Pancreatic Response to Bolus Injection of Cholecystokinin-pancreozymin in Anaesthetized and Conscious Rats Fed Raw Soyaflour

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Abstract

Pancreatic secretion was studied in rats fed raw soyaflour (basal) and after stimulation with cholecystokinin-pancreozymin (CCK) given in either ascending or descending dose orders ranging from 1·25 to 20 or from 20 to 1·25 Crick–Harper–Raper units (CHRU). These results were compared with those reported previously for animals fed a stock cube diet. Two experimental conditions were used: anaesthetized animals were tested immediately after cannulation of the pancreatic duct and conscious animals were tested 48 h after surgery. Basal flow was significantly increased in anaesthetized and conscious rats fed RSF compared with the respective animals fed cubes. Mean basal protein output was also increased, but this difference was not significant. The pancreatic response to the ascending and descending doses of CCK in anaesthetized rats fed RSF was linearly related to the log of the dose of CCK in both animals fed RSF and cubes, though the response to CCK was greater in the rats fed RSF. When ascending doses of CCK were given to conscious rats fed RSF, the protein output increased up to 10 CHRU of CCK but was inhibited by 20 CHRU of CCK, whereas it decreased after the first dose of CCK (1·25 CHRU) in animals fed cubes. When descending doses of CCK were given to animals fed RSF, protein output was greatest after the first dose and no simple relationship between dose and response was seen. Compared with rats fed cubes, the pancreas in rats fed RSF thus appears to respond to a given dose of CCK with increased secretion, and conscious animals fed RSF can tolerate a higher dose of CCK before protein output is inhibited. This is consistent with an increased population of acinar cells in the animals fed RSF, with each hypertrophied cell responding to CCK with increased secretion.

Introduction

When rats are fed raw soyaflour (RSF), marked pancreatic hypertrophy and hyperplasia occur. This effect is probably due to the trophic action of increased circulating levels of endogenous cholecystokinin–pancreozymin (CCK) on the pancreas. Increased release of CCK occurs in response to ingestion of trypsin inhibitor, such as is found in RSF (Khayambashi and Lyman 1969; Brand and Morgan 1981). CCK release is thought to be controlled by intestinal trypsin levels (Green and Lyman 1972; Green et al. 1972) and the removal of trypsin by binding to a trypsin inhibitor is thought to produce increased release of CCK. The enlarged pancreas is both morphologically (Beswick et al. 1971; Folsch et al. 1974) and functionally (Lyman and Lepkovsky 1957; Folsch and Wormsley 1974) different from the normal pancreas.

In a previous paper (Oates and Morgan 1981), pancreatic secretion was studied in rats fed a stock cube diet. Two experimental conditions were used: anaesthetized animals were studied immediately after surgery and conscious rats were tested 48 h after surgery. The response of these two groups was very different. Thus, the
basal flow and protein output were 10- and 14-fold higher, respectively, in rats studied 48 h after the operation compared with anaesthetized animals tested immediately after surgery. Furthermore, when these rats received doses of CCK given in either an ascending or descending order, the anaesthetized rats showed a linear relationship between the log-dose of CCK and the flow, total protein output and enzyme outputs, whereas in conscious rats flow was unaffected by CCK and protein output was greatest after the first dose, whether of an ascending or descending series. It was suggested that in conscious rats even the lowest dose of CCK used caused pancreatic damage and inhibition of protein secretion.

In view of the pancreatic growth seen in animals fed RSF, it is possible that the response to CCK might be modified in rats fed this diet. In particular, it might be predicted that the enlarged pancreas of anaesthetized rats would respond to CCK with an increased flow and protein output and that in the conscious animal the pancreas would tolerate higher doses of CCK before inhibition occurs. The present study, therefore, compares the pancreatic response of rats fed RSF to the same stimuli used previously, with that of animals fed cubes reported in Oates and Morgan (1981).

