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CHOLECYSTOKININ AFFECTS GASTRIC EMPTYING AND STOMACH MOTILITY IN THE RAINBOW TROUT *ONCORHYNCHUS MYKISS*

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Summary

In this study, we describe new methods for recording gastric emptying and *in vivo* measurements of intragastric pressure in fish. Using these methods, we investigated the effects of the sulphated octapeptide of cholecystokinin (CCK8) on gastric emptying and on stomach motility *in vivo* and *in vitro*. Gastric emptying of $^{99}Tc^{m}$-labelled food was measured in swimming fish by using a gamma camera, counting consecutive 2.5 min periods for 18–42 h. After 20 h, 55.3±4.0 % of the labelled food remained in the stomach of the control fish (mean ± S.E.M., *N*=9). Vascular infusion of CCK8 (25 pmol kg^{-1} h^{-1}) delayed gastric emptying so that 70.4±4.8 % of the labelled food remained in the stomach after 20 h (*N*=8). Gastric pressure changes *in vivo* were measured using a balloon surgically fitted into the cardiac or pyloric part of the stomach. In the cardiac part, intra-arterial infusion of CCK8 at 0.1 nmol kg^{-1} h^{-1} resulted in a decrease in the frequency and amplitude of rhythmic contractions, while higher doses started/increased contractions. Atropine blocked much of the basal contractile activity, but did not influence the CCK8-induced inhibition of contractile activity. The pyloric part of the stomach was unaffected by intra-arterial infusion of CCK8 or atropine. *In vitro* perfusion of the stomach (with a balloon placed in the cardiac part to record motility) with CCK8 at high concentrations (10^{-7} mol l^{-1} and above) augmented the spontaneous contractions, while lower concentrations had inconsistent effects. In addition, CCK8 (10^{-7} to 10^{-6} mol l^{-1}) decreased the amplitude of spontaneous contractions in longitudinal strip preparations, usually in combination with an increase in the resting tension. The decrease in amplitude was not affected by the nitric oxide synthase inhibitor N^{G}-nitro-L-arginine methyl ester hydrochloride (L-NAME; 10^{-4} mol l^{-1}). Depending on the concentration and experimental arrangement, CCK8 had either inhibitory or excitatory effects on the cardiac stomach, suggesting the possible presence of different types of CCK receptor. We conclude that the predominant effect of CCK8 *in vivo* may be a slowing down of gastric emptying, presumably coinciding with a release of bile into the duodenum.

Key words: teleost fish, gastric motility, gastric emptying, cholecystokinin, rainbow trout, *Oncorhynchus mykiss*, nitric oxide.

Introduction

In mammals, cholecystokinin (CCK) emanating from endocrine cells of the duodenum and jejunum or from local enteric neurones plays an important role in the control of gut motility and secretion, and in the control of food intake by inducing satiety (see e.g. Furness and Costa, 1987; Solcia et al., 1989). Our previous studies have shown that CCK induces gallbladder motility in the rainbow trout, presumably after release from endocrine cells in the duodenum caused by fat, amino acids or acidification of the duodenal contents (Aldman and Holmgren, 1987, 1995; Aldman et al., 1992). Himick and Peter (1994) reported a decrease in the food intake of goldfish after intraperitoneal or intraventricular injections of CCK. The primary aim of the present study was to continue the studies of the effects of CCK in the fish gut by elucidating the effects of CCK on gastric emptying and stomach motility in the rainbow trout *Oncorhynchus mykiss*. To this end, we also aimed to improve the methods used to measure gastric emptying and intragastric pressure *in vivo* in fish.

The fish stomach is divided anatomically into two parts: the proximal cardiac stomach and the distal pyloric stomach (Jacobshagen, 1913). In the rainbow trout, the cardiac part is the major part; it is characterised by an abundance of gastric glands and by a relatively thin muscular wall. The stomach folds back on itself *via* a transitional or fundic region into the much more muscular pyloric part of the stomach, which lacks glandular tissue. The pyloric part tapers off into the pyloric sphincter without a distinct change in morphology (Jacobshagen, 1913; Ezeasor, 1981). The combined properties of the stomach, the pyloric sphincter and the intestine create a resistance to the flow of chyme from the stomach to the

intestine. Gastric emptying is achieved when this resistance to flow is overcome.

Gastric emptying in fish has received some attention over recent decades (for reviews, see Fänge and Grove, 1979; Jobling, 1987), with emphasis being placed on feeding regimes and food formulae. We were interested in the physiological control mechanisms involved, especially the integrated effects of CCK *in vivo*, and have developed a new method for measuring gastric emptying in fish based on the scintigraphic technique used preferentially in mammalian studies. This method allows for continuous monitoring of the gastric contents and continuous drug infusion during the experiment and avoids the problems associated with repeated anaesthesia.

To elucidate further the mechanisms behind the effects of CCK on gastric emptying, we have performed *in vivo* and *in vitro* motility studies on the cardiac and pyloric stomach. Jensen et al. (1991) presented data from *in vivo* studies of the cardiac stomach of the Atlantic cod *Gadus morhua*. Their surgical method of insertion of an intragastric balloon for pressure recordings was tested and was found to have drawbacks in the rainbow trout because of bleeding. Farmed rainbow trout usually have masses of well-vascularised fat in the body cavity, which easily causes haemorrhages during surgery. We therefore aimed to developed a method that requires a minimum of surgery and leaves the fish in a better post-surgical condition.

Materials and methods

Rainbow trout [*Oncorhynchus mykiss* (Walbaum)] of either sex weighing 700–1600 g were bought from a local hatchery and kept in tanks with aerated recirculating fresh water at 10 °C. The fish were fed once a week until 1 week before experiments began.

Gastric emptying

Surgical procedure

The fish was fitted with a catheter in the dorsal aorta as previously described (Aldman et al., 1992). Briefly, the fish was anaesthetised in MS222 (100 mg l^{-1}) and placed on an operating table with a constant flow of water (containing MS222 50 mg l^{-1}) over the gills. $NaHCO_3$ was added to buffer the water at pH 7.0. A polyethylene catheter (PE50) for administration of NaCl or CCK8 was inserted in the dorsal aorta and secured by two stitches in the dorsal part of the buccal cavity. The catheter was led back through the opercular opening and secured again by a skin stitch.

After surgery, the fish were left to recover for 24 h in a Perspex (2 mm) tank with a continuous flow of fresh water at 10 l min^{-1} and 12 °C. The tank contained 4 l of water and was 5 cm wide, which prevented the fish from moving sideways away from the camera, but allowed some vertical and horizontal movements. The tanks were kept covered to minimise disturbance to the fish.

Feeding

Each fish was fed with 0.5 % body mass of food pellets (EWOS vextra maxi; raw protein 42 %, raw fat 22 %, carbohydrates 16 %, water 10 %; 17 kJ g^{-1}). This is a fair-sized meal although, when feeding voluntarily, fish of this size can consume over 2 % of their body mass (Grove and Holmgren, 1992a). The pellets were labelled with $^{99}Tc^{m}$ (see below) and placed in a soft plastic tube (diameter 10 mm). To prevent air from entering the stomach together with the food, 0.5 ml of water was added per gram of food to fill air pockets between the pellets. The fish was carefully lifted out of the tank, and the feeding tube was gently pushed down into the stomach. The food was pushed out of the tube over a 15–30 s period, the tube was then retracted and the fish was put back into the tank. The time required for feeding (i.e. the time for which the fish was outside the water tank) never exceeded 1 min. Ten fish were infused with CCK octapeptide (CCK8; 25 pmol kg^{-1} h^{-1}, 0.1 ml h^{-1}) for 18–42 h. The choice of dose was based on previous studies on the motility of the gallbladder (Aldman and Holmgren, 1995) and pilot experiments. Ten control fish were infused with 0.9 % NaCl.

