Pancreatic Response of Anaesthetized and Conscious Rats to Bolus Injection of Cholecystokinin-pancreozymin

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Abstract

Pancreatic secretion was studied in anaesthetized rats tested immediately after surgery or in conscious rats tested 48 h after the cannulation of the pancreatic duct. Pancreatic flow, protein output and enzyme output were measured over a 30-min period in the unstimulated state and after the intravenous injection of bolus doses of cholecystokinin-pancreozymin (CCK-PZ) ranging from 1·25 to 20 Crick–Harper–Raper units (CHRU). Each animal received three doses of CCK-PZ, as either ascending or descending doses.

In anaesthetized rats there was a linear relationship between the log-dose of CCK-PZ and the flow, protein and enzyme output with both the ascending and descending doses. In contrast, in conscious rats flow was unaffected by CCK-PZ, and protein output was greatest after the first dose, whether this was given in the ascending or descending doses. At all CCK-PZ levels flow in anaesthetized rats was less than that seen in conscious animals, but at doses of CCK-PZ above 5·00 CHRU protein output was greater in anaesthetized rats than in conscious rats.

Ultrastructural studies of the pancreas showed areas of focal cytoplasmic degeneration and possible blockage of the duct with cellular debris after administration of high doses of CCK-PZ to conscious rats. These changes may be responsible for the reduced protein output with the second and third dose of CCK-PZ in these animals. No such changes were seen in anaesthetized rats after similar doses of CCK-PZ.

These studies show fundamental differences in the response of the pancreas to CCK-PZ in anaesthetized and conscious rats. The mechanism for this difference is not clear, but it may represent a change in the normal response to CCK-PZ in the anaesthetized rats as a result of the effects of acute operative trauma, possibly acting through changes in pancreatic blood flow.

Additional keyword: autophagosome

Introduction

Pancreatic secretion in the rat is affected by the time elapsed since the preparative surgery. In a recent detailed study, Petersen and Grossman (1977) showed a tenfold increase in basal flow in conscious, recovered rats compared with anaesthetized animals tested 3 h after surgery, and a markedly different response to secretin and cholecystokinin-pancreozymin (CCK-PZ) between rats in the two conditions.

Petersen and Grossman stimulated pancreatic secretion by continuous infusion of gastrointestinal hormones, and tested anaesthetized animals at a body temperature of 31–33°C. It has been shown by DeWaele et al. (1974) that pancreatic secretion is depressed at temperatures below normal body temperature (38°C). In the present study bolus injections of CCK-PZ were used to determine whether the response of anaesthetized rats maintained at 37°C more closely resembled that of conscious, recovered rats. The results in general confirm the findings of Petersen and Grossman.
(1977), and suggest that the fundamental difference between the response of the recovered and anaesthetized rats is that in the conscious animal pancreatic secretion is inhibited by high doses of CCK-PZ, whereas in the anaesthetized animal it is stimulated.

Materials and Methods

Male Wistar rats locally inbred for 15 years, weighing 250-350 g, were fed rat cubes supplied by Milne Feed Company, Welshpool, W.A..

Anaesthetized Rats

In these studies food was withheld overnight but the animals were permitted a 0·4% (w/v) sodium chloride solution orally. Urethane (25% w/v) was given intraperitoneally at a dose of 1·25 g/kg. Three-quarters of this dose was given initially and the remainder injected 30 min later. Body temperature was monitored with a rectal thermometer and maintained at 37°C by means of an electric heating pad.

Surgery consisted of cannulating the external jugular vein for CCK-PZ injections using S.P. 31 polyethylene tubing (i.d. 0·5 mm, o.d. 0·8 mm; Dural Plastics, Dural, N.S.W.). A tracheostomy was performed using S.P. 120 tubing (i.d. 1·27 mm, o.d. 2·08 mm). The abdomen was opened and the bile duct doubly ligated at the hilum of the liver above the pancreatic tissue. The pylorus was also ligated. The distal bile-pancreatic duct was cannulated with S.P. 10 tubing (i.d. 0·28 mm, o.d. 0·61 mm). The wound was covered with absorbant gauze soaked in warm saline.

