EFFECTS OF CHRONIC IRRITATION OF SPLANCHNIC NERVES ON PANCREATIC JUICE SECRETION IN DOGS

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SUMMARY

It has been previously shown in the dog that chronic irritation of the splanchnic nerves (CISN) induced inflammatory lesions of the biliary papilla, quite similar to the so-called Odditis or Vaterian stenosis in man.[1-3] We now report the effects of CISN on exocrine pancreatic secretion in the conscious dog. Dose--response curves to secretin and to CCK-PZ were determined in three dogs, both before and three months after inducing CISN by the sterile pumice method.[3] CISN decreased fluid, bicarbonate and protein cumulated outputs by 21-28 %, secreted in response to secretin stimulation. On the contrary, under CCK-PZ stimulation, CISN induced a small (15 %), increase in protein output, in spite of decreased fluid and bicarbonate outputs, similar to those found under secretin. Electronmicroscopy studies showed some discrete modifications of pancreatic acinar cells, which can be interpreted as the result of hyperfunction; duct cells were not altered. It is concluded that the changes in dose-response curves could be due to modification of the pancreatic sensitivity to exogenous hormones, their mechanism(s) remaining to be investigated.

RESUMO

Influência da *irritação crónica* dos nervos esplâncnicos sobre a secreção pancreático no cão

A *Irritação Crónica* dos Nervos Esplâncnicos (CISN) provoca uma reacção inflamatória da papila de Vater histologicamente, semelhante à Oddite, no homem.[1-3] Estudámos agora a influência da CISN sobre a secreção pancreática no cão. A secreção pancreática foi estudada através da determinação das curvas dose-resposta à secretina e colecistoquinina antes e três meses depois do estabelecimento das lesões conducentes à CISN. Em resposta a estimulação pela secretina, a CISN provoca uma diminuição do volume de secreção e dos débitos de bicabornato e proteínas na ordem de 21 a 28 %. Ao contrário e quando sob estimulação pancreática pela colecistoquinina a CISN provoca como resposta um aumento do débito das proteínas (15 %) apesar do volume da secreção e do débito dos bicarbonatos permacerem semelhantes às encontradas sob estimulação pela secretina. O estudo em microscopia electrónica evidencia discretas alterações das células acinosas, interpretadas como de hiperfunção, e completa normalidade das células canaliculares. Conclui-se que as alterações das curvas de dose-resposta poderão ser consequência da modificação da sensibilidade pancreática às hormonas exôgenas, impondo-se contudo, para completo conhecimento deste mecanismo fisiopatológico, posterior e mais profunda investigação.

INTRODUCTION

The nervous control of the exocrine pancreatic secretion, which was demonstrated as early as 1888 by Pavlow, has been underestimated[4] after the discovery of secretion by Bayliss and Starling in 1902,[5] and that of pancreozymin (CCK-PZ) by Harper and Raper in 1943.[6]

The influence of the splanchnic nerves on the exocrine pancreatic secretion has been studied in various animal species under various experimental conditions, with conflicting results. These physiological studies have been devoted to the effects of sectioning and/or stimulating the spanchnic nerves, but we did not find any report dealing with the experimental study of pathological situations, such as progressive compression or chronic irritation of the nerves.

In previous papers from our group it was shown, that chronic irritation of the splanchnic nerves (CISN) induced inflammatory lesions of the biliary papilla, in the dog, resulting in chronic papillitis.[1-3] Moreover it was found that a reflex arch was necessary for the experimental production of odditis. The afferent pathway, i.e. the splanchnic nerve, relayed in the semi-lunar or in the thoracic ganglia, while the vagus nerve constituted the efferent pathway. It was also found that at least two months were necessary for CISN to induce an experimental odditis.[2] Section of vagi prevented the development of Odditis.[3] When odditis was fully established, around three months post-operatively, sectioning of splanchnic or vagus nerves did not decrease the phenomemon, thus ruling out the possibility of a trophic mechanism.[3] A study of splanchnic nerve alterations induced by this method showed typical chronic inflammatory lesions, including sclerosis surrounding nervous fibers, the number of which decreased greatly.[2]

It was thus decided to investigate the effects of CISN on pancreatic juice secretion, since pancreatic innervation closely resembles that of the biliary tract. This is the first paper; next, presently under study, will be the investigation of vagus irritation (L. Gaeta and J. Cl. Sarles).