**Material and Methods**

Male Wistar rats, locally inbred for 15 years, weighing 250–350 g and between 2 and 4 months old, were used.

**Diet**

Rats were fed RSF obtained from Soy Products of Australia Pty Ltd, Bayswater, Vic., supplemented with vitamins and minerals, as recommended by Folsch and Wormsley (1974). Each rat ate 15–20 g of flour each day. The RSF contained inhibitor activity, which inhibited approximately 40 mg of trypsin per gram of flour, as measured by the method of Kakade et al. (1974). The suppliers stated that it consisted of approximately 25% carbohydrate, 40% protein and 20% fat. Rats were fed RSF for at least 1 month, by which time maximal pancreatic growth had occurred (Crass and Morgan 1982; Oates and Morgan 1982). The results with rats fed RSF were compared with the previously published data for animals fed stock cubes (Oates and Morgan 1981). The cubes used in these studies contained only traces of trypsin inhibitor (approximately 500 μg of trypsin inhibited per gram of cubes) and were stated by the manufacturer to consist of 52% carbohydrate, 22% protein and 5% fat. Animals fed cubes and RSF were studied concurrently and were housed under the same conditions.

Rats fed cubes (Oates and Morgan 1981) or RSF (present study) were tested while either anaesthetized or conscious.

**Anaesthetized Rats**

Food was withheld overnight but the animals were permitted a 0·4% (w/v) sodium chloride solution orally. Details of the surgical procedure are given in Oates and Morgan (1981). Surgery involved the cannulation of the external jugular vein and pancreatic duct. The bile duct and pylorus were ligated. A tracheostomy was performed.

Sampling of the pancreatic juice commenced 3 h after the onset of anaesthesia (Love 1957). The procedure to determine the effect of bolus doses of CCK in anaesthetized rats was the same as that for conscious animals (see below).

**Conscious Rats**

The animals were allowed food and 0·4% (w/v) saline, until surgery. Details of the surgical procedure are given in Oates and Morgan (1981). Surgery involved the cannulation of the external jugular vein, duodenum, stomach and pancreatic and bile ducts. After surgery, the rats were restrained in Bollman restraint cages (Bollman 1948). Collection of pancreatic juice commenced 48 h after surgery.
Post-operative Care

Saline was infused intravenously at 1 ml h\(^{-1}\) using a peristaltic pump. An oral electrolyte solution of 2·5\% (w/v) Staminade (Nicholas Pty Ltd, Melbourne) was freely 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. Pancreatic juice recirculation was monitored as described previously (Oates and Morgan 1981). Bile was not recirculated.

As pancreatic enlargement is only maintained while the animals are fed RSF and there is rapid pancreatic involution if the diet is changed to one free of trypsin inhibitor (Crass and Morgan 1981), cannulated conscious rats fed RSF received an intragastric infusion of a 1\% (w/v) extract of trypsin inhibitor (Boscetti and Delay 1977) in an attempt to maintain pancreatic enlargement. Rats fed cubes used in similar studies were given 1\% (w/v) bovine serum albumin (Oates and Morgan 1981). These proteins were dissolved in 0·15 M saline containing 2·5\% (w/v) glucose and were administered 24 h after surgery at 3 ml h\(^{-1}\). The preparation of trypsin inhibitor represented about a fivefold concentration of inhibitor activity in RSF as estimated by the method of Kakade et al. (1968). The infused trypsin inhibitor therefore represented about one-third of the total trypsin inhibitor ingested per day by rats eating RSF. On the day of the experiment, the pancreatic–duodenal connection was broken and the intragastric and i.v. infusions were stopped 30 min before juice was collected.

