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MECHANISMS CONTROLLING STOMACH VOLUME OF THE ATLANTIC COD (*GADUS MORHUA*) FOLLOWING GASTRIC DISTENSION

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Summary

1. Inflation of the cardiac stomach of the cod induced rhythmic contractions of the muscles and a slow increase in stomach volume towards a maximum. After deflation, the stomach remained relaxed and easily distensible for one or more hours. Section of the vagal tracts to the stomach did not change the response.

2. Inflation *in vitro* produced a somewhat faster relaxation and a much faster recovery to the pre-distended state than occurred *in vivo*. Stimulation of the cut ends of the vagus raised gastric tone and increased resistance to distension, an effect mediated by cholinergic nerves.

3. Tetrodotoxin and atropine relaxed the stomach so that distension was rapid and the maximal volume increased, revealing slower, possibly myogenic, contractions.

4. The 5-hydroxytryptamine (5-HT) antagonist methysergide, vasoactive intestinal polypeptide (VIP), met-enkephalin and neurotensin did not affect the responses to distension. Somatostatin abolished spontaneous contractions in the resting stomach and lowered gastric tone, but did not further affect the responses to distension.

5. In conclusion, cholinergic nerves maintain gastric tone in the cod. 5-HT neurones are absent in the cod stomach, and there are no indications of a 5-HT/VIP-controlled mechanism operating during distension. The effect of somatostatin differs from that in rainbow trout.

6. For comparison with trout and cod, responses to *in vivo* gastric distension are also described for the flatfish *Scophthalmus maximus*, *Scophthalmus rhombus*, *Limanda limanda* and *Pleuronectes platessa*.

Introduction

We have recently reported that distension of the cardiac stomach in the rainbow

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trout (*Oncorhynchus mykiss*), caused by inflating an intragastric balloon, led to a sustained increase in compliance of the gastric smooth muscles (Grove and Holmgren, 1992). The large decrease in stomach tone following distension appeared largely to be caused by changes in activity of excitatory neurones within the cardiac stomach wall itself. Reflex, rhythmic contractions (dependent on cholinergic and, to some extent, serotonergic nerves) were readily suppressed by somatostatin (SST). The overall tone of the muscle wall, which limited the rate of distension, depended primarily on activity in serotonergic nerves; vasoactive intestinal polypeptide (VIP) suppressed their effect.

The question remains whether similar processes are found in other fish. We elected to study *in vivo* and *in vitro* preparations of the stomach of the cod *Gadus morhua*, since, like the trout, some information on the functioning of its gastrointestinal system is already available (Nilsson and Fänge, 1969; Larsson and Rehfeld, 1977; Holstein, 1979, 1982, 1983; Holstein and Cederberg, 1984, 1986; Holmgren *et al.* 1985; Jensen and Holmgren, 1985; Jensen *et al.* 1987, 1991; Jönsson *et al.* 1987; Holmgren and Jönsson, 1988). A brief report is also included, for comparison with cod and trout, of *in vivo* responses to gastric distension obtained from four species of marine flatfish.

Materials and methods

The methods used were essentially those reported in our previous study on rainbow trout (Grove and Holmgren, 1992). Atlantic cod *Gadus morhua* (body mass 280–900 g) were held in the laboratory in recirculating, aerated sea water at 12 °C. In addition, several individuals of each of the following flatfish were held at 14 °C for preliminary examination: plaice (*Pleuronectes platessa*, 300–500 g), sand dab (*Limanda limanda*, 200–400 g), brill (*Scophthalmus rhombus*, 500–600 g) and turbot (*Scophthalmus maximus*, 500–700 g).

For *in vivo* study, fish were anaesthetized in MS222 (1:20 000) and held on an operating table whilst their gills were irrigated with anaesthetic in sea water. An intragastric balloon, connected to a movable, weighed water-filled reservoir, was inserted into the cardiac stomach by way of the mouth. Vagotomy in the cod was performed by cutting the intestinal branch of the vagus nerve on each side near the gills.

For *in vitro* study, the stomach and associated blood vessels were removed and mounted in an organ bath. Perfusion of the stomach with cod Ringer at constant pressure (Holmgren and Nilsson, 1974; Holmgren *et al.* 1985) allowed drugs to be tested, at known concentrations and during distension by the intragastric balloon, in the absence of extrinsic nerve reflexes. Electrical stimulation of the intestinal branches of the vagus nerve was undertaken using a Grass model S7 stimulator with bipolar platinum electrodes.

Calculations

Calculations were performed as described previously (Grove and Holmgren,

1992). When analyzing the rates of entry of fluid into the intragastric balloon after the hydrostatic pressure had been raised, the slower expansion in phase 2 has been treated as an exponential process between V_{res} and V_{max}. Distension rate is expressed as an instantaneous rate ($K\,min^{-1}$) to allow comparisons between treatments and with findings on the rainbow trout (Grove and Holmgren, 1992).

Immunohistochemistry

Immunohistochemical examination of tissues from the cod gastrointestinal tract to detect serotonergic nerves were made using the whole-mount technique of Costa *et al.* (1980). The stomach and intestine were cut open, pinned to dental wax and fixed for 18 h by floating in Zamboni's fixative (2 % formaldehyde and 15 % picric acid in $0.1\,mol\,l^{-1}$ phosphate buffer, pH 7.4), rinsed in 80 % ethanol, dehydrated, treated with xylene for 30 min and rehydrated. The samples were kept in phosphate-buffered saline (PBS, pH 7.4) until further processing (within 24 h). The tissues were peeled so that samples from submucosa, circular muscle and longitudinal muscle could be incubated with primary antiserum (serotonin antiserum 932, Immuno Nuclear Corporation, diluted 1:100) for 18 h. They were rinsed in PBS for 1 h and incubated with secondary antiserum (swine anti-rabbit IgG conjugated with fluorescein–isothiocyanate; DAKO Immunoglobulins a/s, Copenhagen, Denmark diluted 1:10) for 1.5 h. After rinsing in PBS for 1 h, the preparations were mounted in glycerol–carbonate buffer (1:1, pH 8.4) and viewed with a Leitz Dialux fluorescence microscope.