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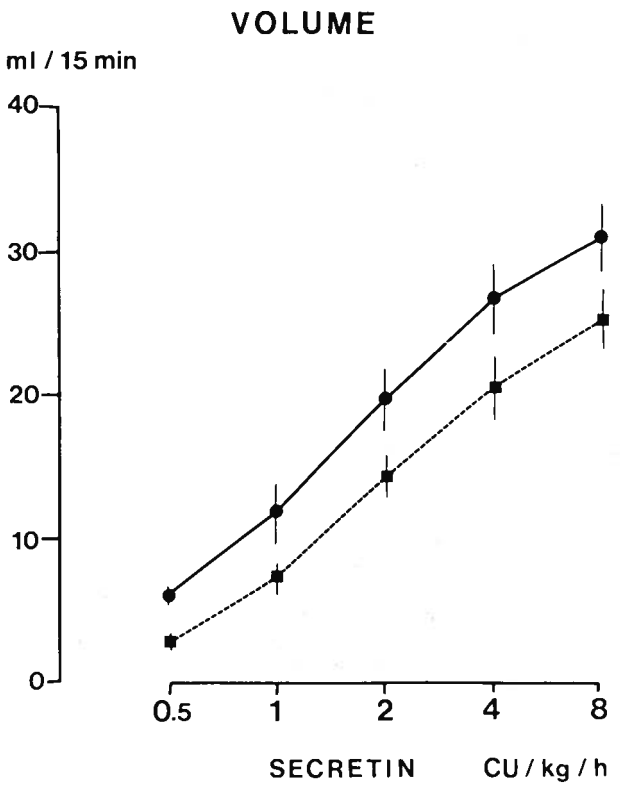


Figure 1: Volume outputs in response to graded doses of secretin: symbols are mean values before (●) and after (■) CISN; bars represent SEMs. The difference (– 27 %) was found to be significant ($p < 0.05$); the parallel line assay showed a 47 % decrease in sensitivity.

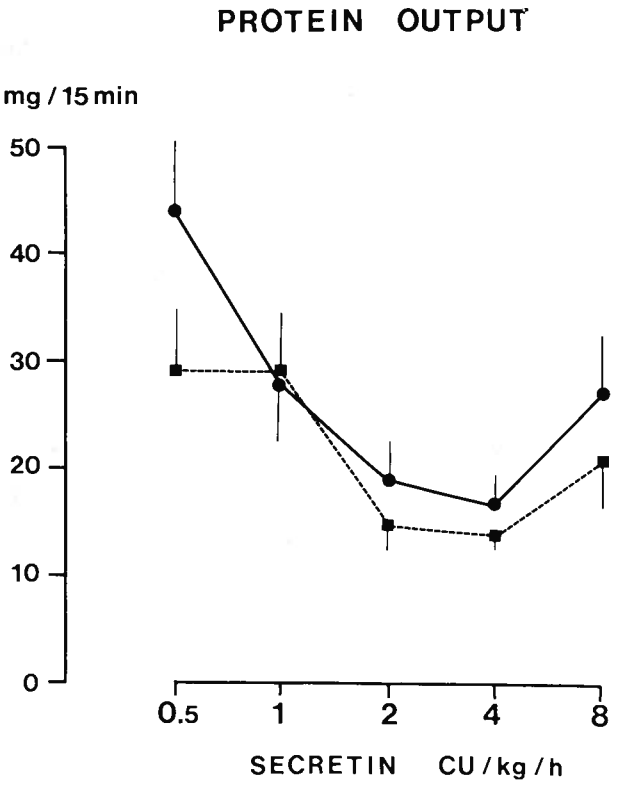


Figure 2: Protein outputs in response to graded doses of secretin, before and after CISN; symbols as in Fig. 1. The difference was not significant.