The food was labelled with 20 MBq of $^{99}Tc^{m}$ (half-life 6 h, photon energy 140 keV) macroalbumin aggregates (LyoMAA Byk-Malinchkrodt) because of its high binding affinity to proteins. $^{99}Tc^{m}$–MAA aggregates have been used to label Swedish pancakes for human tests (Hermansson, 1991), and it was shown that the choice of the isotope-binding complex MAA is important for stable isotope labelling to be achieved.

Measurement of gastric emptying

The tank containing the fish was placed in front of a gamma camera (General Electric MAXI I) equipped with a medium-energy parallel-hole high-resolution collimator and connected to a computer system (NUD Gamma-II MTT system). The energy window was ±10 % around the energy peak. Data acquisition started immediately after the fish had been fed, and the gamma camera counted consecutive 2.5 min periods for 18–42 h. Fig. 1 shows examples of the data images obtained during one experiment.

A region of interest was defined around the stomach and was further divided into two areas, the cardiac and pyloric stomach. The amount of radioactivity remaining in the stomach was measured continuously, and values were taken from a frame every 10 min. The curves were normalised to the time when the highest count was measured (30–60 min after the start of the experiment). All counts were corrected for radioactive decay and background radiation. Compensations for movements of the fish were made for each picture by moving the region of interest.

In vivo *studies of gastric motility*

Surgical procedures

The fish were anaesthetised in MS222 (100 mg l^{-1}) and placed on an operating table with a constant flow of water (MS222 50 mg l^{-1}) over the gills. $NaHCO_3$ was added to buffer the water at pH 7.0.

To record motility from the cardiac stomach, the fish were laparotomised on the right-hand side approximately 1 cm oral to the buccal fin. A Perspex tube (diameter 10 mm) was gently

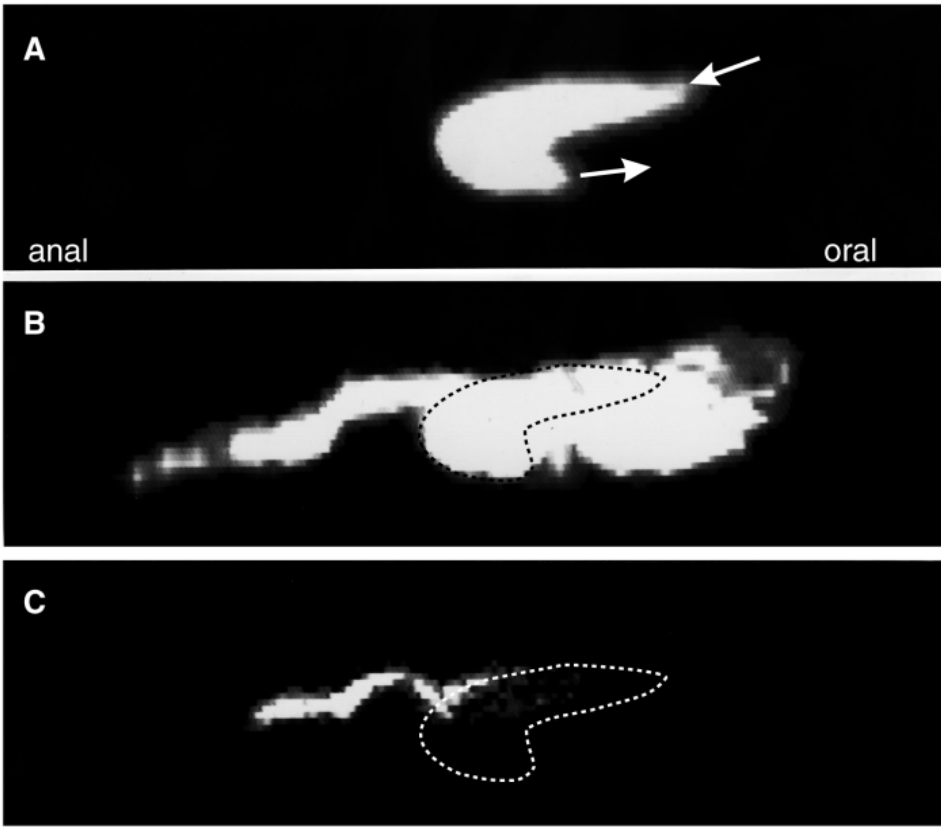


Fig. 1. Gastric emptying in an individual rainbow trout (*Oncorhynchus mykiss*). The images show the distribution of $^{99}Tc^{m}$-labelled food in different parts of the gastrointestinal tract at different times after feeding. (A) At 1 h after feeding, all the food is found in the stomach. The arrows indicate the direction of food transport. (B) After 10 h, the stomach (outlined by the dotted line) has started to empty and some of the food is found in the intestine. (C) After 25 h, no food remains in the stomach, while some food is still left in the distal part of the intestine.

pushed down through the mouth and oesophagus into the stomach. A thin rod of steel (diameter 1 mm) with a sharpened tip was introduced into the opening through the gut wall and penetrated through the stomach wall into the Perspex tube. The rod was then pushed forward and out through the mouth, and the tube was removed. A PE50 catheter, with a latex balloon (volume 5 ml) connected to it, was attached to the oral tip of the rod, and the rod was gently retracted through the stomach wall and the opening in the body cavity wall, leaving the balloon in the stomach with the catheter leading out through the stomach and body cavity walls. A small plastic cap, with a central hole 0.1 mm smaller than the outer diameter of the PE50 catheter, was pushed over and down the catheter against the stomach wall to avoid leakage from the stomach into the peritoneal cavity. The plastic cap was kept in position throughout the experiment by friction against the catheter (controlled *post mortem* in all fish). The catheter was then tunnelled through the body wall and secured using a skin stitch. The initial opening in the body wall was closed using silk sutures, and the balloon was inflated with 1 ml of water.

To record the motility of the pyloric stomach, the fish was opened laterally on the right-hand side starting approximately 2 mm behind the operculum. A Perspex tube (diameter 4 mm), bent 2 cm from the tip at an angle of 120 °, was gently introduced into the stomach and placed with the opening in the pyloric part of the stomach. A pyloric caecum that opened into the duodenum close to the pyloric sphincter was cut open, and a PE50 catheter was pushed through the caecum and through the pyloric sphincter into the Perspex tube and out through the mouth. The tube was removed, and the oral end of the catheter was attached to another PE50 catheter with a latex balloon (volume 3 ml) connected to it. The catheters were retracted, leaving the balloon in the lower part of the stomach. To secure the balloon in the appropriate place, a small plastic disc, with a central hole with a diameter 0.1 mm smaller than the outer diameter of the PE50 catheter, was pushed over and down the catheter against the pyloric caecum. The catheter was tunnelled out through the body wall and secured using a skin stitch.

