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THE ENDOCRINE REGULATION OF EXOCRINE PANCREAS IN PRERUMINANT MILK-FED CALVES

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Résumé

RÉGULATION ENDOCRINIENNE DU PANCRÉAS EXOCRINE CHEZ LE VEAU PRÉRUMINANT NOURRI AU LAIT. — Nous avons utilisé 15 veaux mâles Holstein × Frisons, nourris avec un lait de remplacement, pesant 65 ± 3 kg, porteurs d’un cathéter réentrant (implanté de façon chronique à l’âge de 10 jours) sur le canal pancréatique.

Au cours de six expériences successives, nous avons étudié l’influence d’une perfusion intraveineuse de sécrétine, cholécystokinine-pancréozymin (CCK-PZ), vasoactive intestinal peptide (VIP), gastrine et somatostatine sur le débit pancréatique et l’excrétion de protéines, calcium, magnésium, phosphore, zinc et activité amylasique dans le suc pancréatique. Au cours de deux autres expériences, nous avons étudié l’influence d’une perfusion de somatostatine administrée seule ou simultanément avec de la sécrétine ou de la CCK-PZ. Chaque expérience débute 5 h après le repas, et aucun aliment n’est distribué aux animaux pendant la durée de l’expérience.

Les résultats obtenus indiquent que la régulation du pancréas exocrine du jeune veau présente de nombreuses analogies avec celle des monogastriques. La sécrétine et la CCK-PZ entraînent une augmentation de l’excrétion pancréatique d’eau, de minéraux, de protéines et d’amylase. La somatostatine et le glucagon inhibent les effets des deux hormones précédentes. La gastrine augmente légèrement le débit sans toutefois modifier significativement l’excrétion de protéines et d’amylase. Le VIP n’a provoqué aucune variation significative.

Cependant, la régulation du pancréas exocrine du jeune veau se caractérise essentiellement par la stimulation intense de l’excrétion des protéines et d’amylase sous sécrétine.

The hormonal control of pancreatic exocrine secretion in mammals mainly depends from the duodenal release of secretin and pancreozymin. Secretin primarily stimulates the secretion of water and bicarbonate (Scratcherd and Case, 1973) and also exerts slight but indeniable ecbolic effects (Wormsley, 1968; Dockray, 1972). Pancreozymin preferentialy increases enzyme secretion and secondary the movement of water and ions (Henriksen, 1968).

However, other gastrointestinal hormones play also a role in the regulation of exocrine pancreas. Recently, a vasoactive intestinal peptide (VIP) has been isolated from hog duodenum and shown to be related in structure to
secretin and glucagon (Said and Mutt, 1972). The physiological role played by VIP is uncertain but this peptide is known to have vasodilator and hypotensive actions in the dog (Said and Mutt, 1970). It seems as potent as secretin in stimulating the flow of pancreatic juice in the cat (Said and Mutt, 1972).

Somatostatin (somatotrophin-release inhibiting factor : SRIF) extracted from ovine hypothalamus (Brazeau et al., 1973) but also present in the islets of Langherans (Dubois, 1975) inhibits exocrine pancreatic secretion in man (Dollinger et al., 1976).

Fourteen of the 27 amino acids present in the secretin molecule have the same position as in glucagon (Bromer et al., 1956). However glucagon inhibits pancreatic exocrine secretion in vivo (Dyck et al., 1969, 1970; Steffen et al., 1972).

The C terminal pentapeptide Gly-Try-Met-Asp-Phe-NH₂ is common to both gastrin and pancreozymin sequences (Mutt and Jorpes, 1967). Gastrin stimulates water, minerals and enzyme excretion in the pancreatic juice (Stenning and Grossman, 1969).

As far as we are aware until now, nobody has studied the influence of these hormones on pancreatic exocrine secretion in young calves. This was the purpose of the work reported here.

**Materials and Methods**

Fifteen male Holstein × Frisian calves, weighing 65 ± 3 kg were used.