METHODS

Under general anesthesia, three mongrel dogs (17-24 kg) were equipped with duodenal and gastric cannulae,[7] the accessory pancreatic duct being ligated. The dogs were allowed to recover over a four week period. The exocrine function of the pancreas was then tested (see protocols).

Chronic irritation or splanchnic nerves

After being thus tested, each dog was submitted to chronic irritation of the splanchnic nerves (CISN) by the method previously used in the study of experimental odditis.[1]

In the same way to equip the dogs with Thomas's canulae they had a general anaesthesia: after induction with i.v. pentobarbital sodium (2 mg/kg) the animals had tracheal intubation and anesthesia was maintained by a mixture of oxigen and fluothane using a *Celog 2* machine. A large median laparotomy was performed to gain access to the splanchnic nerves. These nerves were identified at the point where they leave the diaphragm and were dissected free over about 2 cm, up to the semilunar ganglia. A small quantity of sterile pumice powder was then deposited in contact with the splanchnic nerves and the posterior parietal peritoneum was closed. The laparotomy was closed with a non-reabsorbable material and penicillin, 1 000 000 units day^{-1}, was given for a week. Three months later, the exocrine function of the pancreas was again tested similarly. After completing the second protocol, and before being sacrificed, dogs were submitted to a pancreatic biopsy (see microscopy).

Protocols

Dose-response curves to secretin and to CCK-PZ were determined three times in each dog, both before and after CISN. Tests were performed every other day.

Before each test, dogs were denied food, but not water for 18 hours. After cleaning the duodenal cannula, a glass cannula was inserted into the papilla, and the pancreatic juice was continously collected in ice-chilled graduated tubes, the gastric cannula being kept open to prevent the release of endogenous hormones.

Pancreatic stimualtion was performed by infusing graded doses of hormones dissolved in normal saline, through a leg vein. Peristaltic pumps were used to maintain the 100 $ml.h^{-1}$ infusions.

Each dose was given for three 15-min periods, the response being the mean value of the two last periods at that dose. The dose was then doubled. Five doses were administered per test: 0.5-8 CU. kg^{-1} .h^{-1} secretin (S) and 1.5-24 CHR $U.kg^{-1}$ .h^{-1} CCK-PZ superimposed on a constant 1 CU. kg^{-1} .h^{-1} S background. Both hormones were purchased from G.I.H. laboratory (Karolinska Institute).

Chemical determinations

Volumes were read to the nearest 0.1 ml. Bicarbonate (HCO_3) was determined by the method of Van Slyke. Protein concentration was determined after appropriate dilution, by absorbance at 280 nm, using the extinction coefficient $E^{1\%}_{1\,cm} = 20$ (Holochrom spectrophotometer, Gilson, Middleton, Wisc.).

For each period, et each dose, outputs were then calculated for each parameter, and expressed per 15-min periods.

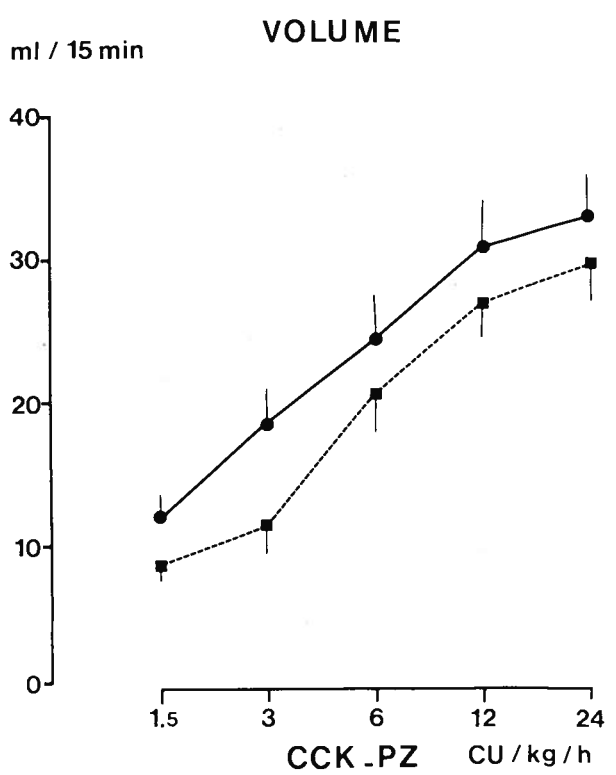


Figure 3: Volume outputs in response to graded doses of CCK-PZ superimposed on a secretin background (1 $U.kg^{-1}.h^{-1}$), before and after CISN; symbols as in Fig. 1. The difference was not significant; the parallel line assay showed a 42 % decrease in sensitivity.