Sampling of the pancreatic juice commenced 3 h after the first urethane injection (Love 1957) and when residual bile had cleared from the pancreatic cannula. The experimental protocol to determine the effect of the bolus injections of CCK-PZ in anaesthetized rats was the same as that for conscious animals (see below).

Conscious Rats

In these studies rats were allowed food and water until surgery. The animals were anaesthetized with a 5-7% (w/v) solution of chloral hydrate intraperitoneally, 0·5 ml per 100 g body weight. An external jugular cannula was inserted and led through a subcutaneous channel to the back of the neck. The wound was sutured with 3·0 silk. Through a midline incision, cannulae (S.P. 31) were placed in the gastric fundus and in the duodenum through an incision on the antimesenteric border of the duodenum. The bile duct was cannulated immediately below the liver and the pancreatic duct at its distal end, as the duct passed through the duodenal wall. The duodenal, bile and pancreatic cannulae consisted of silicone tubing (0·5 mm i.d.) tipped with S.P. 10 polyethylene. The gastric and duodenal cannulae were led outside via a stab wound in the left flank while the bile and duodenal cannulae were passed through a stab wound in the right flank. The silicone tubing was looped in the peritoneal cavity to allow for movement when the animal regained consciousness, and the abdomen closed with a double layer of sutures.

Following surgery the rats were restrained in Bollman restraint cages (Bollman 1948).

Postoperative Care

The jugular cannula was kept open by the i.v. infusion of saline at 1 ml/h using a peristaltic pump. Oral fluid in the form of a glucose-electrolyte solution (2·5% w/v Staminade, Nicholas Pty Ltd, Vic.) was made available. After surgery the pancreatic and duodenal cannulae were connected to permit recirculation of pancreatic juice to the duodenum at all times except during sampling. Injections of 0·3-0·5 ml warm saline were made via the duodenal cannula at approximately 4-hourly intervals during the day after surgery to prevent any obstruction of the cannula by intestinal contents. Blockage of the duodenal cannula occurred occasionally during the first day after operation, but was not a problem when the duodenum had cleared of food debris. Pancreatic flow was steady from the time of breaking the connection to the duodenum in all conscious rats at 48 h, indicating no obstruction to the return of juice in these animals. Bile was not recirculated. Twenty-four h after surgery an intragastric infusion of 1% (w/v) bovine serum albumin and 2·5% (w/v) glucose in 0·9% saline was started at 3 ml/h. On the day of the experiment the pancreatic-
duodenal connection was broken. The intragastric and i.v. infusions were stopped 30 min before juice collection.

**Dose Schedule**

CCK-PZ was obtained from the Boots Co. Ltd (Nottingham, England). The effect of the i.v. injections of 1·25, 2·50, 5·00, 10·00 and 20·00 Crick–Harper–Raper units (CHRU) of CCK-PZ on pancreatic function were studied. Each rat received only three doses of CCK-PZ. These doses were given in either an ascending or a descending dose schedule. A low dose range of 1·25, 2·50 and 5·00 CHRU was used in four rats as the ascending dose schedule with a high dose range of 5·00, 10·00 and 20·00 CHRU in a further four animals. The order of these doses was reversed in two further groups of four animals in the descending schedule. Thus in all 32 rats were used. The CCK-PZ preparation was dissolved in saline, so that the lowest dose was contained in 25 μl, 5 min prior to injection. CCK-PZ was injected over a standard 10-s period using a microsyringe (Gastight No. 1725, Hamilton Co. Inc., Whittier, Calif.) and flushed into the circulation with 0·3 ml of saline.