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Origin</th>
<th>Dose used (per kg body weight/15 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Secretin</td>
<td>porcine¹</td>
<td>1.5 Clinical unit</td>
</tr>
<tr>
<td>CCK-PZ</td>
<td>porcine¹</td>
<td>1.1 Ivy dog unit</td>
</tr>
<tr>
<td>VIP</td>
<td>porcine¹</td>
<td>300 ng</td>
</tr>
<tr>
<td>Gastrin</td>
<td>porcine GI + GI²</td>
<td>1.5 µg</td>
</tr>
<tr>
<td>Glucagon</td>
<td>bovine²</td>
<td>40 µg</td>
</tr>
<tr>
<td>Somatostatin</td>
<td>ovine synthetic⁴</td>
<td>6 µg</td>
</tr>
</tbody>
</table>

2. Eurorga, Paris, France.
3. Eli Lilly Laboratories, Chicago, Illinois, U.S.A.
red during sampling, which avoided disturbing the feed-back regulation of the exocrine pancreas by pancreatic enzymes released in the intestine (Corring, 1974; Davicco et al., 1979).

The calves were fed twice daily with a commercial milk replacer (0.4 kg powdered milk/kg body weight/day) whose composition is given in table 1.

Each experiment was performed 5 h after calves feeding. No milk was given to the animals during the time of sampling.

**Hormonal infusions.**

In six successive experiments, we have observed the influence of an intravenous infusion of secretin, cholecystokinin-pancreozymin (CCK-PZ), vasoactive intestinal peptide, glucagon, gastrin and somatostatin on exocrine pancreatic secretion. The dose used for each hormone is given in table 2. In each experiment, basal pancreatic secretion was collected for at least 30 min before initiation of hormonal infusion, while saline (0.9% NaCl) was infused at a rate of 40 ml/h into the jugular vein. The hormone was then added to the saline and infused for 15 min. Pancreatic juice was collected during hormonal infusion and for 90 min after stopping it.

In two experiments, we have studied the interaction of secretin or CCK-PZ with somatostatin (SRIF). Then after collection of basal pancreatic secretion for 30 min, as in preceding experiments, SRIF (6 µg/kg body weight) and secretin (0.6 Ivy Dog Unit/kg body weight) or CCK-PZ (1.1 Unit/kg body weight) were added to the saline and infused into the right jugular vein for 15 min. 60 min after the end of this infusion, secretin (0.6 Ivy Dog Unit/kg body weight) or CCK-PZ (1.1 Unit/kg body weight) were dissolved in saline and infused into the left jugular vein for 15 min. Pancreatic juice was collected for 60 min after stopping this second infusion.

**Analysis.**

On each thawed sample of pancreatic juice we measured total proteins, amylase and minerals: calcium (Ca), magnesium (Mg), inorganic phosphorus (PO₄) and zinc (Zn). The proteins were measured by Biuret's colorimetric reaction adapted to a Technicon autoanalyzer (Michel, unpublished data). For the
amylase assay, we used a volume of pancreatic juice reacting with a starch solution for a given length of time in a water bath at 39 °C. The sugars released after incubation were extracted by 85° alcohol and measured by the sulphuric orcinol method (Besle, 1974). Ca, Mg, Zn were measured by atomic absorption spectrophotometry (Perkin-Elmer 400) and PO₄ was measured by colorimetry.

In order to correct any individual variations of pancreatic juice in the animals, the results were expressed as a percentage of those obtained before each infusion. Each animal thus served as its own control and the significance of the differences between the values was computed by the Student’s t test.

Results

Secretin increased significantly (P < 0.01) the excretion of water (+ 457 ± 70 %), calcium (+ 398 ± 18 %), magnesium (+ 331 ± 44 %) and amylase activity (+ 1023 ± 286 %) in the pancreatic juice during 15 min after starting the infusion (fig. 1). The excretion of inorganic phosphorus (+ 150 ± 5 %), zinc (+ 285 ± 200 %) and proteins (+ 93 ± 60 %) was significantly increased between the 15th and 30th minute after starting the infusion (fig. 1).