For drug delivery, a PE50 catheter was placed in the dorsal aorta using the method described previously by Aldman et al. (1992). The fish was transferred to an experimental tank (10 l) with a continuous flow of fresh water (10 l min^{-1}) at 10 °C and left to recover for 40–50 h.

Experimental procedure

The pressure in the stomach was recorded *via* a pressure transducer (Statham P23) on a Grass (model 7) polygraph recorder, and the signals were collected in a computer for statistical calculations. Sampling frequency was set to 15 samples s^{-1}, and a mean on-line value was calculated and stored every 15 s to construct trends over time. From these values, changes in stomach pressure were monitored as a mean value (the area under the trace but above the baseline) over 10 min periods. The transducer was calibrated against a water column.

CCK8 was infused at rates of 0.1, 1 and 10 nmol kg^{-1} h^{-1} for consecutive 30 min periods. Atropine was given at 1.2 mg kg^{-1} as a slow infusion on the second day of the experiment, and CCK8 was infused 45 min later, as on the previous day.

In vitro *perfusion studies*

Surgical procedure

The fish were stunned by a blow to the head and injected with heparin (0.5 ml kg^{-1}; 5000 i.u. ml^{-1}) into the caudal vein before they were killed and prepared essentially as described by Holmgren et al. (1985). The fish was opened on its right side, and a cannula was inserted in the mesenteric artery for vascular perfusion of the stomach using a constant-pressure perfusion system (Nilsson and Grove, 1974). Rainbow trout Ringer's solution, bubbled with a gas mixture of O_2 (97 %) and CO_2 (3 %), was used for the perfusion (Holmgren, 1983). A cannula was inserted into the hepatic portal vein to measure the outflow of the vascular perfusate from the stomach. All branches of the caeliac artery and the portal vein not involved in the supply of blood to, or the drainage of blood from, the stomach were ligated. The stomach was dissected free and placed in an organ bath containing Ringer's solution at 10 °C.

A water-filled rubber balloon was inserted into the stomach *via* the oesophagus for measurements of the muscle activity of the stomach wall. The intragastric balloon was connected *via* a tube to an open reservoir suspended from a Grass FT03 isometric transducer. Contractions of the stomach expelled fluid into the reservoir, and this increased the mass of the reservoir, which was recorded on a Grass (model 7) polygraph recorder. The water level in the reservoir was set to approximately 2 cm above the level of the stomach.

Experimental procedure

The preparations were left to recover for at least 1 h before the start of the experiments and then CCK8 (10^{-12} to 10^{-5} mol l^{-1}) was slowly injected into the perfusion line in bolus doses of 1 ml.

Strip preparations

Surgical procedure

The fish was killed by a blow to the head, the stomach was dissected out and longitudinal and/or circular muscle strips (approximately 2 mm×10 mm) were prepared from the cardiac part. The preparations were mounted in organ baths containing 5 ml of Ringer's solution (see above) and stretched to an initial tension of 10 mN. The tension was recorded on a Grass (model 7) polygraph recorder *via* a Grass FT 03 force transducer.

Experimental procedure

The strip preparations were left to recover until they showed stable spontaneous contractions (for a minimum of 60 min). Cumulative doses of CCK8 were added to the bath, yielding increasing concentrations in the bath from 10^{-9} to 10^{-6} mol l^{-1}. To examine the possible involvement of nitric oxide, strips were preincubated with the L-arginine analogue L-NAME (N^G-nitro-L-arginine methyl ester hydrochloride; 10^{-4} mol l^{-1}) for 20 min before the first dose of CCK8 was given. This concentration and incubation time for L-NAME have been shown previously to be effective in blocking an induced relaxation of, for example, the urinary bladder of the Atlantic cod *Gadus morhua* (Olsson and Holmgren, 1996). The results were expressed as a percentage of the activity before the addition of CCK8.

Drugs

Sulphated cholecystokinin octapeptide (CCK8) was obtained from Cambridge Research Biochemicals, UK, and 3-aminobenzoic acid ethyl ester (MS222), atropine sulphate and N^G-nitro-L-arginine methyl ester hydrochloride (L-NAME) were obtained from Sigma Chemicals, USA.

Statistics

Mean values are given ± S.E.M. For statistical calculations, Wilcoxon's signed rank sum test (two-tailed) for paired and unpaired observations was used. In cases of repeated testing, a sequentially rejective Bonferroni test (Holm, 1979) was used to eliminate, as far as possible, any type I error. Differences were considered significant where $P<0.05$.

Results

Gastric emptying

One fish in the control group (NaCl infusion) and two in the CCK8-treated group were excluded because of vomiting during the experiment. After 10 h, 83.8±2.0 % and 92.2±1.7 % (significant difference, $P<0.01$) of the labelled food remained in the stomach of the control fish ($N=9$) and of the CCK8-treated group ($N=8$), respectively (Fig. 2). After 20 h, 55.3±4.0 % of the radioactivity was left in the control group, while 70.4±4.8 % (significant difference, $P<0.05$) remained in the CCK8-treated fish, demonstrating an inhibitory effect of CCK8 on the rate of stomach emptying. There were, however,

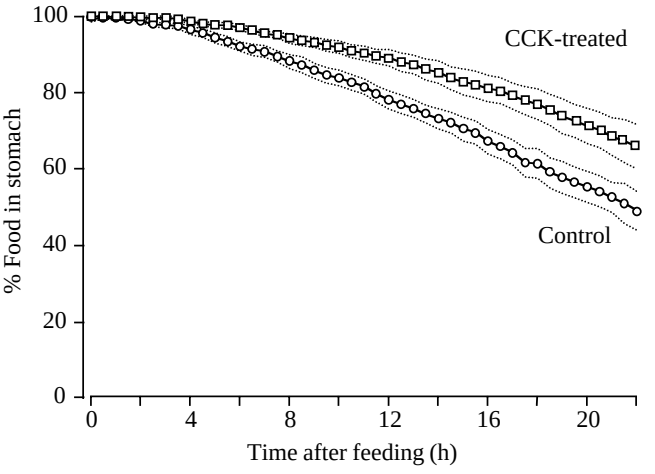


Fig. 2. The effect of vascular infusion of CCK8 on gastric emptying in the rainbow trout *Oncorhynchus mykiss*. Circles, control fish (0.9 % NaCl; $N=9$); squares, CCK8-treated fish (25 pmol kg^{-1} h^{-1}, 0.1 ml h^{-1}; $N=8$). The percentage of $^{99}Tc^m$-labelled pellets remaining in the stomach at different times after feeding is higher in the CCK8-treated fish than in the control fish, indicating that CCK8 delays gastric emptying. Values are means and the dotted lines show S.E.M.