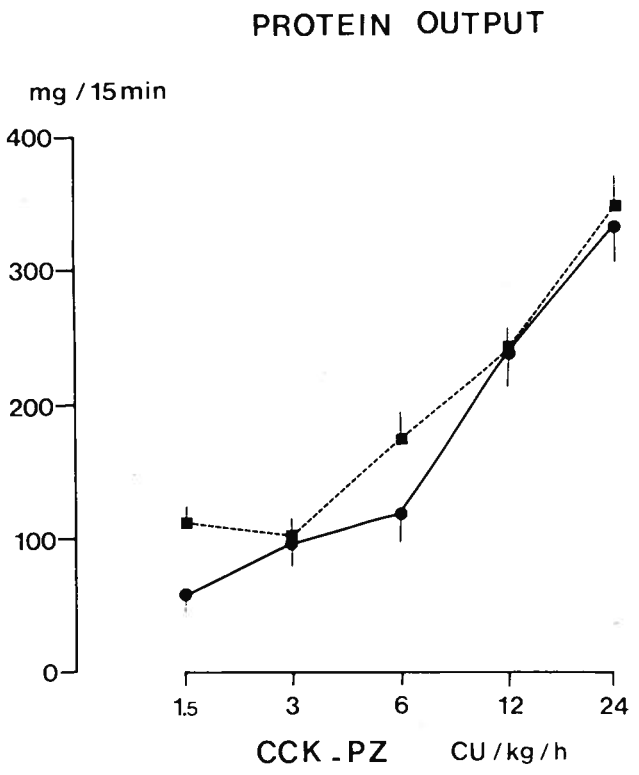


Figure 4: Protein outputs in response to graded doses of CCK-PZ, superimposed on a secretin background (1 $U.kg^{-1}.h^{-1}$), before an after CISN; symbols as in Fig. 1, but note an 8-fold change of scale as compared to Fig. 2. The only CISN-induced difference was in response to the lowest dose.

Data Analysis

After calculation of means and standard errors of the means (SEM's), several statistical procedures were used for analyzing the data. All calculations were performed on a P652 Olivetti microcomputer.

To get an overall view of CISN effects on pancreatic responses to S and to CCK-PZ, the classical Hofstee transformation,[8] i.e. response vs response/dose, or R vs R/D, was used to calculate the maximal responses (calculated maximal responses, or CMRs) and the doses eliciting half the CMRs, i.e. D_{50}'s, for each hormone. By comparing the regressions before and after CISN, it was then possible to assess the effects of CISN on the dose-response curve to each hormone.

Another statistical procedure, the 3 + 3 parallel line assay[8] was also used to compare the responses to a same hormone, before and after CISN; this procedure was found to be useful to demonstrate a change in pancreas sensitivity to exogenous hormones. Finally, to compare the effects of CISN at any particular dose the Scheffé's method for analysis of contrasts was used.[8]

Microscopy

After completing the second protocol, and before being sacrificed, dogs were submitted to pancreatic biopsy. Tissue samples were fixed by a 2.5 % glutaraldehyde, 2 % paraformaldehyde, in 0.1 M cacodylate buffer (pH 7.2) at 4 °C for 2 hours. Osmium tetroxide 1 % in 0.1 M phosphate buffer (pH 7.2) was used for post-fixation. Tissue samples were then dehydrated through successive ethanol baths, then embedded in Epon 812. Semithin sections (2 μm) were stained with toluidine blue, and ultrathin sections were stained with uranyl acetate, then with lead citrate.

RESULTS

As expected, dose-response curves were found in all dogs, for both hormones. Moreover, the effects of CISN obeyed to the same patterns in all dogs.