![Diagram showing the number of rats used and the range of doses (CHRU) of CCK given to either anaesthetized or conscious rats fed RSF. Thirty-two rats fed the stock diet were also given the same doses of CCK as the groups fed RSF (Oates and Morgan 1981).](image)

Dose Schedule

CCK was purchased from the Boots Co. Ltd (Nottingham, England). Two batches (Nos 90025 and 90192) were used. Tests on rats fed cubes or RSF were performed concurrently and both groups were tested with both batches of CCK. There was no difference in the response to the two batches.

Each rat received three doses of CCK. Two groups of four rats were given, in an ascending order, either a lower dose range (1·25, 2·50 and 5·00 CHRU of CCK) or an upper dose range (5·00, 10·00 and 20·00 CHRU of CCK). The order of these doses was reversed in another two groups of four rats in the descending dose schedule. In all, therefore, two groups of 32 rats were used. The various conditions and treatments are summarized in Fig. 1. The CCK was dissolved in saline, so that the lowest dose was contained in 25 \(\mu\)l, 5 min before the first injection.

Collection of Pancreatic Juice in Anaesthetized and Conscious Rats

Pancreatic juice was collected in calibrated silicone capillary tubes in anaesthetized rats, and in preweighed sampling tubes in conscious animals. Twelve 10-min collections were made. The first
three collections were taken before CCK stimulation and represented the basal output (Love 1957). At 29, 59 and 89 min, a dose of CCK was injected intravenously over a 10-s period and flushed into the circulation with 0·3 ml of saline. The collection tube was changed 1 min later. At the completion of the secretion studies, the animals were killed by an overdose of anaesthetic and then autopsied. The results from conscious rats that showed pancreatic damage attributable to the surgery were discarded.

Determinations

In anaesthetized rats, the flow of pancreatic juice was calculated from the length of tubing filled, and in conscious rats by the change in weight of the collection tubes, assuming a density of 1·0 for the juice. The volume of each sample was made up to 3 ml with cold saline and the sample stored on ice. Protein estimation was performed by the method of Schacterle and Pollack (1973).

Statistics

Differences between individual means were analysed by Student's t-test for unpaired samples. Significance was considered to be \( P < 0·05\% \). Dose-response curves were compared by analysis of covariance (Snedecor and Cochran 1980).

Results

Standard Basal Output

In each group tested, pancreatic secretions were the same for the first 30 min irrespective of whether the rats were given ascending or descending doses of CCK. It was, therefore, possible to combine the basal outputs in rats given ascending and descending doses of CCK to obtain a 'standard basal output' for anaesthetized and conscious rats.

<table>
<thead>
<tr>
<th>State of rat</th>
<th>Flow (µl)</th>
<th>Protein output (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaesthetized, fed:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cubes</td>
<td>23 ± 2</td>
<td>0·47 ± 0·21</td>
</tr>
<tr>
<td>raw soyaflour</td>
<td>35 ± 5</td>
<td>0·76 ± 0·18</td>
</tr>
<tr>
<td>Conscious, fed:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cubes</td>
<td>225 ± 7</td>
<td>6·9 ± 1·0</td>
</tr>
<tr>
<td>raw soyaflour</td>
<td>353 ± 31</td>
<td>7·3 ± 0·7</td>
</tr>
</tbody>
</table>

In rats fed RSF, standard basal flow and protein outputs were both tenfold higher in conscious rats compared to anaesthetized animals (Table 1). There was a significant increase in basal flow in anaesthetized and conscious rats fed RSF compared to the respective rats fed cubes. Mean protein output was increased, although this difference was not significant.

Stimulated Pancreatic Secretions

Anaesthetized rats

In anaesthetized rats tested with either the ascending or descending dose order, the response to the 5·00 CHRU of CCK was not significantly different regardless of whether the dose was given as the first dose or the last dose in the order. The
data could, therefore, be combined to allow the calculation of a dose-response relationship for the range 1·25-20 CHRU for both ascending and descending dose schedules.