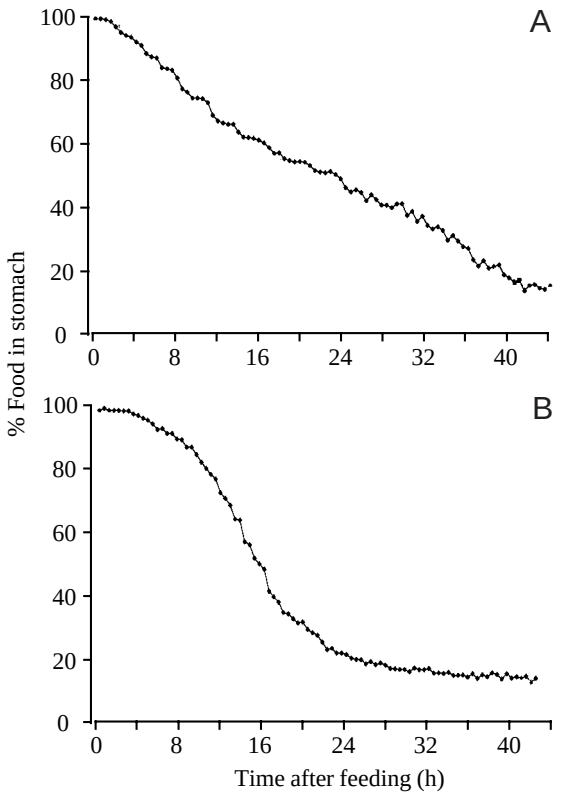


Fig. 3. Gastric emptying in two untreated fish demonstrating individual differences. After an initial lag phase, emptying starts in both fish. Emptying is linear in the fish in A, but emptying follows an exponential curve in the fish in B.

large differences between individuals in both groups. The lag phase from feeding until the stomach started to empty varied between zero and approximately 5 h. Some fish appeared to demonstrate linear emptying after the lag phase (Fig. 3A), while in others the emptying curve had an exponential shape (Fig. 3B). In addition, some fish showed pulsatile emptying of the stomach.

In vivo *studies of stomach motility*

The cardiac stomach adopted a basal resting tension giving an intraluminal pressure of 1.45±0.32 kPa (*N*=9). Superimposed single contractions occurred in an irregular manner throughout the experimental period (up to 5 days) interspersed by periods of quiescence typically lasting 1–5 min (Fig. 4). The contractions had a mean frequency of 0.57±0.07 min^{-1} and gave a mean (calculated over 10 min) total intraluminal pressure of 2.04±0.37 kPa (Fig. 5A). The resting pressure increased slightly in four of seven fish after atropine treatment, but mean total pressure was unaffected (Fig. 5A), since atropine at the same time decreased the frequency and amplitude of the superimposed contractions (Figs 4, 5B) and totally abolished these contractions in four fish.

CCK8 did not affect the resting (=baseline) tension but altered the contractile activity. Infusion of CCK8 (0.1 nmol kg^{-1} h^{-1}) decreased both the frequency (to 0.14±0.63 min^{-1}) and amplitude of contractions, which caused a decrease in mean total pressure to 1.61±0.37 kPa (Fig. 5A). An increase in the concentration of CCK8 (to 1 nmol kg^{-1} h^{-1}) induced contractions in three of the nine fish. These contractions were of lower amplitude but higher frequency than the contractions before CCK8 infusion (Fig. 4). CCK8 (10 nmol kg^{-1} h^{-1}) induced contractions in all fish and caused an increase in mean total pressure compared with the lowest concentration of CCK8 (Fig. 5A).

In fishes still showing spontaneous contractions after atropine treatment, CCK8 (0.1 nmol kg^{-1} h^{-1}) tended to decrease the frequency and amplitude of contractions further, but did not affect the resting tension. Higher doses of CCK8 induced contractions of low amplitude in five atropine-treated animals (Fig. 4).

The pyloric stomach adopted a basal resting tension giving an intraluminal pressure of 1.65±0.40 kPa (*N*=6). Superimposed single contractions occurred in an irregular manner with a mean frequency of 0.50±0.09 min^{-1}, giving a mean total intraluminal pressure of 3.02±0.74 kPa (Fig. 6A). CCK8 had no significant effects on the contractile activity, and atropine treatment gave inconclusive results (Fig. 6A,B).

In vitro *perfusion studies*

Infusion of CCK8 (10^{-7} mol l^{-1} and above, *N*=12) caused a brief increase in the frequency and amplitude of spontaneous contractions of the cardiac stomach. Lower concentrations had variable and inconclusive effects on motility, increasing or decreasing the activity but never completely abolishing the spontaneous contractions.

Strip preparations

The longitudinal strip preparations from the cardiac stomach (*N*=9) adopted a basal resting tension interrupted by spontaneous contractions (Fig. 7). CCK8 did not affect the total work performed by the strip preparations (Fig. 7C), although high concentrations (10^{-8} to 10^{-6} mol l^{-1}) of CCK8 decreased the amplitude of the contractions (Fig. 7D). In contrast, 10^{-6} mol l^{-1} CCK8 caused an increase in the resting tension to 136±12 % of the control value. The effect on the frequency was inconsistent, although a small increase was seen at 10^{-9} mol l^{-1} (Fig. 7E). The response of circular muscle (*N*=7) preparations to CCK8 varied greatly between individuals, and no consistent conclusions could be drawn.

Pretreatment with L-NAME (10^{-4} mol l^{-1}) altered neither the resting tone nor the effects of CCK8 on the amplitude, frequency or total work (Fig. 7).

Discussion

In this study, we decribe new methods for measuring gastric emptying and of making *in vivo* recordings of intragastric pressures in the rainbow trout. The scintigraphic technique used preferentially in studies of gastric emptying in mammals (see Ziessman, 1992) has been modified to suit studies in fish.

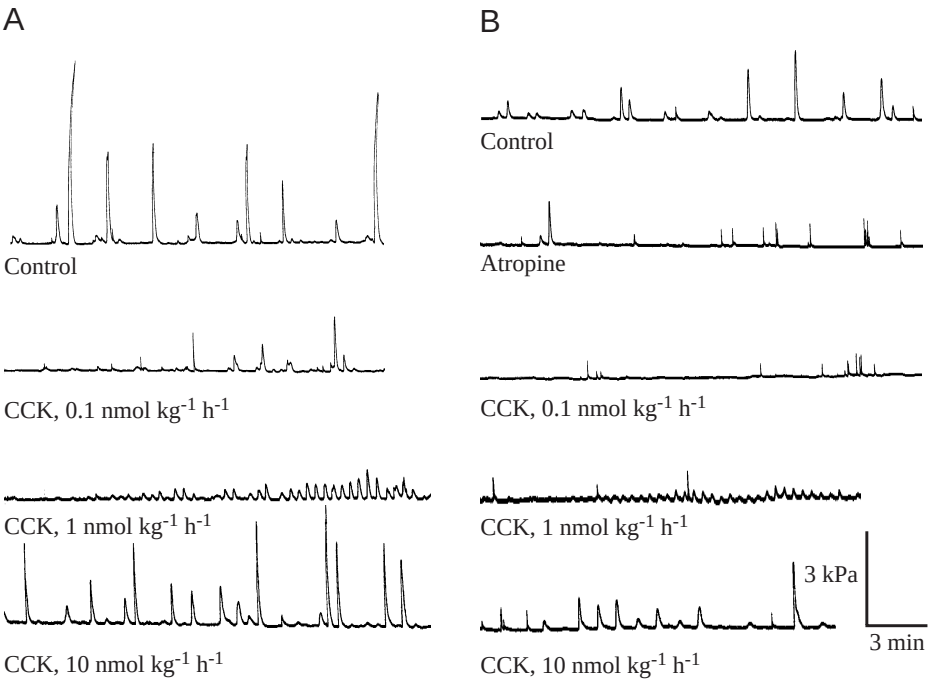


Fig. 4. The effects of intra-arterial infusion of increasing doses of CCK8 on the intragastric pressure and rhythmic activity of the cardiac stomach of the rainbow trout *Oncorhynchus mykiss*. Consecutive tracings from day 1 (A, untreated fish) and day 2 (B, atropine-treated fish) are shown from one experiment. There is approximately 10 min between each tracing within each panel, and approximately 20 h between A and B. Note that atropine reduces the activity of the control stomach, that a low dose of CCK8 (0.1 nmol kg^{-1} h^{-1}) reduces the activity of both the control and the atropine-treated stomach, and that higher concentrations of CCK8 (1 and 10 nmol kg^{-1} h^{-1}) stimulate the activity of the stomach.