CISN effects on pancreatic response to secretin

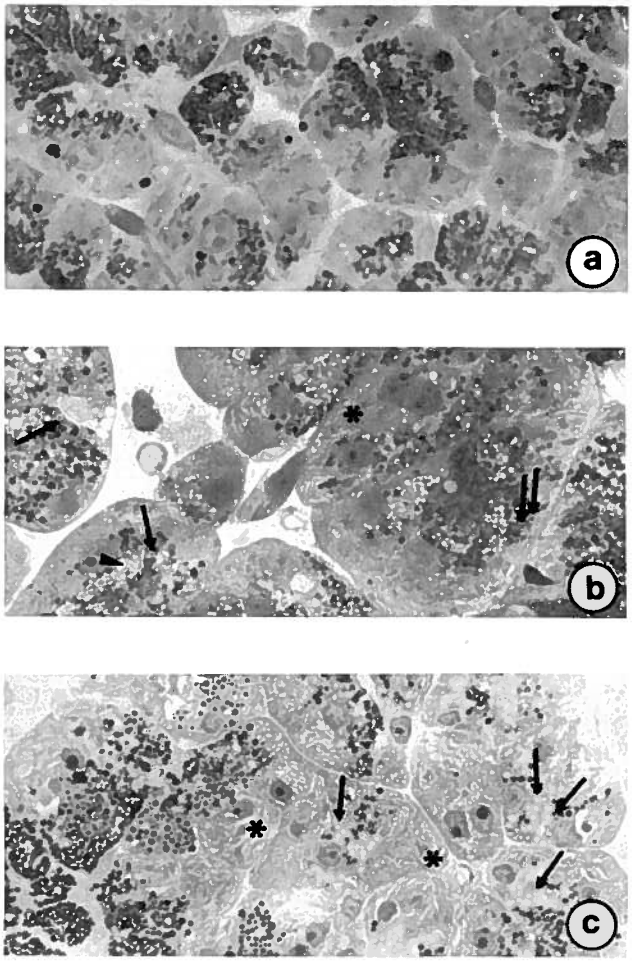
The comulated *fluid output*, secreted in response to the five S doses, decreased by 27 % (Figure 1). This decrease was found to be significant by the Scheffé's method ($p < 0.05$). The CMR decreased by 12 % from 38.4 ± 1.4 to 33.9 ± 1.0 ml/period, while the D_{50} exhibited a 45 % increase from 1.9 ± 0.2 to 2.7 ± 0.2 $U.kg^{-1}.h^{-1}$. The parallel line assay showed a 47 % decrease in pancreas sensitivity, a figure which agrees with the D_{50} increase.

HCO3 concentration was not altered, while the effects of CISN on *HCO3 output* (not shown) followed a pattern similar to those on fluid secretion. The 28 % decrease was found to be significant by the Scheffé's method ($p < 0.05$). the CMR decreased by 9 % from 5.1 ± 0.3 to 4.6 ± 0.4 nmol/period, while the D_{50} exhibited a 77 % increase from 1.4 ± 0.3 to 2.4 ± 0.6 $U.kg^{-1}.h^{-1}$. The parallel line assay showed a 50 % decrease, a figure close to that found for the fluid output.

The mean *protein concentration* did not exhibit any alteration, but the cumulated *protein output* decreased by

21 %, a decrease similar to those of both fluid and HCO_3 outputs (Figure 2). As expected, no dose-response to S was found.

In summary, CISN effects on the pancreatic response to secretin were characterized by small (21-28 %), but significant, decreases in fluid, HCO_3 and protein cumulated outputs. The concentrations, and the HCO_3-to-protein ratio, remained unchanged.