Conscious rats

In conscious rats, flow and protein output were the same for 5·00 CHRU when this was given in the lower and upper dose ranges of the ascending dose schedule. When descending doses were given, the response to 5·00 CHRU was significantly greater when given as the first dose of the lower dose order than when given as the last dose of the upper dose order. Therefore, in the results that follow for conscious rats, the lower and upper dose orders have been combined for ascending doses but assessed independently for the descending schedule.

Figs 2 and 3. Basal pancreatic juice flow (B) and flow in response to ascending (Fig. 2) and descending (Fig. 3) doses of CCK in anaesthetized (a) and conscious (b) rats fed RSF (●) or stock cubes (○). The data in these figures and Figs 4 and 5 for rats fed cubes are taken from Oates and Morgan (1981). Values are means ± s.e. of eight (5·00 CHRU) and four (other doses) rats.

Flow

Anaesthetized rats

In anaesthetized rats fed RSF or cubes, flow increased with increasing doses of CCK (Fig. 2a). With both diets, the relationship between the doses of CCK and the flow was approximately log-linear for the ascending schedule. Similarly in anaesthetized rats fed cubes, the pancreas responded to decreasing doses of CCK with decreasing flow and the response for the entire range 20·00-1·25 CHRU was
also log-linear. However, with this dose order in rats fed RSF, the pancreatic response was log-linear only between 10·00 and 1·25 CHRU of CCK (Fig. 3a). Nevertheless, in both dose schedules, flow for each dose was greater in anaesthetized animals fed RSF than in rats fed cubes, and the dose-response curves for ascending doses over the entire range and descending doses between 10 and 1·25 CHRU were significantly higher in animals fed RSF.

Conscious rats

When ascending doses of CCK were given to conscious rats fed cubes, flow did not change, whereas in conscious rats fed RSF flow decreased after 2·50 CHRU of CCK (Fig. 2b). However, in conscious rats fed RSF or cubes and tested with the descending doses of CCK, the responses to the upper and lower dose ranges were dissociated. Flow was greater when doses of CCK were given in the lower order than in the upper order (Fig. 3b).

Protein Output

Anaesthetized rats

In anaesthetized rats fed cubes or RSF, the response of protein output to the ascending schedule was similar to that seen for flow. Thus, a linear relationship existed between the log of the dose of CCK and the protein output over the range 1·25–20·00 CHRU (Fig. 4a). In anaesthetized rats fed cubes and given descending doses of CCK, protein output was similar to flow (Fig. 5a), there being a log-linear decrease in protein output with the decreasing schedule. In anaesthetized
rats fed RSF, protein output was less for 20·00 than for 10·00 CHRU of CCK, though between 10·00 and 1·25 CHRU the response was approximately log-linear. The dose-response curves for the ascending doses over the entire range and for descending doses between 10·00 and 1·25 CHRU were significantly higher in animals fed RSF.

**Conscious rats**

In conscious rats fed cubes and tested with ascending doses, protein output was highest after the first dose but at no dosage level did protein output change significantly from the basal value (Fig. 4b). In conscious animals fed RSF, protein output was less at 1·25 CHRU than that for animals fed cubes, the same at 2·50 CHRU and significantly greater above 5·00 CHRU of CCK. When the dosage schedule reversed was in a separate group of animals, the responses to the upper and lower dose orders were dissociated (Fig. 5b). Protein output was highest for the first dose given (20·00 or 5·00 CHRU of CCK) and decreased for lower doses. In both diets with descending doses, the response of protein output was greater for the lower dose order than for upper doses of CCK.

**Discussion**

These studies have investigated the response of the pancreas to bolus injection of Boots CCK. Bolus injection rather than continuous infusion of CCK was used because preliminary studies in this laboratory showed that a reproducible log-linear dose-response curve could be readily obtained with this technique. Within 30 min of each dose, output had returned to basal, whereas with continuous infusion a complex response is seen (Folsch and Wormsley 1974), suggesting that continuous exposure to the hormone leads to changes in the sensitivity of the organ to the hormone. Other technical problems of the continuous infusion (blocked cannulae, leaking connections, absorption of hormone to infusion tubing) were also avoided by bolus injection.