This technique opens new possibilities for research in this area. Two advantages of this method are that the fish is awake during the experiment and that the course of events can be studied continuously. A disadvantage of the method is that the anatomy of the rainbow trout gastrointestinal tract may cause the amount of food remaining in the stomach to be overestimated. This is not a major problem when, as in the present study, the aim is to compare an untreated and a treated group rather than to establish absolute rates of emptying. The procedure of force feeding may also affect the rate of gastric emptying (Windell, 1966; Grove et al., 1978; Dos Santos and Jobling, 1988; Grove and Holmgren, 1992a) in both groups of fish and could be the cause of the vomiting seen in some of the fishes.

Both the cardiac and the pyloric parts of the stomach of the rainbow trout maintained a basic muscle tonus, interrupted by irregular contractions, throughout the *in vivo* experiment. Since the fish in the experiment were not fed during the week preceding surgery, the activity of the stomach could be compared with the interdigestive stage of the mammalian and avian stomach. In these animals, the interdigestive stage is characterised by cyclic patterns of migrating motor (myoelectric) complexes (MMCs), consisting of a basic state of quiescence (phase I), interrupted by periods of irregular single contractions (phase II) leading into a phase (phase III) of intensive muscular contractions (Szurszewski, 1969; Code and Marlett, 1975; Mueller et al., 1990). The stomach of the rainbow trout does not exhibit the long quiescent periods characteristic of phase I of the MMC and also lacks the characteristic variation between the low activity of phase II and the regular high activity of phase III. As in the rainbow trout, the cardiac stomach and the intestine of the Atlantic cod *Gadus morhua* demonstrate spontaneous contractions (Jensen et al., 1991; Karila and Holmgren, 1996). The contractions of the intestine moved anally at approximately the same speed (3.5±1.0 cm min^{-1}) as the MMC in mammals (Karila and Holmgren, 1996). In addition, the contractions were tetrodotoxin-sensitive, indicating that they are dependent on the (enteric) nervous system.

Gastric emptying in the rainbow trout involves a lag phase before the stomach starts to empty. This has been described previously in other fish species (turbot *Scophthalmus maximus*, Grove et al., 1985; plaice *Pleuronectes platessa*, Basimi and Grove, 1985; sandbar shark *Carcharhinus plumbeus*, Medved, 1985). The subsequent emptying varies between individual trout, being linear in some specimens and exponential in others. In the present study, we show that gastric emptying is clearly delayed by the presence of exogenous CCK. This effect of CCK is in accord with results from several studies in mammals (Chey et al., 1970; Debas et al., 1975; Moran and McHugh, 1982; Liddle et al., 1986; Smith et al., 1989; Jin et al., 1994). It should, however, be noted that, in contrast to the present study, liquid meals were used in most of the mammalian studies.

The site of action of CCK on gastric emptying is not clear, but the proximal stomach, the pyloric sphincter and centrally mediated actions have been proposed. In mammals, CCK receptors have been demonstrated on circular smooth muscles in the pyloric sphincter (Smith et al., 1984) and also on afferent vagal neurones (Corp et al., 1991; Lin and Miller, 1992). In the rainbow trout, CCK8 decreases the motility of the cardiac stomach and contracts the pyloric sphincter (present study; C. Olsson, G. Aldman and S. Holmgren, unpublished observations). The inhibitory effects of CCK8 on the amplitude and on the frequency of contractions *in vivo* are in agreement with studies on other vertebrates. In sheep, CCK8 decreases

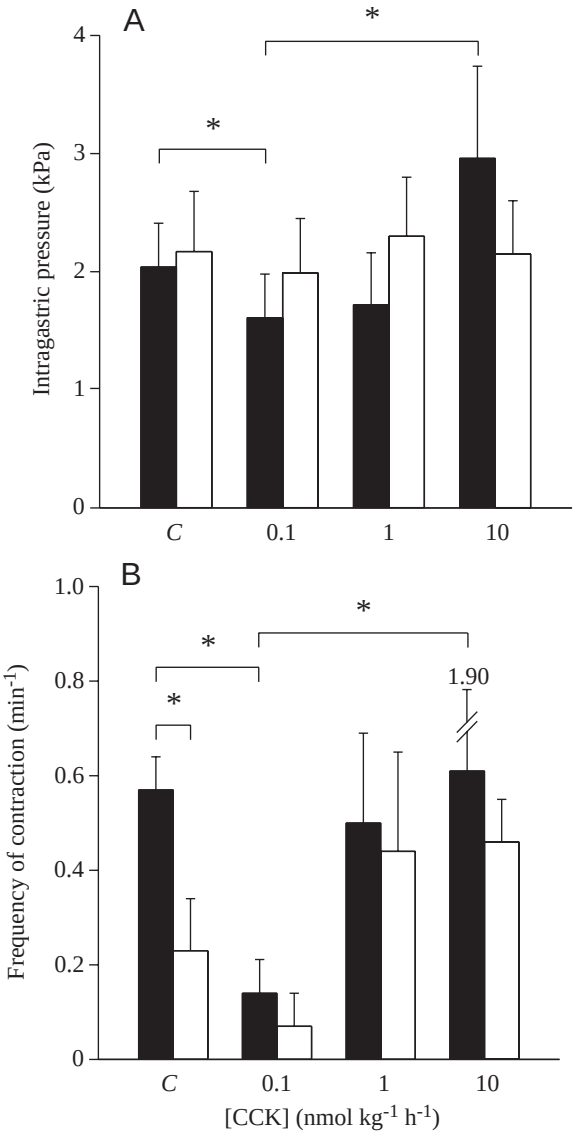


Fig. 5. The effect on mean intragastric pressure (A) and the frequency of contraction (B) of the cardiac stomach after *in vivo* intra-arterial infusion of CCK8 in the rainbow trout *Oncorhynchus mykiss*. Filled columns, control (N=9); open columns, after atropine treatment (N=7). Statistical significance is denoted by an asterisk ($P \leqslant 0.05$). (A) Note that a low dose of CCK8 (0.1 nmol kg^{-1} h^{-1}) reduces the intragastric pressure, while a high dose of CCK8 (10 nmol kg^{-1} h^{-1}) increases the mean pressure. (B) Atropine reduces the contraction frequency during the control period (*C*), the low dose of CCK8 reduces the contraction frequency, while higher doses of CCK8 increase the contraction frequency. Values are means + S.E.M.