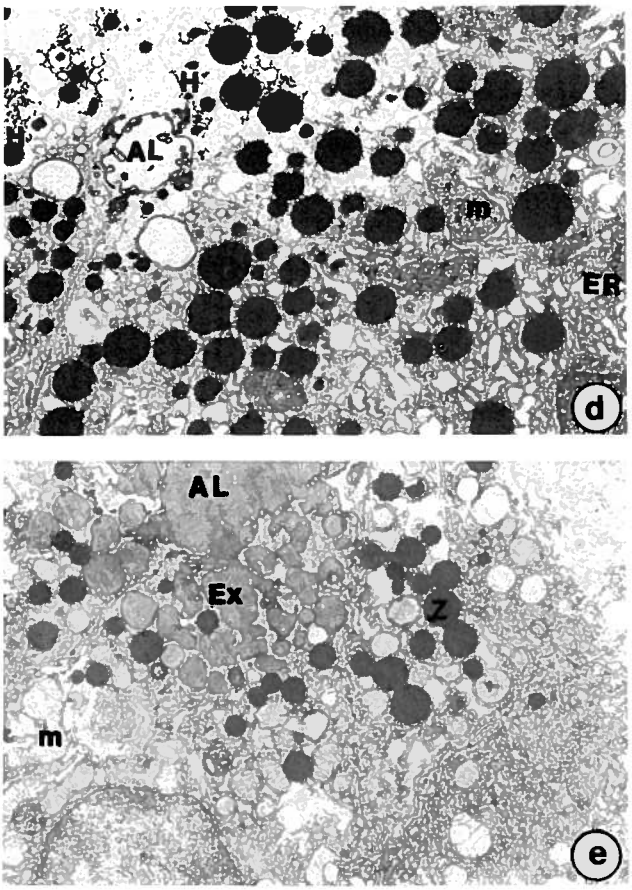


Figure 5: Morphological modifications of acinar pancreatic cells: control dog (5a) and CISN dogs (5b-5e). **Semithin Sections**: Compared to control dog (5a), CISN dogs (5b—5) exhibited dilatation of endoplasmic reticulum (*), halolike secretory granules (arrows) and more numerous clear Golgian vacuoles (arrowheads); magnification X 1000. **Ultrathin Sections**: (Fig. 5d) the halo-like secretory granules (H) appeared to be immature secretory granules; endoplasmic reticulum (ER) cisternae were well dilated; mitochondria (m) were entirely normal; Magnificantion X 1000; (Fig. 5e) Exocytosis (Ex) was a frequently observed feature; Z = zymogen granule; AL = acinar lumen; Magnification X 10000.

CISN effects on pancreatic response to CCK-PZ

The cumulated *fluid output* decreased by 18 % (Figure 3). This decrease was not found to be significant by the Scheffé's method. Similarly, the CMR decreased by 5 %, from 38.1 ± 0.9 to 36.2 ± 4.0 ml/period, a non-significant decrease. On the contrary, the D_{50} exhibited a 57 % increase, from 3.3 ± 0.2 to 5.2 ± 1.1 $U.kg^{-1}.h^{-1}$. Moreover, the parallel line assay showed a 42 % decrease ($p < 0.01$) in pancreas sensitivity, a figure which agrees with the D_{50} increase.

HCO3 concentration was not altered, while the effects of CISN on *HCO3 output* (not shown) exhibited a pattern similar on fluid secretion. Again, the 19 % decrease was not found to be significant by the Scheffé's method. Similarly, the CMR decreased by 7 %, from 5.7 ± 0.4 to 5.3 ± 0.8 nmol/period, a non-significant decrease. On the contrary, the D_{50} exhibited a 69 % increase from 3.5 ± 0.5 to 5.9 ± 1.6 $U.kg^{-1}.h^{-1}$. The parallel line assay showed a 45 % decrease ($p < 0.01$), a figure close to that found for the fluid output.

The mean *protein concentration* increased by 41 %, while the cumulated *protein output* increased by 15 % (Figure 4). As expected, a dose-response curve to CCK-PZ was observed. The overall increase was not found to be significant by the Scheffé's method. Similarly, the CMR did not increase significantly from 240 ± 12 to 253 ± 23 mg/period (+5 %), the D_{50} remaining unchanged at 12.0 ± 1.8 vs. 11.5 ± 0.9 $U.kg^{-1}.h^{-1}$. The only CISN-induced difference was in the response to the lowest dose (t test, $p < 0.05$).

In other words, CISN effects on the pancreatic response to CCK-PZ were characterized by small (18-19 %) decreases in fluid and HCO_3 outputs, which did not prevent a simultaneous increase (15 %) in protein output. The HCO_3-to-protein ratio decreased by 43 %.