Boots CCK was used rather than the purer Kabi (G.I.H.) preparation because of its ready availability as a diagnostic agent. Subsequent studies have shown that the two preparations have essentially equal cholecystokinin activity (1 CHRU = 1IDU) (Brand 1981) and stimulate pancreatic protein output equivalently (Crass and Morgan, unpublished data). However, the Boot's preparation is a much more potent stimulant of pancreatic flow than Kabi CCK, presumably because of a substantial contamination with secretin in the Boot's material, and variation between batches of the Boot's preparation does occur. The studies reported in this paper were all performed with two batches of CCK that showed no detectable difference in activity.

In both the rats fed RSF reported here and the animals fed cubes reported earlier (Oates and Morgan 1981), basal pancreatic flow and protein output were increased approximately 10-fold in the conscious compared with the anaesthetized animal. The increase in basal secretions with time after surgery is probably a result of recovery from the surgical trauma, as previously suggested (Oates and Morgan 1981). Surgical trauma affects gastrointestinal function (Woolley and Simmonds 1959; Morgan 1966) and blood flow (Hiley et al. 1978; Granger et al. 1980) and depressed pancreatic function caused by reduced blood flow appears likely. When basal pancreatic
secretions were studied at various intervals after surgery, in rats fed a normal diet, flow and protein output increased gradually up to 48 h (De Waele et al. 1974). Such a graded increase in pancreatic secretions with time after surgery is compatible with recovery from the post-operative trauma, possibly acting through blood flow.

In anaesthetized and conscious rats fed RSF, basal flows were increased by approximately 55% compared to the respective rats fed cubes. Mean protein outputs were slightly increased in the groups fed RSF, though these differences were not significant. Basal secretions have been shown to be increased in rats fed RSF (Green and Lyman 1972) and in rats with external pancreatic fistulae (Green et al. 1973; Petersen and Grossman 1977). In both circumstances, secretion is probably increased by the same mechanism. Pancreatic enzyme secretion is largely controlled by CCK levels (Petersen and Grossman 1977). CCK release is thought to be inhibited by the presence of trypsin in the small intestine (Green and Lyman 1972). However, in the presence of trypsin inhibitor or after diversion of pancreatic juice to the exterior, the inhibition of CCK release by trypsin is lost and CCK release is increased. A reduction in intestinal CCK levels has been shown after trypsin inhibitor has been given orally (Brand and Morgan 1981), and increased pancreozymin activity in the plasma has been shown after feeding on RSF (Khayambashi and Lyman 1969). Since CCK is trophic for the pancreas (Petersen et al. 1978; Solomon et al. 1978; Dembenski and Johnson 1980; Morisset 1980) feeding on RSF results in hyperplastic and hypertrophic enlargement of the gland (Melmed 1976; Crass and Morgan 1982; Oates and Morgan 1982).

When CCK was injected intravenously in anaesthetized rats fed RSF, the pancreatic responses were similar to those seen in the respective groups of rats fed cubes, though for each dose of CCK the response from the enlarged gland was greater. With feeding on RSF, the hypertrophied acinar cell has an increased rate of protein synthesis (Konijn et al. 1970; Dijkhof et al. 1977) and in the fasting state contains more zymogen granules than in rats fed a normal diet (Folsch et al. 1974). It could be predicted, therefore, that these cells would respond to each dose of CCK with an increased protein discharge.