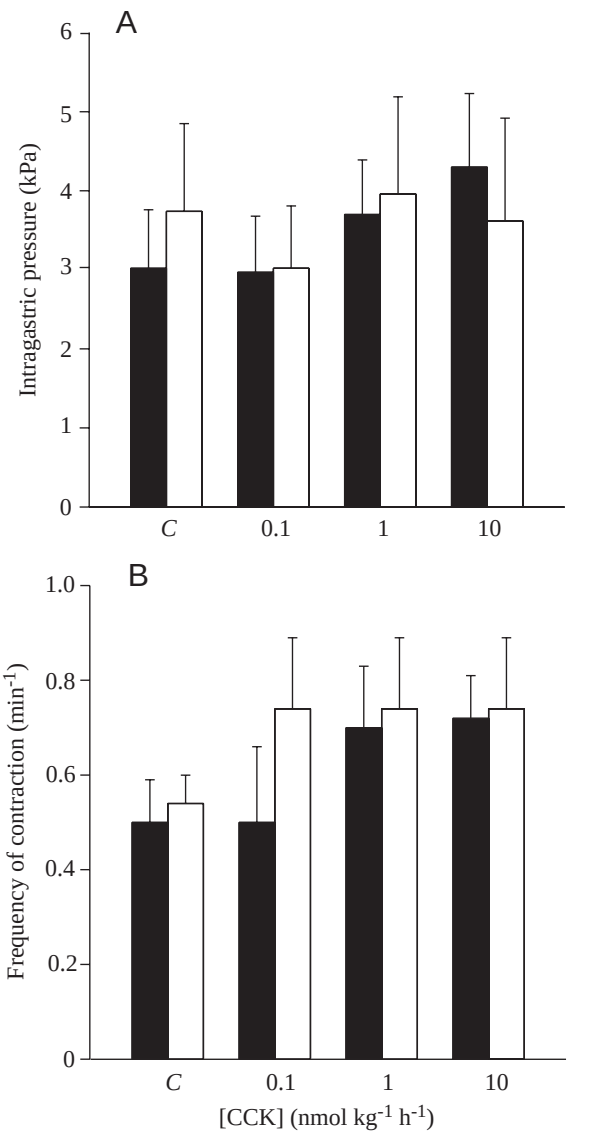
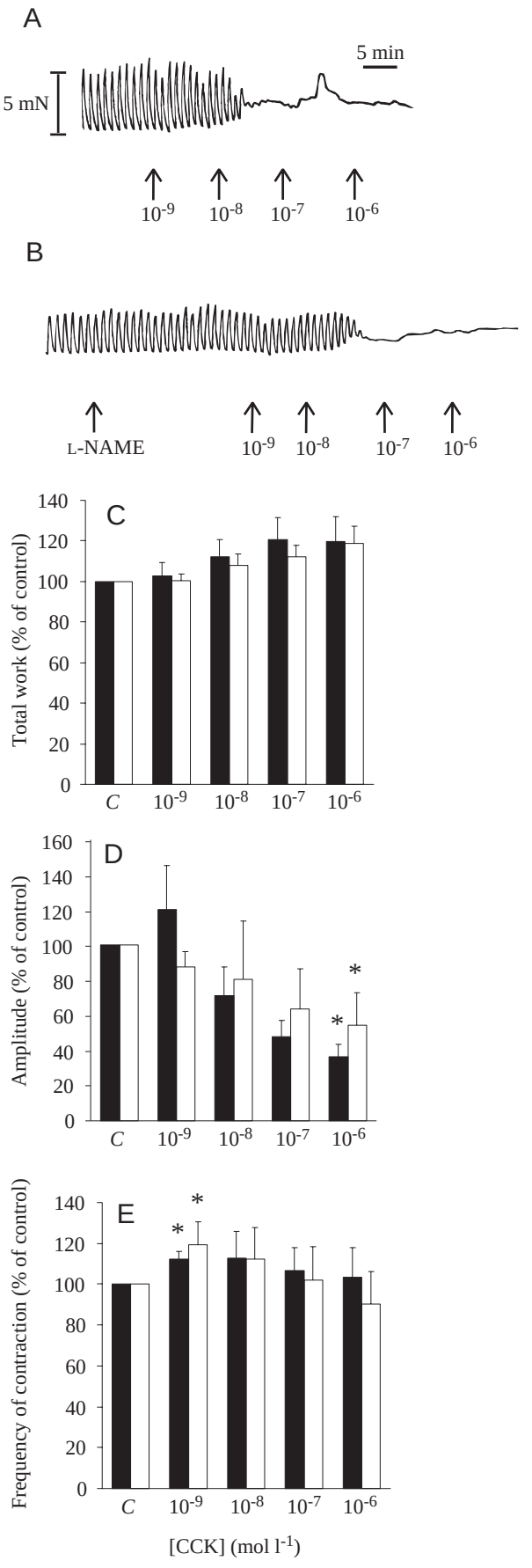


Fig. 6. The lack of effect on the mean intragastric pressure (A) and the frequency of contraction (B) of the pyloric stomach after *in vivo* intra-arterial infusion of CCK8 in the rainbow trout *Oncorhynchus mykiss*. Filled columns, control (N=6); open columns, after atropine treatment (N=6). Statistical significance is denoted by an asterisk ($P \leqslant 0.05$). Values are means + S.E.M. *C*, control period before CCK8 treatment.

the electrical spike frequency of the rumen (Kermani and Rezaiee, 1993), and in the cat stomach, it decreases the amplitude of contractions (Roche et al., 1993). Vagotomy abolishes the inhibitory effects of CCK8 on gastric emptying in the rat (Raybould and Taché, 1988; Schwartz et al., 1993) and on gastric electrical activity in the chicken (Martinez et al., 1993). In the rat, CCK8 has also been found to increase the electrical activity in the vagal afferent fibres (Schwartz et al., 1997). It has been suggested that CCK acts *via* a vago-vagal reflex to inhibit gastric motility, and Martinez et al. (1993) demonstrated that the effect of CCK in the chicken is mediated mainly by nitric oxide. The absence of inhibition by CCK at lower concentrations in the rainbow trout *in vitro* (when extrinsic pathways are cut) provides evidence for an indirect inhibitory effect involving extrinsic pathways *in vivo*.

The observation that longitudinal strip preparations showed a decrease in the amplitude of contraction at higher concentrations of CCK suggests that other inhibitory CCK receptors may be present. In dogs, L-NAME abolishes the inhibitory responses to CCK *in vitro* (Daniel et al., 1994).



Since L-NAME did not affect the CCK-induced decrease in contraction amplitude in the present study, this effect of CCK is probably not mediated by nitric oxide. Instead, it may to some extent depend on the increase in resting tension. L-NAME had no effect on the resting tone either, indicating that the rainbow trout stomach probably lacks a nitrergic inhibitory tone. This is in contrast to, for example, the cod intestine (Karila and Holmgren, 1996).

