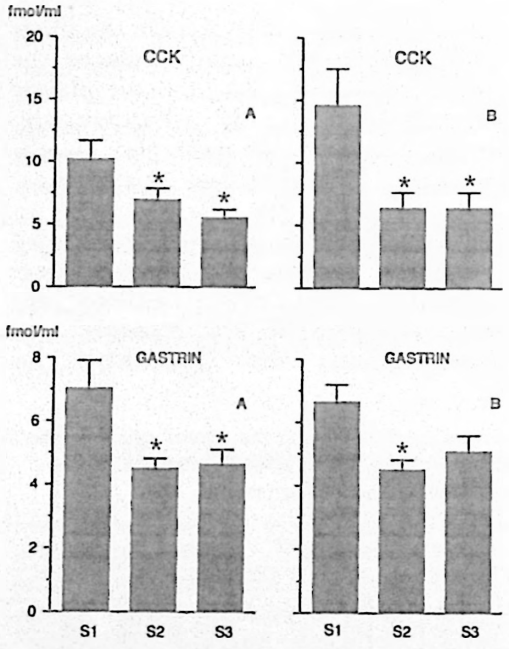

Fig 3. *Portal plasma levels of CCK and gastrin (fmol/ml) in animals with an intravenous infusion of atropine (A) or hexamethonium (B).*
Legend as in figure 1.

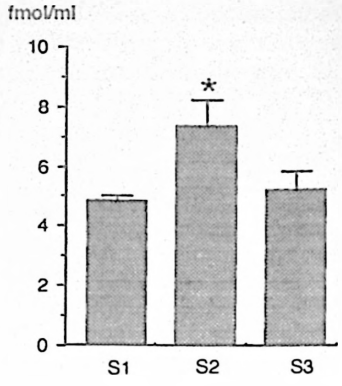

Fig. 4. *Portal plasma levels of VIP in animals with an intravenous infusion of hexamethonium.*
Legend as in figure 1.

On the contrary, protein output was significantly reduced in the second sample after drug administration (S3), whereas the first sample (S2) after drug administration was similar to the basal value (S1) (fig. 2B). Finally, hexamethonium also significantly ($p < 0.05$) diminished CCK release (fig. 3B) and, to a lesser extent, gastrin release (fig. 3B).

Discussion

The intraduodenal perfusion of PBS in the anesthetized rabbit produced an increase in protein output from exocrine pancreas in the interdigestive state that could be ascribed to the effect of CCK.

The increase observed in protein output after intraduodenal perfusion of PBS could indicate the existence of a yet unknown intraduodenal factor that inhibits the protein secretion of the rabbit in the interdigestive state. In this respect it is interesting to emphasize that the rabbit has a relatively high basal secretion, demonstrated with in vitro experiments (19), and therefore it seems convenient to avoid an unneccesary entry of pancreatic enzymes to the intestine.

The situation is completely different from that found in the rat where a stimulant duodenal factor (CCK releasing peptide) has been demonstrated (15, 18) and this is probably related to the different digestive behaviour of this species. Moreover, it is remarkable that both stimulatory (in the rat) and inhibitory (in the rabbit) factors are related to CCK, the stimulatory one producing the release of this peptide while the inhibitory one decreasing, by a mechanism not yet known, its release. Furthermore, the effect of PBS perfusion is specific to protein secretion since the flow rate remained constant. This is in agreement with the findings obtained for the other peptides (secretin and VIP) whose levels also remained unchanged. Bearing in mind that the control of the exocrine pancreatic secretion from the intestine in the interdigestive state is a general mechanism described in many species (6, 7, 11, 12, 17) (though with some differences among them), our results confirm this for the rabbit and also demonstrate important interspecific differences.