In conscious rats fed cubes and given increasing doses of CCK, protein output decreased after 1·25 CHRU of CCK but in animals fed RSF protein output increased up to 10·00 CHRU before falling at 20·00 CHRU of CCK. The enlarged pancreas thus requires larger doses of CCK before inhibition of protein output occurs. In Oates and Morgan (1981) it was suggested that inhibition of protein output in conscious animals fed cubes was a result of basal secretion being close to maximum and that CCK stimulation produced damage to the pancreatic acinar cells, which in turn led to decreased protein discharge. It appears that in the rat fed RSF, higher doses of CCK are required before such damage occurs. A number of mechanisms may be involved in this increased resistance of the pancreas of the conscious rats fed RSF to damage by exogenous CCK. Thus, the increased protein synthetic rate and zymogen-granule contents of these cells would probably allow a considerably increased secretion in response to CCK before this mechanism is overloaded and cellular damage occurs. Furthermore, the enlarged pancreas may include an enlarged ductular system. In autoradiographic studies, increased labelling in duct cells during the first and second week after feeding on RSF has been seen (Oates and Morgan 1982). In support of this is the study by Petersen et al. (1978), which showed that when CCK was given three times daily for 15 days pancreatic weight increased by 57% and that the pancreas
responded to secretin with an increased bicarbonate release compared to control animals. This suggests that the duct-cell compartment is increased in the CCK-treated pancreas, since the action of secretin is predominately on duct cells and has little influence on secretion by acinar cells. If the ductal system is enlarged it would be less susceptible to the blockage by cellular debris seen in conscious rats fed cubes (Oates and Morgan 1981). Finally in rats fed RSF, the acinar cells are chronically exposed to high levels of endogenous CCK. Under these circumstances, some down-regulation of the sensitivity of individual cells to CCK might be expected, and higher doses of endogenous CCK would be required to produce damage. If such an effect does occur, it must be more than compensated for by the increased cell number since the output for a given dose of exogenous CCK is higher in the rats fed RSF.

In the study reported here, pancreatic secretion did not return during the experimental period. As discussed earlier, pancreatic diversion leads to an increased pancreatic flow, probably because of increased release of endogenous CCK. This effect may be seen within 1 h of diversion (Petersen and Grossman 1977). Since, in the present study, juice collection was continued for 2 h after diversion, an increase in release of endogenous CCK may occur. Nevertheless, in anaesthetized animals, flow and protein output returned to values close to the initial basal secretion between injections and in the final collection period, indicating that any change in the release of endogenous CCK over the experimental period had little effect on secretion. Whether this effect of pancreatic diversion in increasing release of CCK might be a factor in the high basal output and resistance to exogenous CCK in conscious animals is unknown. However, if it is a factor, its relative importance would seem to be less in the animal fed RSF than in rats fed cubes, since rats fed RSF responded to exogenous CCK with an increased secretion whereas animals fed cubes did not.

When decreasing doses of CCK were given to anaesthetized rats fed RSF, the flow and protein-output responses to 20·00 CHRU were less than those to 10·00 CHRU of CCK. Such an effect was not seen for rats fed cubes. The reason for this difference is not apparent. However, it seems unlikely that extensive cellular damage occurs with the highest dose in the anaesthetized animals fed RSF since the response 30 min later to 10 CHRU of CCK was greater than the initial response. The doses of CCK used in these studies were chosen because they produced a reproducible dose-response curve in anaesthetized rats. Nevertheless, they almost certainly result in plasma levels of CCK considerably above the physiological range, though no measurements of levels of plasma CCK in the rat have been reported, and indeed reliable measurements of this hormone in man have only recently become available (Byrnes et al. 1981).

It is not suggested that the inhibition of pancreatic secretion seen in conscious rats with the higher doses of CCK is a physiological response, but the difference between rats fed cubes and RSF in this regard does indicate a functional difference in the pancreas in these two groups. The lowest dose of CCK used in the present study is equivalent to about 4 CHRU per kilogram whereas the recommended dose for studies of pancreatic secretion and stimulation in the human is 1–2 CHRU per kilogram.

These findings are consistent with the pancreas of the rat fed RSF having an increased number of hypertrophied cells with each cell possibly responding to a dose of CCK with an increased output.
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