It is noteworthy that CCK does not influence the tonus of the rainbow trout stomach *in vivo*. In several mammals, CCK is reported to decrease the intragastric pressure (Yamagishi and Debas, 1978; Tani and Muto, 1985; Raybould et al., 1987), and this is thought to contribute to the delay in gastric emptying after CCK treatment (Liddle, 1989). It may be argued that the present experimental conditions do not allow further relaxation of the stomach of the rainbow trout. However, the stomach creates a positive pressure throughout the experiment and should be able to relax in response to an appropriate stimulus. Furthermore, *post mortem* analysis revealed that the stomach was not dilated in any of the animals, whether infused with drugs or not. It is possible that a relaxation in response to CCK occurs only in fed fish, where other factors, both hormonal and neural, are presumably activated. This activation could produce an increased tonus that could be antagonised by CCK.

Atropine decreases the frequency of spontaneous contractions *in vivo*, indicating that these contractions are under cholinergic control. A cholinergic excitatory innervation of the teleost fish stomach like that of the stomach of most vertebrates has long been established (see Fänge and Grove, 1979; Nilsson, 1983), although the origin of these fibres may be splanchnic rather than truly vagal in some fish species, including the rainbow trout (Campbell, 1975; Holmgren and Nilsson, 1981). However, few studies in fish have concerned the identification of the physiological motility patterns that involve these cholinergic neurones. Cholinergic neurones are involved in the reflex initiation of rhythmic activity after distension of the stomach wall in both the cod and the rainbow trout (Grove and Holmgren, 1992a,b), and the present study

Fig. 7. (A,B) Original tracings showing the effect of increasing concentrations of CCK8 (10^{-9} to 10^{-6} mol l^{-1}) on longitudinal strip preparations from the cardiac stomach of the rainbow trout *Oncorhynchus mykiss*. (A) Addition of CCK8 abolished the rhythmic contractions but caused an increase in the resting tension. (B) Preincubation for 20 min with the nitric oxide synthase antagonist L-NAME (10 μmol l^{-1}) did not alter the response to CCK8. (C–E) The effect of CCK8 on the total work (C), mean amplitude (D) and frequency of contraction (E). Filled columns, control; open columns, after L-NAME (10^{-4} mol l^{-1}) treatment (N=9). A high concentration of CCK8 (10^{-6} mol l^{-1}) lowers the amplitude of contractions, but does not affect the mean total work or the frequency of contraction. A low concentration of CCK8 (10^{-9} mol l^{-1}) causes a small, but significant, increase in the frequency of contractions. The response to CCK8 is not affected by the presence of the nitric oxide synthase antagonist L-NAME. The asterisks denote a significant difference compared with the control value ($P \leqslant 0.05$). Values are means + S.E.M. *C*, control period before L-NAME treatment.

suggests that, in the rainbow trout, these neurones also stimulate the rhythmic activity during the interdigestive stage. In fish that still showed contractions of the stomach after atropine treatment, it was also observed that atropine treatment *per se* did not impair the inhibitory effect of CCK8. However, the excitatory effect of higher doses of CCK8 was reduced or abolished by atropine treatment. This suggests that the excitatory effect of CCK8, but not the inhibitory effect, may be mediated at least partly *via* a cholinergic pathway.

In conclusion, CCK may be involved in the control of food processing in the gut of the rainbow trout by integrated actions on stomach motility, gastric emptying and the release of bile from the gallbladder. Results from other fish species also include effects on the pancreatic secretion of digestive enzymes and on satiety. In the present study, CCK8 delayed gastric emptying, probably *via* an indirect (possibly vago-vagal) non-cholinergic pathway. The excitatory effects of CCK8 at higher concentrations are at least partly mediated by cholinergic pathways.

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References

Aldman, G., Grove, D. J. and Holmgren, S. (1992). Duodenal acidification and intra-arterial injection of CCK8 increase gallbladder motility in the rainbow trout *Oncorhynchus mykiss*. *Gen. Comp. Endocr.* **86**, 20–25

Aldman, G. and Holmgren, S. (1987). Control of gallbladder motility in the rainbow trout, *Salmo gairdneri*. *Fish Physiol. Biochem.* **4**, 143–155.

Aldman, G. and Holmgren, S. (1995). Intraduodenal fat and amino acids activate gallbladder motility in the rainbow trout, *Oncorhynchus mykiss*. *Gen. Comp. Endocr.* **100**, 27–32

Basimi, R. A. and Grove, D. J. (1985). Gastric emptying rate in *Pleuronectes platessa* L. *J. Fish Biol*. **26**, 545–552.

Campbell, G. (1975). Inhibitory vagal innervation of the stomach in fish. *Comp. Biochem. Physiol.* **50**C, 169–170.

Chey, W. Y., Hitanant, S., Hendricks, J. and Lorber, S. H. (1970). Effect of secretin and cholecystokinin on gastric secretion in man. *Gastroenterology* **58**, 820–827.

Code, C. F. and Marlett, J. A. (1975). The interdigestive myoelectric complex of the stomach and small bowel of dogs. *J. Physiol., Lond.* **246**, 289–309.

Corp, E. S., McQuade, J., Moran, T. H. and Smith, G. P. (1991). Characterization of type A and B CCK receptor binding sites in the rat vagus nerve. *Brain Res.* **623**, 161–166.

Daniel, E. E., Vergara, P., Mao, Y. K. and Fox-Threlkeld, J. A. E. T. (1994). CCK8, a neuromodulator of NO, VIP and ACh release in canine intestine: functional and ligand binding studies. *Biomed. Res.* **15**, 51–56.

Debas, H. T., Farooq, O. and Grossman, M. I. (1975). Inhibition of gastric emptying is a physiological action of cholecystokinin. *Gastroenterology* **68**, 1211–1217.

Dos Santos, J. and Jobling, M. (1988). Gastric emptying in cod, *Gadus morhua* L.: effects of food particle size and dietary energy content. *J. Fish Biol*. **33**, 511–516

Ezeasor, D. N. (1981). The fine structure of the gastric epithelium on the rainbow trout, *Salmo gairdneri*, Richardson. *J. Fish Biol*. **19**, 611–627.

Fänge, R. and Grove, D. J. (1979). Digestion. In *Fish Physiology* (ed. W. S. Hoar and D. J. Randall), pp. 161–260. London, New York: Academic Press.

Furness, J. B. and Costa, M. (1987). *The Enteric Nervous System*. Edinburgh: Churchill Livingstone.

Grove, D. J. and Holmgren, S. (1992a). Intrinsic mechanisms controlling cardiac stomach volume of the rainbow trout (*Oncorhynchus mykiss*) following gastric distension. *J. Exp. Biol.* **163**, 33–48.

Grove, D. J. and Holmgren, S. (1992b). Mechanisms controlling stomach volume of the Atlantic cod (*Gadus morhua*) following gastric distension. *J. Exp. Biol.* **163**, 49–63.

Grove, D. J., Loizides, L. and Nott, J. (1978). Satiation amount, frequency of feeding and gastric emptying rate in *Salmo gairdneri*. *J. Fish Biol.* **12**, 507–516.

Grove, D. J., Moctezuma, M. A., Flett, H. J. R., Foott, J. S., Watson, T. and Flowerdew, M. W. (1985). Gastric emptying and the return of appetite in juvenile turbot, *Scophthalmus maximus* L. fed on artificial diets. *J. Fish Biol.* **26**, 339–354.