The infusion of porcine CCK-PZ increased significantly (P < 0.05) the excretion of water, proteins, and amylase activity (fig. 2). But this increase in flow rate is significantly lower (P < 0.05) than after secretin infusion (+ 457 ± 70 %). Similarly after CCK-PZ infusion, proteins excretion (+ 50 ± 25 %), calcium excretion (+ 110 ± 60 %) and amylase activity (+ 380 ± 92 %) increased less (P < 0.05) than after secretin infusion (proteins excretion : + 180 ± 70 %; calcium excretion : + 300 ± 54 %; amylase activity : + 900 ± 87 %). When infused alone, somatostatin decreased significantly the excretion of water (− 33 ± 8 %), minerals (Ca : − 46 ± 12 %; Mg : − 49 ± 18 %; PO₄ : − 25 ± 7 % and Zn : − 34 ± 7 %), proteins (− 72 ± 14 %) and amylase activity (− 58 ± 11 %) in the pancreatic juice 15 min after stopping the infusion (fig. 3).

When infused alone, somatostatin decreased significantly the excretion of water (− 33 ± 8 %), minerals (Ca : − 46 ± 12 %; Mg : − 49 ± 18 %; PO₄ : − 25 ± 7 % and Zn : − 34 ± 7 %), proteins (− 72 ± 14 %) and amylase activity (− 58 ± 11 %) in the pancreatic juice 15 min after stopping the infusion (fig. 3). When SRIF was given simultaneously

![Fig. 3. — The effect of SRIF on pancreatic juice flow rate and on the excretion of proteins and calcium, magnesium, inorganic phosphorus, zinc and amylase activity in the pancreatic juice of 5 calves (mode ± S.E.).](image)

![Fig. 4. — The effect of secretin infused alone or together with SRIF on pancreatic juice flow rate and on the excretion of proteins and calcium, magnesium, inorganic phosphorus, zinc and amylase activity in the pancreatic juice of 5 calves (mode ± S.E.).](image)
with secretin, the flow of pancreatic juice and amylase activity decreased significantly ($-8 \pm 7\% ; P < 0.01$) between the 30th and the 45th min after this infusion. Sixty minutes after stopping secretin + SRIF, the infusion of secretin alone increased significantly ($P < 0.05$) pancreatic excretion of water ($+219 \pm 15\%$), proteins ($+205 \pm 10\%$) and amylase activity ($+257 \pm 72\%$) (fig. 4). When SRIF was given simultaneously with CCK-PZ, the excretion of water ($-30 \pm 13\%$), proteins ($-29 \pm 7\%$) and amylase activity ($-30 \pm 4\%$) decreased significantly ($P < 0.01$) 15 min after stopping the infusion. The infusion of CCK-PZ alone 60 min later, increased pancreatic flow ($+123 \pm 11\%$), proteins excretion ($+195 \pm 24\%$) and amylase activity ($+182 \pm 15\%$) without effect on the excretion of minerals (fig. 5).

The infusion of porcine VIP increased significantly ($P < 0.05$) the excretion of water ($+226 \pm 15\%$) and calcium ($+129 \pm 17\%$) (fig. 6). The infusion of gastrin increased significantly ($P < 0.05$) the flow of pancreatic juice ($+123 \pm 15\%$), proteins ($+195 \pm 24\%$), calcium ($+129 \pm 17\%$), magnesium ($+120 \pm 14\%$) and amylase activity ($+182 \pm 15\%$) (fig. 7).

**Fig. 5.** The effect of CCK-PZ infused alone or together with SRIF on pancreatic juice flow rate and on the excretion of proteins and calcium, magnesium, inorganic phosphorus, zinc and amylase activity in the pancreatic juice of 5 calves (mode ± S.E.).

**Fig. 6.** The effect of VIP on pancreatic juice flow rate and on the excretion of proteins and calcium, magnesium, inorganic phosphorus, zinc and amylase activity in the pancreatic juice of 5 calves (mode ± S.E.).

**Fig. 7.** The effect of gastrin on pancreatic juice flow rate and on the excretion of proteins and calcium, magnesium, inorganic phosphorus, zinc and amylase activity in the pancreatic juice of 5 calves (mode ± S.E.).
138 ± 24 %) during 15 min after starting this infusion, but had no influence on excretion of minerals proteins and amylase activity (fig. 6).