The decrease in protein output after atropine treatment is a logical consequence of the blockade of muscarinic receptors, which coincides with a decrease of CCK and gastrin levels. Though for many years the positive influence of vagal stimulation on gastrin secretion has been known (20), CCK release has only recently been described affected by the cholinergic nervous system (14). On the other hand, no effect of atropine on secretin and VIP levels was found, which is in agreement with what was observed in the flow rate since it did not suffer any change after atropine injection.

Hexamethonium caused a significant increase in the pancreatic flow rate; this stimulating effect is specific of hydroelectrolyte secretion because, although the protein output of S2 is practically the same as that of S1, due to a washout effect of the high flow rate, this protein secretion significantly decreases in S3 (fig. 2B). As regards CCK and gastrin levels, their decrease can be explained in terms of the same reasons offered above for atropine treatment.

On the other hand the significant increase observed in plasma VIP levels after hexamethonium treatment, which was also previously found in dogs (16), may account for the observed increase in pancreatic flow rate. This involves the existence of a tonic inhibitory effect on the release of this peptide and also on hydroelectrolyte exocrine pancreatic secretion under basal conditions in the rabbit. According to our previous results using different α and β adrenergic antagonists this might explain the increase in flow rate described after adrenergic blockade (4, 8). However, whatever the mechanism involved, it seems clear that VIP has important implications in the regulation of exocrine pancreas in the rabbit. Furthermore, our results are in agreement with those of SOLOMON *et al.* (21), who described a nicotine-induced inhibition of pancreatic exocrine secretion of water and bicarbonate in the rabbit, probably mediated by catecholamines.

Our results demonstrate that, in the interdigestive state, the exocrine pancreatic secretion of the rabbit is tonically inhibited. This inhibition can be split into two completely different mechanisms: on the one hand, there is a decrease in enzyme secretion produced by a duodenal factor and mediated by CCK and muscarinic mechanisms; on the other, there is inhibition of hydroelectrolyte secretion with no duodenal participation which is probably controlled by nervous non-muscarinic mechanisms and VIP involvement.

As far as we know nothing similar to this complex regulation of secretion in the interdigestive state has yet been described in any species, which does not mean that its possible existence in other animals should be ruled out.

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Resumen

Se estudia el efecto del lavado duodenal con solución salina tamponada sobre la secreción pancreática exocrina y los niveles plasmáticos de secretina, VIP, gastrina y CCK, así como la posible regulación nerviosa de dicha secreción utilizando atropina y hexametonio. El lavado duodenal produce un incremento significativo en la secreción pancreática enzimática (35,0 µg/min en el grupo control sin perfusión duodenal y 72,15 en el grupo de animales perfundidos) y en los niveles plasmáticos de CCK (5,2 fmol/ml frente a 13,17). Estos incrementos son reducidos por la administración intravenosa de atropina (de 78,19 µg/min a 32,45 en proteínas y de 10,1 fmol/ml a 5,55 en CCK plasmática). No se observa ningún cambio en el resto de parámetros del grupo de animales perfundidos intraduodenalmente. La administración de hexametonio incrementa significativamente la secreción pancreática hidroelectrolítica (6,99 µl/min frente a 15,15) y los niveles plasmáticos de VIP (de 4,8 fmol/ml a 7,3), reduciendo significativamente la secreción de proteínas por el páncreas (de 61,47 µg/min a 30,75) y los niveles plasmáticos de CCK (14,56 frente a 6,25 fmol/ml). Se concluye que en conejo, en el período interdigestivo, la secreción pancreática exocrina está tónicamente inhibida, proponiéndose dos mecanismos diferentes: 1) Inhibición de la secreción pancreática enzimática producida por la presencia de un factor duodenal y mediada por CCK y por receptores muscarínicos; 2) Inhibición de la secreción pancreática hidroelectrolítica, probablemente controlada por mecanismos nerviosos no muscarínicos, con la participación del VIP y no mediada por el duodeno.

Palabras clave: Colecistoquinina, Colinérgico, Páncreas exocrino, Secretina, Péptido intestinal vasoactivo.

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