**Sampling Procedure for Anaesthetized and Conscious Studies**

After the appropriate reservoir (preweighed sampling tubes for conscious studies or calibrated silicone capillary tubes for anaesthetized studies) had been attached to the pancreatic cannula, 12, timed, 10-min collections were made. The first three samples were taken before stimulation and represented the basal output (Love 1957). At 29, 59 and 89 min a dose of CCK-PZ was injected via the jugular cannula and the sampling reservoir changed 1 min later.

At the end of the experiment conscious animals were killed with an overdose of anaesthetic and the abdomen opened. The pancreas was examined for signs of duct blockage or pancreatitis (oedema, fat necrosis, inflammation) and the gastric and duodenal cannulae checked for leakage. None of the animals which survived to the end of the 48-h studies showed overt signs of pancreatic damage, but a number of animals had to be killed at earlier times because of peritonitis from leakage around the gastrointestinal tubes or technical faults with the jugular or pancreatic cannulae.

**Determinations**

Pancreatic flow was measured by either the change in weight of the sampling tubes, assuming a density of 1·0 for the juice (conscious rats), or by the measurement of the length of the silicone tubing filled (anaesthetized rats). Samples were then made up to 3 ml with saline and stored on ice. Protein estimation was performed by the method of Schacterle and Pollack (1973). α-Amylase activity (EC 3.2.1.1) was determined by the method of Dahlenqvist (1962). Trypsin (EC 3.4.21.4) content was determined by the procedure of Preiser et al. (1975). Triacylglycerol lipase (EC 3.1.1.3) activity was estimated by the method of Verduin et al. (1973).

**Histology**

Pancreatic morphology was studied in four cannulated anaesthetized and four cannulated conscious rats after the combined dose of 5·00, 10·00 and 20·00 CHRU of CCK-PZ. These animals were surgically prepared, maintained and tested in the same way as for anaesthetized and conscious animals used in secretion studies. A further four conscious animals received saline in place of CCK-PZ and provided unstimulated control tissue.

Conscious rats were anaesthetized by the intravenous injection of 0·3 ml of 50% (w/v) urethane in saline at the 95th minute of sampling, 6 min after the injection of the 20·00-CHRU dose of CCK-PZ. This anaesthetic dose was sufficient to immobilize the animal within 20 s. The rat was then removed from the restraint cage and an additional 0·2 ml of urethane given before the abdomen was opened. With the heart still contracting 100–200 mg of the splenic region of the pancreas was removed and quickly immersed in a solution of 2·5% (w/v) gluteraldehyde (TAAB Laboratories, Reading, England) in 0·1 M sodium cacodylate buffer (B.D.H. Chemicals, Poole, England), pH 7·4. The animal was then killed by intravenous injection of air. The excised tissue was cut into 1-mm cubes while immersed in fixative and fixed in fresh fixative at room temperature. The specimens were processed on an automatic processor (Sukara REM 20B, Sukara Fine Technical Co., Tokyo) for Araldite embedding. Thin sections were viewed on a Phillips 300 electron microscope.
Tissue from anaesthetized rats was removed, fixed and processed in the same manner as tissue from conscious rats.

Statistics
Data was analysed by Student’s t-test.

Results
Standard Basal Output
In both anaesthetized and conscious rats three collections were made before any CCK-PZ was given, as an estimate of basal output. Since in each group treatment was the same up to the first injection, the basal outputs of rats assigned to ascending and descending schedules were combined to obtain a ‘standard basal output’ for the two test conditions.

Standard basal flow and protein output were 10 and 14 times higher respectively in conscious rats compared with anaesthetized rats. Standard basal output of the enzymes tested showed similar differences, but the concentration of protein and of individual enzymes were not significantly different under the two conditions (Table 1).