The infusion of gastrin increased significantly the excretion of water (+ 156 ± 20 % ; P < 0.01) during 30 min after starting the infusion, without effect on excretion of proteins and amylase (fig. 7).

Glucagon infusion inhibited significantly (P < 0.01) pancreatic flow rate (− 13 ± 3 %), minerals excretion (calcium : − 19 ± 6 % ; magnesium : − 14 ± 10 % ; phosphore : − 15 ± 11 % and zinc : − 20 ± 4 %), proteins excretion (− 15 ± 7 %) and amylase activity (− 10 ± 5 %) during 60 min after stopping this infusion (fig. 8).

Discussion

Results obtained after CCK-PZ infusion confirmed previous work, on rats which demonstrate that not only secretin but also CCK-PZ induces an increase of pancreatic flow rate (Dockray, 1972 ; Kanno and Nishimura, 1976). On the other hand, secretin increases proteins excretion and amylase activity significantly more that CCK-PZ. But in dog, Meyer et al., (1971) have shown that endogenous CCK-PZ as well as exogenous CCK-PZ strongly potentiates bicarbonate secretion stimulated by endogenous or exogenous CCK-PZ. Secretin can also stimulate amylase release *in vivo* (Kanno and Yamamoto, 1977) or on isolated acinar cells from rat pancreas. In the presence of CCK-PZ a synergistic action was observed (Lee, 1979). The increase of proteins excretion persisted during 45 min after secretin infusion. However the rise in amylase activity occurred 15 min before that observed for protein excretion (fig. 1) while after CCK-PZ infusion a concomitant increase in protein and amylase excretion is observed (fig. 2). Thus, our results do not allow to decide wether the effect of secretin on protein excretion in our animals is a « washing out » phenomenon.

Recent reports on the action of secretin and CCK-PZ suggest that secretin stimulates pancreatic secretion by increasing cyclic AMP levels in acinar cells (Robberecht et al., 1974 ; Robberecht, 1976), while CCK-PZ stimulates guanosine 3’5’ monophosphate (cyclic GMP) in acinar cells with concomitant increase of Ca²⁺ efflux from these cells (Robberecht et al., 1974 ; Sjodin and Gardner, 1977). Gardner and Jackson (1977) have demonstrated that these two pathways can interact in stimulating amylase secretion in pancreatic acinar cells from guinea pigs.

Several investigations have reported that SRIF inhibits both basal and CCK-PZ or secretin-stimulated enzyme secretion and pancreatic juice flow in men (Dollinger et al., 1976) and dogs (Boden et al., 1975 ; Kayasseh et al., 1977). However in anaesthetized cats (Albinus et al., 1976) and rats (Fölsch et al., 1978 ; Charriot et al., 1978). SRIF inhibited caerulein-stimulated protein secretion, but had not effect on secretion stimulated by exogenous and endogenous secretin. It has also been demonstrated that SRIF inhibits gastric hydrochloric acid secretion (Bloom et al., 1974) which stimulates release of secretin in fasted conscious dogs ; this inhibition is rapid in onset and quickly reversible (Boden et al., 1975). In humans, SRIF also decreases the release of endogenous secretin (Hanssen et al., 1977). The existence of the same sequence of four amino acid residues in the two peptides suggests that somatostatin activation of the exocrine pancreas depends on its interaction with secretin receptors (Robberecht et al., 1975). Moreover, in dogs, the kinetic analysis shows that the interaction between SRIF and secretin affecting pancreatic bicarbonate...
secretion possesses the characteristics of competitive inhibition (Konturek et al., 1977).

Indeed, the inhibitory action of SRIF on CCK-PZ stimulated enzyme secretion ceased 15 min after stopping somatostatin infusion, reflecting the short half life (about 3 min) of SRIF (Redding and Coy, 1974; Sheppard et al., 1979) and demonstrating the reversibility of the inhibition (Fölsch et al., 1978).

In calves, VIP appears as a potent stimulant of pancreatic juice flow rate, but seems to have no effect on proteins excretion and amylase activity. Similar results have been reported in dogs in which VIP was found to have a lower efficacy than secretin (Konturek et al., 1975; Makhlof et al., 1974) and inhibited secretin-induced pancreatic excretion of water (Konturek et al., 1976). Whereas in rats, secretin and VIP showed equal efficacy, and their combination exhibited an increasing action on pancreatic bicarbonate secretion (Said and Mutt, 1972).

