

+ 3.64 $\mu\text{g}\cdot\text{kg}^{-1}$ b.w. of cimetidine were injected and in the fourth trial 36.36 $\mu\text{g}\cdot\text{kg}^{-1}$ b.w. of histamine + 0.90 $\text{mg}\cdot\text{kg}^{-1}$ b.w. of Diphergan (promethazinum hydrochloricum) were injected. All injections were administrated intramuscularly. Pancreatic juice was analysed for protein content according to the method of Bradford (Bradford, Anal. Biochem. 72 (1976) 248–254). Proteolytic activity was determined spectrophotometrically using casein as a substrate. The trypsin activity was measured using a micromodification of the original method of Erlanger et al. (Erlanger et al., Arch. Biochem. Biophys. 95 (1961) 271–278). Histamine administration evoked a significant increase in pancreatic juice volume from 17.45 ± 1.94 mL/15 min to 20.54 ± 3.31 mL/15 min, $P < 0.01$. Protein content decreased slightly from the initial value 4.84 ± 1.15 $\text{mg}\cdot\text{kg}^{-1}$ to 3.84 ± 1.19 $\text{mg}\cdot\text{kg}^{-1}$ 180 min after histamine administration. Proteolytic activity increased significantly from the basal values 58.48 ± 5.78 $\text{U}\cdot\text{mg}^{-1}$ of protein to 76.17 ± 8.43 ($P < 0.05$) $\text{U}\cdot\text{mg}^{-1}$ of protein after 90 min of histamine administration and remained at higher levels over the course of 1.5 h (70.88 ± 6.61 ; $P < 0.05$). Basal trypsin activity amounted to 5.25 ± 0.98 $\text{U}\cdot\text{mg}^{-1}$ of protein and slightly increased showing the highest value 6.68 ± 0.63 $\text{U}\cdot\text{mg}^{-1}$ of protein ($P < 0.05$) 180 min after histamine injection. These results indicate that histamine can modify the release of pancreatic enzymes in pre-ruminant calves.

Communication no. 4

Regulation of chymotrypsin and amylase expression by gastrin and its pancreatic CCK_B/gastrin receptor type.

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The predominance of CCK_B/gastrin receptors in the pancreas of high mammals (human, calf, pig) is now accepted, but their physiological function has not yet been well characterized. Indeed, most studies regarding the biological function of the pancreas have been performed in mice and rats, both exclusively expressing CCK_A receptors. We recently demonstrated, *in vivo*, the involvement of CCK_B/gastrin receptors in exogenous CCK- and gastrin-stimulated exocrine pancreatic response in the calf (Le Dréan (1997) Ph.D. thesis, Université de Rennes-I, 193 pp.). In order to elucidate the role of CCK_B/gastrin receptors in the normal pancreas, we carried out *in vitro* studies on pancreatic acini from mice expressing transgenic CCK_B/gastrin receptors in the exocrine pancreas. Using this new tool, we investigated whether gastrin, via CCK_B/gastrin receptors, could regulate the secretion, transcription and synthesis of pancreatic enzymes in normal pancreatic cells.

All experiments were performed in isolated acini prepared by collagenase digestion with increasing doses of gastrin (sulphated [¹¹Nle¹¹]gastrin 13) in the presence of SR27897 (1.8 μM), a specific antagonist of CCK_A receptors. After 30 min of incubation with gastrin, secretion of chymotrypsin from pancreatic acini showed a typical dose–response curve and maximal secretion was obtained with 1 nM of gastrin. Analysis by northern-blotting, using ³²P-labelled probes of rat pancreatic chymotrypsin and amylase, revealed an increase in mRNAs after 3 and 20 h of incubation with 300 and 10 nM of gastrin, respectively. Gastrin-stimulated protein synthesis in isolated acini was evaluated by 20 min ³⁵S-methionine incorporation into total TCA-precipitable pancreatic protein. After 1 h of incubation with gastrin, total protein synthesis was increased to a maximum with 30 pM of gastrin and inhibited with higher doses (≥ 1 nM). The dose–response curves for gastrin-stimulated protein synthesis and enzyme secretion from transgenic mouse acini were similar to those obtained with

CCK stimulation on rat acini (Korc et al. *Am. J. Physiol.* 241 (1981) G116–G121; Matozaki et al., *Am. J. Physiol.* 257 (1989) G594–G600).

In conclusion, these data clearly demonstrate that in transgenic mouse acini the activation of pancreatic CCK_B/gastrin receptors with gastrin stimulates crucial biological functions of the pancreas.

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Histological changes in the upper gut by CCK-A receptor antagonist administration in neonatal calves.

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Little is known on the role of CCK in the development of the upper gut in mammalian species. The aim of the present study was to investigate the role of CCK in exocrine pancreatic secretion and in the upper gut micro structure in neonatal calves assessed by a repetitive intraduodenal administration of a selective CCK-A receptor antagonist (FK-480, Fujisawa Pharmaceuticals, Osaka, Japan). The experiment was performed on ten neonatal calves (six control and four treated with CCK-A receptor antagonist) surgically prepared with an accessory pancreatic duct catheter as well as duodenal and duodenal bulb cannulas. Surgery was performed under halothane + rompun general anaesthesia soon after birth. The secretion of pancreatic juice was measured everyday before and after the morning and evening meals and the juice was analysed for vol-

ume and trypsin output. The perfusions with FK480 were made during the first 6 days of life before morning and evening colostrum (days 1 and 2) and milk (days 3–6) feeding. CCK-A receptor antagonist was administered for 1.5 h into the duodenal bulb cannula with a peristaltic pump (0.5 mg/kg/h). On day 7, 3-cm-long whole thickness segments of the small intestine (duodenal bulb, mid-duodenum, proximal jejunum and terminal ileum) were fixed in Bouin's solution. After hematoxylin and eosine staining, the depth of crypts, length and width of villi and thickness of tunica mucosa and muscularis mucosa were measured (mean + SEM).

The periprandial pancreatic juice secretion was significantly reduced in FK480-treated calves. This reduction in juice volume and trypsin activity mostly concerned preprandial secretion and the secretion during feeding (i.e. cephalic phase). The depth of the crypt in the duodenal bulb in FK480-treated calves was significantly greater than that in control calves (257 ± 4 versus $232 \pm 4 \mu\text{m}$, $P < 0.0001$). In contrast, the depth of crypt in the mid-duodenum, jejunum and ileum of FK480-treated calves was significantly smaller than that in control calves. The length of villi in FK480-treated calves did not differ from control calves besides in the jejunum (848 ± 12 versus $568 \pm 22 \mu\text{m}$, $P < 0.0001$). The width of villi was increased in the duodenal bulb and reduced in the other parts of the intestine. The mucosa thickness was reduced in the mid-duodenum (1005 ± 17 versus $916 \pm 14 \mu\text{m}$, $P = 0.0012$), and increased in the jejunum (883 ± 20 versus $1115 \pm 13 \mu\text{m}$, $P < 0.0001$) in FK480-treated calves. The thickness of the tunica muscularis was not significantly different in the duodenum, whereas it was increased in the jejunum (264 ± 8 versus $284 \pm 7 \mu\text{m}$, $P = 0.0012$), and reduced in the ileum (322 ± 9 versus $295 \pm 10 \mu\text{m}$, $P = 0.036$).

The present results suggest that the blockade of CCK-A receptors by FK480 during the first 6 days of life reduced the pancreatic