Table 1. Standard basal secretions

<table>
<thead>
<tr>
<th>Secretion</th>
<th>Acutely anaesthetized</th>
<th>Chronic conscious</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow (µl per 30 min)</td>
<td>23 ± 2</td>
<td>225 ± 7</td>
</tr>
<tr>
<td>Protein (mg per 30 min)</td>
<td>0·5 ± 0·21</td>
<td>6·9 ± 1·0</td>
</tr>
<tr>
<td>(g/l)</td>
<td>20 ± 4·7</td>
<td>31·0 ± 8·1</td>
</tr>
<tr>
<td>Amylase (units per 30 min)</td>
<td>54 ± 12</td>
<td>524 ± 95</td>
</tr>
<tr>
<td>(units/ml)</td>
<td>2·3 ± 0·3</td>
<td>2·3 ± 0·4</td>
</tr>
<tr>
<td>Lipase (units per 30 min)</td>
<td>1·4 ± 0·3</td>
<td>21·8 ± 3·0</td>
</tr>
<tr>
<td>(units/ml)</td>
<td>0·06 ± 0·01</td>
<td>0·09 ± 0·01</td>
</tr>
<tr>
<td>Trypsin (milliunits per 30 min)</td>
<td>81 ± 14</td>
<td>628 ± 91</td>
</tr>
<tr>
<td>(units/ml)</td>
<td>3·4 ± 0·4</td>
<td>2·7 ± 0·3</td>
</tr>
</tbody>
</table>

Stimulated Pancreatic Secretion
In the anaesthetized rats there was no significant difference between the response to the 5·00-CHRU dose of CCK-PZ whether this was given in the lower or upper dose ranges for either the ascending or descending schedules. It was therefore possible to calculate a mean 5·00-CHRU response for all parameters and to combine the responses of the lower and upper dose ranges in the analysis which follows.

In the conscious rats the responses to 5·00 CHRU of CCK-PZ were the same in the lower and upper dose ranges only for the ascending doses. With the descending schedule in these animals the response to 5·00 CHRU when this was given as the last dose of the upper dose range (20·00, 10·00 and 5·00 CHRU) was significantly lower than when it was given as the first dose of the lower dose range (5·00, 2·50 and 1·25 CHRU). In the analysis of the results in the conscious rats, therefore,
the responses to the upper and lower dose ranges were combined for the ascending doses but treated separately for the descending doses.

Fig. 1. Pancreatic juice flow in the unstimulated rat (B) and in anaesthetized (○-○) and conscious (■-■) rats in response to increasing (a) or decreasing (b) doses of CCK-PZ. Values are means ± s.e. of eight (5·00 CHRU) or four (other doses) rats.

Flow

In anaesthetized rats the flow increased with increasing doses of CCK-PZ over the range studied. This response was independent of the order of dosage. The relationship between the dose and response was approximately log-linear for both ascending and descending doses though the slope of the line was higher with the descending doses (Fig. 1). The calculated log-linear regressions and parameters of these lines were as follows:

<table>
<thead>
<tr>
<th>Dose schedule</th>
<th>Ascending</th>
<th>Descending</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow</td>
<td>[ y = 74 \cdot 5x + 30 \cdot 1 ]</td>
<td>[ y = 102 \cdot 2x + 22 \cdot 0 ]</td>
</tr>
<tr>
<td></td>
<td>[ r = 0 \cdot 986 ]</td>
<td>[ r = 0 \cdot 979 ]</td>
</tr>
<tr>
<td>Protein output</td>
<td>[ y = 9 \cdot 9x + 0 \cdot 4 ]</td>
<td>[ y = 12 \cdot 2x + 1 \cdot 1 ]</td>
</tr>
<tr>
<td></td>
<td>[ r = 0 \cdot 922 ]</td>
<td>[ r = 0 \cdot 976 ]</td>
</tr>
<tr>
<td>Protein concentration</td>
<td>[ y = 56 \cdot 1x + 32 \cdot 2 ]</td>
<td>[ y = 57 \cdot 1x + 27 \cdot 8 ]</td>
</tr>
<tr>
<td></td>
<td>[ r = 0 \cdot 973 ]</td>
<td>[ r = 0 \cdot 992 ]</td>
</tr>
</tbody>
</table>