Hermansson, G. (1991). Tc-labelled pancake for studies of gastric emptying of solids. *Nucl. Med. Commun.* **12**, 973–981.

Himick, B. A. and Peter, R. E. (1994). CCK/gastrin-like immunoreactivity in brain and gut and CCK suppression of feeding in goldfish. *Am. J. Physiol.* **267**, R841–R851.

Holm, S. (1979). A simple sequentially rejective multiple test procedure. *Scand. J. Statist.* **6**, 65–70.

Holmgren, S. (1983). The effects of putative non-adrenergic, non-cholinergic autonomic transmitters on isolated strips from the stomach of the rainbow trout, *Salmo gairdneri*. *Comp. Biochem. Physiol.* **74**C, 229–238.

Holmgren, S., Grove, D. J. and Nilsson, S. (1985). Substance P acts by releasing 5-hydroxytryptamine from enteric neurons in the stomach of the rainbow trout, *Salmo gairdneri*. *Neuroscience* **14**, 683–693.

Holmgren, S. and Nilsson, S. (1981). On the non-adrenergic, non-cholinergic innervation of the rainbow trout stomach. *Comp. Biochem. Physiol.* **70**C, 65–69.

Jacobshagen, E. (1913). Untersuchungen über das Darmsystem der Fische und Dipnoer II. *Jena. Zeitschr.* **49**, 25–811.

Jensen, J., Axelsson, M. and Holmgren, S. (1991). Effects of substance P and vasoactive intestinal polypeptide on gastrointestinal blood flow in the Atlantic cod *Gadus morhua*. *J. Exp. Biol.* **156**, 361–374.

Jin, H. O., Lee, K. Y., Chang, T. M., Chey, W. Y. and Dubois, A. (1994). Physiological role of cholecystokinin on gastric emptying and acid output in dogs. *Digest. Diseases Sci.* **39**, 2306–2314

Jobling, M. (1987). Influences of food particle size and dietary energy content on patterns of gastric evacuation in fish: test of a physiological model of gastric emptying. *J. Fish Biol.* **30,** 299–314.

Karila, P. and Holmgren, S. (1996). Enteric reflexes and nitric oxide in the fish intestine. *J. Exp. Biol.* **198**, 2405–2411.

Kermani, R. Z. and Rezaiee, A. (1993). The effects of intravenous cholecystokinin, secretin and pentagastrin on electromyographic activity of the rumen in sheep. *Regul. Pept.* **45**, 371–377.

Liddle, R. A. (1989). Integrated actions of cholecystokinin on the

gastrointestinal tract: use of the cholecystokinin bioassay. *Gastroenterol. Clin. North America* **18**, 735–756.

Liddle, R. A., Morita, E. T., Conrad, C. K. and Williams, J. A. (1986). Regulation of gastric emptying in humans by cholecystokinin. *J. Clin. Invest.* **77**, 992–996.

Lin, C. W. and Miller, T. R. (1992). Both CCK-A and CCK-B/gastrin receptors are present on rabbit vagus nerve. *Am. J. Physiol.* **263**, R591–R595.

Martinez, V., Jimenez, M., Gonalons, E. and Vergara, P. (1993). Mechanism of action of CCK in avian gastroduodenal motility: evidence for nitric oxide involvement. *Am. J. Physiol.* **265**, G842–G850.

Medved, R. J. (1985). Gastric evacuation in the sandbar shark, *Carcharhinus plumbeus*. *J. Fish Biol.* **26**, 239–253.

Moran, T. H. and McHugh, P. R. (1982). Cholecystokinin suppresses food intake by inhibiting gastric emptying. *Am. J. Physiol.* **242**, R491–R497.

Mueller, L. R., Duke, G. E. and Evanson, O. A. (1990). Investigations of the migrating motor complex in domestic turkeys. *Am. J. Physiol.* **259**, G329–G333.

Nilsson, S. (1983). *Autonomic Nerve Function in the Vertebrates*. Berlin, Heidelberg, New York: Springer Verlag.

Nilsson, S. and Grove, D. J. (1974). Adrenergic and cholinergic innervation of the spleen of the cod: *Gadus morhua*. *Eur. J. Pharmac.* **28**, 135–143.

Olsson, C. and Holmgren, S. (1996). Involvement of nitric oxide in inhibitory innervation of urinary bladder of Atlantic cod, *Gadus morhua*. *Am. J. Physiol.* **270**, R1380–R1385.

Raybould, H. E., Roberts, M. E. and Dockray, G. J. (1987). Reflex decreases in intragastric pressure in response to cholecystokinin in rats. *Am. J. Physiol.* **253**, G165–G170.

Raybould, H. E. and Taché, Y. (1988). Cholecystokinin inhibits gastric motility and emptying *via* a capsaicin-sensitive vagal pathway in rats. *Am. J. Physiol.* **255**, G242–G246.

Roche, M., Descroix-Vagne, M., Benouali, S. and Chayvialle, J. A. (1993). Effect of some gastrointestinal hormones on motor and electrical activity of the digestive tract in the conscious cat. *Br. J. Nutr.* **69**, 371–384.

Schwartz, G. J., Berkow, G., McHugh, P. R. and Moran, T. H. (1993). Gastric branch vagotomy blocks nutrient and cholecystokinin-induced suppression of gastric emptying. *Am. J. Physiol.* **264**, R630–R637.

Schwartz, G. J., Moran, T. H., White, W. O. and Ladenheim, E. E. (1997). Relationships between gastric motility and gastric vagal afferent responses to CCK and GRP in rats differ. *Am. J. Physiol.* **272**, R1726–R1733.

Smith, G. P., Greenberg, D., Falasco, J. D., Avilion, A. A., Gibbs, J., Liddle, R. A. and Williams, J. A. (1989). Endogenous cholecystokinin does not decrease food intake or gastric emptying in fasted rats. *Am. J. Physiol.* **257**, R1462–R1466.

Smith, G. T., Moran., T. H., Coyle, J. T., Kuhar, M. J., O'Donahue, T. L. and McHugh, P. R. (1984). Anatomic localization of cholecystokinin receptors to the pyloric sphincter. *Am. J. Physiol.* **246**, R127–R130.

Solcia, E., Usellini, L., Buffa, R., Rindi, G., Villani, L., Aguzzi, A. and Silini, E. (1989). Endocrine cells producing regulatory peptides. In *Regulatory Peptides* (ed. J. M. Polak), pp. 220–246. Basel, Boston, Berlin: Birkhäuser Verlag.

Szurszewski, J. H. (1969). A migrating electric complex of the canine small intestine. *Am. J. Physiol.* **217**, 1757–1763.

Tani, S. and Muto, N. (1985). Effects of gastrointestinal hormones and their related compounds on gastric motility in the rat. *J. Pharmacobiodyn.* **8**, 793–799.

Windell, J. T. (1966). Rate of digestion in the bluegill sunfish. *Invest. Indiana Lakes Streams* **7**, 185–214.

Yamagishi, T. and Debas, H. T. (1978). Cholecystokinin inhibits gastric emptying by acting on both proximal stomach and pylorus. *Am. J. Physiol.* **234**, E375–E378.

Ziessman, H. A. (1992). Scintigraphy in the gastrointestinal tract. *Curr. Opin. Radiol.* **4**, 105–116.