CISN effects on pancreatic cell structure in the resting state

By comparison with control pancreas (Figure 5a), three features were found regularly in pancreatic acinar cells after CISN. First, endoplasmic reticulum cisternae were dilated (Figure 5b and 5c). Second, numerous zymogen granules exhibited a halo-like structure (Figure 5b and 5c). Third, clear vacuoles were abundant in the Golgian area (Figure 5b and 5c) and in the apical zone (Figure 5c).

These observations, already apparent at the optical level, were confirmed at the ultrastructural level (Figure 5d and 5e). It was noted that the halo-like granules previously observed were in fact immature zymogen granules (Figure 5d). Moreover it appears that exocytosis pictures were frequent after CISN (Figure 5e).

Intrapancreatic nerves exhibited a quite normal structure (not shown). No alterations were observed in duct cells, as it is usually the case for these cells which are deprived with specific organelles (not shown).

DISCUSSION

These effects of CISN on exocrine pancreatic secretion are characterized by the different behavior of both fluid and protein secretions in response to the hormonal stimulations. Both types of secretion were decreased in response to secretin, while under CCK-PZ stimulation the fluid secretion only was decreased, the protein secretion remaining unchanged. It can be postulated that CISN induced a moderate degree of duct cell inhibition.

These effects of CISN on exocrine pancreatic secretion seem to be mediated by (a) nervous mechanism (s) since other mechanisms can be excluded: i) no trophic mechanism was demonstrated in the experimental study of odditis;[1-3] ii) a vascular mechanism would be followed by a similar effect on both kinds of secretion.[9-12]

This led us to the conclusion that (a) nervous mechanism (s) is (are) responsible for CISN effects on exocrine pancreatic secretion. In the following discussion it will be emphasized that these effects do not match completely the literature reports on sectioning or stimulating the splanchnic nerves, nor those on pharmacological studies of alpha-and beta-adrenergic agonists and antagonists. But part of the discrepancies may be linked to the differences in experimental conditions, such as animal species, anaesthesia, basal secretion, while our experiments were performed on conscious dogs submitted to hormonal stimulations.

The effects of sectioning the splanchnic nerves on pancreatic secretion were investigated in three animal species: rabbit,[13] cat,[14] and dog.[15] In these experiments, the section induced an increase in basal,[13, 14] in secretin-stimulated [14] or in meal-stimulated [15] pancreatic secretion. Note that the increase in pancreatic secretion due to the section of splanchnic nerves was partly inhibited by vagotomy.[10] Moreover, the effects of alpha and of beta-adrenergic blockers on pancreatic secretion have been studied in the conscious dog. Both phenoxybenzamine and propranolol increased fluid and protein outputs,[16, 17] and these findings agree well with the effects of splanchnic nerve section.

On the other hand, the effects of electrical stimulation of the peripheral end of splanchnic nerves have been studied in several animal species. In the rabbit, this stimulation induced an inhibition of pancreatic secretion, later on followed by an increase.[13] At least, three studies were performed on cats.[10, 12, 14] It was first found that basal and secretin-stimulated secretions were decreased with respect to both fluid and protein outputs.[12] This inhibition of pancreatic secretion was linked to a decrease in pancreatic blood flow.[14] Finally, a detailed study showed that the biphasic effect of splanchnic nerve stimulation on pancreatic secretion was also linked to a biphasic effect on pancreatic blood flow.[10] In the pig, the stimulation of splanchnic nerves had no effect on basal secretion, but decreased the response to vagal stimulation.[18] Moreover, the effects of alpha- and beta-adrenergic agonists have been tested in vitro on the rabbit pancreas [19] and in vivo on the conscious dog.[20] In both studies, adrenergic agonists induced a decrease in pancreatic secretion, results which agree with the effects of in vivo stimulation.

Thus the exact mechanism by which CISN induced alterations of the pancreatic secretion is not clear. Indeed, they do not resemble the effects of splanchnic nerve section or adrenergic blockade nor do they completely match those of splanchnic nerve stimulation. Further studies will be necessary to elucidate this point.

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