By contrast, in conscious rats flow did not change significantly when CCK-PZ was given in the ascending doses. Nevertheless, flow at all times was significantly greater than the highest flow seen in the anaesthetized animals. In conscious rats tested with the descending doses flow was constant within the upper and lower dose schedules, but these responses were dissociated (Fig. 1). No regression lines were calculated for the responses in conscious rats.

Protein output and protein concentration

As was the case with flow, in anaesthetized rats a linear relationship between the log of the dose of CCK-PZ and the protein output was found over the range 1·25–20·00 CHRU of CCK-PZ. This response was independent of the order of dosage. However, over the dose range used (1·25–20·00 CHRU) protein output increased sevenfold while flow increased only threefold. Protein concentration therefore also increased with increasing doses of CCK-PZ (Fig. 2).

In conscious rats tested with the ascending doses the highest protein output was seen with 1·25 CHRU of CCK-PZ, but at no dosage level was output significantly greater than basal. At 5·00 units of CCK-PZ protein output in these animals was
the same as that seen in anaesthetized rats tested with 5·00 CHRU of CCK-PZ for the ascending doses, with the response in conscious animals significantly greater than that in anaesthetized animals at doses below 5·00 CHRU and significantly less than that in anaesthetized animals at doses higher than 5·00 CHRU.

![Fig. 2. Protein concentration in pancreatic juice from unstimulated rats (B) and from anaesthetized (○--) and conscious (•••) rats stimulated with increasing (a) or decreasing (b) doses of CCK-PZ. Values given are means ± s.e.](image)

Since flow and protein output were constant in conscious rats tested with the ascending schedule, protein concentration was also constant (Fig. 2). At all doses of CCK-PZ greater than 1·25 CHRU protein concentration in these rats was significantly lower than that seen in anaesthetized rats tested with ascending doses.

In conscious rats tested with the descending schedule the responses to the upper and lower dose ranges were dissociated (Fig. 2). Mean protein output was highest after the first doses given, but a high standard deviation was seen, and the response was not significantly different from basal at any of the doses used. When protein output in these rats was compared with that in anaesthetized rats tested with the descending doses, a relationship similar to that seen with the ascending doses was found, protein output being higher in anaesthetized rats tested with 20·00 and 10·00 CHRU of CCK-PZ, the same at 5·00 CHRU and lower in anaesthetized rats tested with 2·50 and 1·25 CHRU.

In conscious rats tested with the descending doses, protein concentration was highest after the first dose of CCK-PZ (20·00 or 5·00 CHRU) and fell with later doses. At all dose levels in the upper dose range protein concentration in the conscious rats was significantly lower than the corresponding values for anaesthetized rats tested with the descending schedule. However with the lower dose range the protein concentration in the conscious rats was the same as that found in anaesthetized animals at all three doses of CCK-PZ (Fig. 2).

**Enzyme output and concentration**

Trypsin, lipase and amylase outputs in the anaesthetized rats showed a linear relationship between the log of the dose and the response, which was independent of the dosage order. The enzymes dose–response relationship was thus similar to that of the protein output. In conscious rats, the enzyme response to the ascending doses also mirrored the protein output, with enzyme outputs maximal at 1·25 CHRU and falling at higher doses. The upper and lower dose ranges of the descending doses in conscious rats were again dissociated, with the lower dose range producing greater enzyme output than the upper dose range.
Histology

Fig. 3 shows the electron microscopic appearance of the pancreas from an unstimulated conscious rat. The apical cytoplasm of the acinar cells are filled with mature zymogen granules, while the lumen of the gland is circular and largely free of electron-dense material. When anaesthetized rats were stimulated with the upper range of the ascending schedule and killed during the response to 20·00 CHRU of
CCK-PZ this appearance changed to that shown in Fig. 4. In these animals the apical cytoplasm contained a large number of condensing vacuoles and the mitochondria and rough endoplasmic reticulum appeared swollen, probably as a result of increased cellular activity. The acinar lumen was elongated and filled with an electron-dense staining material, but was free of debris.