These results might be explained by the interaction of VIP and secretin, two chemically related peptides (Said and Mutt, 1972) on a common receptor site on the exocrine pancreas (Konturek et al., 1976).

In calves, as in cats (Wizeman et al., 1974), humans (Dyck et al., 1970; Fontana et al., 1976) and dogs (Dyck et al., 1969), glucagon inhibited the excretion of water, proteins and amylase. When glucagon was administered in humans following a stimulatory dose of CCK-PZ or secretin, the stimulation induced both by secretin and CCK-PZ desappeared (Dyck et al., 1970; Steffen et al., 1972). It was also postulated that glucagon exhibits a direct effect on the ductular and acinar cells. The similar structure of glucagon and secretin may account for an interference of both hormones at the secretin receptor mediated through adenylate cyclase (Rutten et al., 1972).

In vitro, using isolated rat pancreatic lobules Adler (1977) has observed an inhibition of proteins synthesis and enzyme content in the pancreas after a 30 min glucagon infusion. This effect was absent after longer infusion periods of up to six hours. After 12 to 24 h infusions, a marked degranulation and decrease in enzyme content was observed. These results suggest a biphasic response of the pancreas to prolonged glucagon infusion: the initial inhibition exerted by the action of glucagon itself; the later effects are the results of the release of a variety of hormones from the gastrointestinal tract which override the glucagon effect and finally lead to a stimulation of the pancreas. Similar conclusions were drawn by Fujita et al. (1976).

In calves, gastrin infusion increases pancreatic flow rate and mineral concentration but amylase activity and proteins secretion do not varied significantly. Studies of the effect of pentagastrin in man have lead to conflicting results (Petersen and Berstad, 1971; Valenzuela et al., 1976). However, on the human pancreas, pentagastrin brings no change in the average of bicarbonate output, but it inhibits the enzymatic secretion caused by the association of secretin and CCK-PZ. The effect is proportional to the dose of CCK-PZ used, and evokes a mechanism of non competitive inhibition (Duffaut et al., 1972). Our experiments were done 5 hours after feeding, thus endogenous secretin and CCK-PZ were then released in the duodenum (Grossman, 1976). So, in our experimental conditions in calves, conclusions similar to that of Duffaut et al. (1972) can be drawn.

In conclusion, our results indicate that the endocrine regulation of the exocrine pancreas in young calves is similar to that described in other mammals. However our animals seem to be characterized by a more intense secretion of proteins and amylase in the pancreatic juice following secretin than after cholecystokinin-pancreozymin infusion.

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**Summary**

Fifteen male Holstein x Frisian calves, weighing 65 ± 3 kg and fed a milk replacer twice daily were fitted with a return cannula loop, chronically implanted at 10 days of age in the pancreatic duct, were used.
In six successive experiments we have observed the influence of an intravenous infusion of secretin, cholecystokinin-pancreozymin (CCK-PZ), vasoactive intestinal peptide (VIP), glucagon, gastrin and somatostatin on pancreatic juice flow rate, and on the excretion of proteins, calcium, inorganic phosphorus, zinc and amylase activity of pancreatic juice. In two other experiments, we have studied the interaction of secretin (or CCK-PZ) with somatostatin (SRIF). Each experiment was performed 5 h after calves feeding and no milk was given to the animals during the time of sampling.

Our results indicate that the endocrine regulation of the exocrine pancreas in young calves is similar to that described in other mammals. Secretin and CCK-PZ increased significantly excretion of water, calcium, magnesium, inorganic phosphorus, zinc, proteins and amylase activity. Somatostatin and glucagon inhibited effects observed with two precedent hormones. Gastrin increased pancreatic juice flow rate but excretion of protein and amylase activity do not varied significantly. VIP showed no significant effect on pancreatic excretory water, minerals, proteins and amylase activity.

However, our animals seem to be characterized by a more intense excretion of proteins and amylase in the pancreatic juice following secretin than after CCK-PZ infusion.

References