In conscious rats the electron microscopic appearance of the unstimulated pancreas was similar to that seen in anaesthetized animals and Fig. 3 is representative of both unstimulated conditions. Stimulation in the conscious rats, however, resulted in extensive cellular damage. Acinar cells from conscious rats after administration of 5·00, 10·00 and 20·00 CHRU of CCK-PZ showed frequent areas of focal cytoplasmic degeneration characterized by the formation of autophagic vacuoles. These autophagic vacuoles contained myelin bodies (Fig. 5) which appeared to be derived from mitochondrial and endoplasmic reticulum. Similar profiles were found in the acinar lumen of conscious rats stimulated with CCK-PZ (Fig. 6).

Discussion

This study shows marked differences in the pancreatic secretions of anaesthetized and conscious rats, both basally and after bolus injections of CCK-PZ. The basal values are similar to those reported by others, as tabulated by Petersen and Grossman (1977), with a tenfold higher flow and twentyfold higher protein output in conscious rats. The mechanism behind the difference in basal secretion is not known but may reflect an effect of anaesthesia or of the surgical trauma associated with cannulation. The latter seems the most likely, possibly through alterations in blood flow.

Surgical trauma affects gastrointestinal function (Woolley and Simmonds 1959; Morgan 1966; DeWaele et al. 1974) and blood flow (Hiley et al. 1978; Granger et al. 1980). A reduction in gastrointestinal blood flow could lead to a fall in pancreatic secretion by an effect on the organ itself or by reducing either the release of stimulatory hormones from the intestinal mucosa (Petersen and Grossman 1977) or the delivery of these hormones to the pancreas. In addition increased sympathetic activity and decreased parasympathetic activity occur in trauma and could lead directly to reduced secretion and indirectly to reduced responsiveness of the pancreas to circulating hormones.

An effect of the anaesthetic agent itself on the pancreas may also occur. However, DeWaele et al. (1974) showed that if rats were allowed to recover from surgery for 48 h, re-anaesthetization with urethane had little effect on pancreatic secretion; these findings have been confirmed in this study. In addition, in a study to be reported elsewhere, pancreatic secretions were still grossly reduced 12 h after surgery under chloral anaesthesia, and were not significantly different from the secretions in anaesthetized rats, despite the fact that the animals appeared fully conscious and alert. Furthermore, Hiley et al. (1978) noted that the hepatosplanchnic blood flow during urethane anaesthesia was not significantly different from that in conscious rats, suggesting that urethane alone does not affect the haemodynamic environment of the pancreas.

Profund differences in the response to CCK-PZ injection were seen in the conscious and anaesthetized animals. These differences could be due to either a difference in the response of the acinar cells to CCK-PZ in the two states or to differences in the delivery of the hormone to the pancreas. It is possible that shock or the anaesthetic
agent could affect the normal nervous and hormonal influences to the pancreas, or directly affect pancreatic acinar cell metabolism and thus modify the response of these cells to exogenous CCK-PZ. However, there is little evidence either for or against this hypothesis. Again we would favour an effect on blood flow. At all dose levels of CCK-PZ, the flow of pancreatic juice was lower in anaesthetized than in conscious rats, but CCK-PZ stimulated flow severalfold in the anaesthetized rats whereas it had no effect on flow in the conscious rats. Such an effect could result from a greatly reduced blood supply to the pancreas in the unstimulated anaesthetized rats, with a graded increase in flow when increasing doses of CCK-PZ were given. A number of workers have reported that CCK-PZ stimulates pancreatic blood flow (Frogge et al. 1970; Papp et al. 1973; Tsuruoka 1976). In the conscious rats pancreatic blood flow would be unimpaired and would therefore not be stimulated to the same extent by CCK-PZ at the dose levels used here.

When increasing doses of CCK-PZ were given, there was a marked increase in both protein output and concentration in anaesthetized rats. In contrast there was a fall in protein output of conscious animals as CCK-PZ levels increased. These effects in the anaesthetized rats would be consistent with the theory presented above—increasing doses resulting in increasing blood levels—together with an increased delivery of CCK-PZ to the acinar cells as a result of vasodilation. However, the failure of the conscious rats to respond to increasing doses of CCK-PZ must be due to some other mechanism. The histological studies in these animals showed that in conscious rats the higher doses of CCK-PZ produced acinar cell damage. These effects of large doses of CCK-PZ have been frequently reported by other workers (Ribet et al. 1969; Goebell et al. 1976; Lampel and Kern 1977). No such damage was visible in the anaesthetized rats in which vasoconstriction may have slowed the delivery of the CCK-PZ to the acini, and thus protected the cells. In conscious rats therefore a relatively constant flow and output with increasing doses of CCK-PZ may result from the opposing effects of increased stimulation by CCK-PZ and increased inhibition by cellular damage.

The damage to the conscious rats seen histologically consists of the appearance of large autophagosomes in the cells and the extrusion of myelin bodies into the acinar lumen where they appear to block the duct (Fig. 6). If such an effect occurred after large doses of CCK-PZ, it might be expected that the response to later lower doses would be greatly reduced. This would explain the dissociated response to the high and low dose ranges in the descending dose studies with conscious rats. A single dose of 20·00 CHRU of CCK-PZ causes such profound damage to the cell that the response to that dose and any later dose is grossly depressed. After a single dose of 5·00 CHRU the damage is less great, and the response when 5·00 CHRU is given as the first dose in a series is therefore significantly greater than when it is given as the third dose in the 20·00, 10·00, 5·00-CHRU series.

No significant stimulation of protein output was seen at any dose of CCK-PZ in the conscious rats. Such an effect was also found by Petersen and Grossman (1977) in conscious animals in which pancreatic juice was not returned, and it was suggested by these authors that in these animals the pancreas was already being maximally stimulated by exogenous CCK-PZ. If this is true, any dose of CCK-PZ might be expected to inhibit pancreatic flow by causing acinar cell damage in studies of this type. It would be of interest, therefore, to study the effect of very much lower doses of CCK-PZ than those used here in conscious rats to determine if this is the case.
Petersen and Grossman (1977) also showed inhibition of output in unconscious animals at the highest rate of infusion. In view of this, higher doses of CCK-PZ should be tried in anaesthetized rats by the technique used here. However, extremely large doses may be needed and pilot studies have shown no inhibition with doses up to 40·00 CHRU.

The preparation of CCK-PZ used in these studies contains secretin and other peptides as contaminants. Some secretin activity to stimulate flow is probably needed for maximal secretion in response to CCK-PZ (Reggio et al. 1971) but the two hormones are synergistic (Grossman 1972) and the pancreatic flows and protein outputs recorded in this paper are probably higher than would be found with equivalent doses of pure CCK-PZ. Nevertheless, there is no reason to expect that the profound differences seen in the response of anaesthetized and conscious rats were related to the presence of impurities in the CCK-PZ preparation. Petersen and Grossman (1977) used the purest commercial preparation of CCK-PZ available (~20% pure, G.I.H. Research Unit, Karolinska Institute, Stockholm, Sweden, now distributed by Kabi Diagnostica, Stockholm, Sweden). While the differences between the technique used in their study and that employed here are too great to allow a detailed comparison of the results, both studies showed fundamentally different responses to CCK-PZ in anaesthetized and conscious animals.

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References


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