Sections of the gut were also examined. After fixation as above, the preparations were put in sodium cacodylate containing 20 % sucrose for at least 24 h, embedded in Tissue-Tek (Miles Scientific, Naperville, Illinois) and snap-frozen in liquid nitrogen. Sections (10 μm) were cut on a cryostat, collected on slides and incubated and treated as above.

Drugs

The drugs used in the present study were atropine sulphate (Sigma); phentolamine hydrochloride (Sigma); MS 222 (tricaine methane sulphonate, Sigma); met-enkephalin (Sigma); methysergide (1-methyl-lysergic acid butanolamine bimaleate, Sandoz); neurotensin (Sigma); synthetic somatostatin-14 (Sigma); tetrodotoxin (Sigma); natural porcine vasoactive intestinal polypeptide (VIP, V. Mutt, Stockholm).

Results

In vivo *studies*

In Fig. 1, the response of the cod cardiac stomach to inflation at low pressures is shown. Application of minimal hydrostatic pressure (0.2 kPa) was followed, as in the rainbow trout, by an immediate inflow (phase 1) of 2–3 ml of water (V_{res}). Thereafter, contractions of the muscles began and the stomach slowly relaxed (phase 2). Tests showed that rather greater pressures (0.4–0.5 kPa) were required

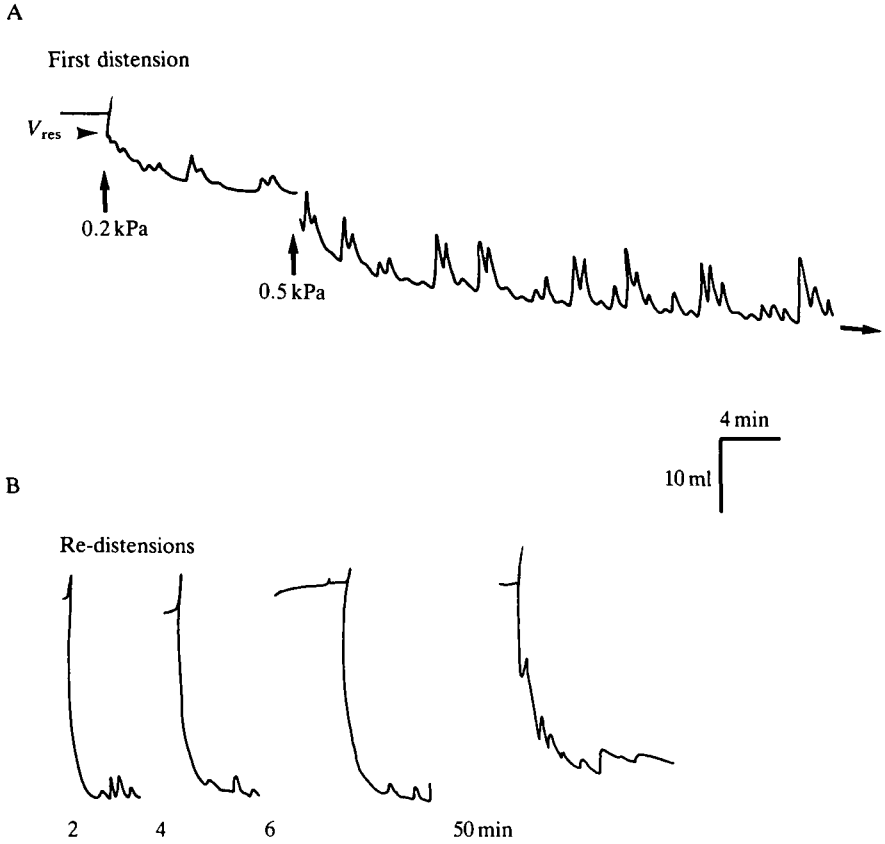

Fig. 1. *In vivo* responses of the cod stomach to distension. (A) Low inflation pressure (0.2 kPa) led to the inflow of a small volume of water (V_{res}, arrowhead) followed by the induction of contractions and a slow increase in volume towards maximal volume (V_{max}). Increasing the pressure to 0.5 kPa led to faster relaxation combined with increased reflex contractile activity. (B) Re-distensions showed that the stomach was fully compliant, despite long periods of deflation for recovery (up to 50 min), but that reflex contractions reappeared before the initial tone was restored. Numbers represent the deflation period (min).

to produce full distension, and these were used throughout subsequent studies. In Fig. 1, this pressure was applied after 14 min of phase 2. It was followed by an increase in both amplitude and frequency of muscular contractions and by the slow relaxation of the stomach towards its maximum volume (V_{max}). V_{max} was usually 6–7.5 ml per 100 g body mass, although occasional cod (2 out of 7) had lower cardiac stomach volumes than expected. The stomach volume may reflect the previous feeding history of the fish before study (Flowerdew and Grove, 1979; Treasurer, 1988).

Following the initial distension, the relaxation process in phase 2 took more than 30 min to complete. The instantaneous rate of relaxation (K) *in vivo* in phase 2 was 0.02–0.07 min^{-1}, values close to those described for the trout. Fig. 1 also shows, from experiments in which deflation of the stomach is followed by re-

inflation a few minutes later, that the stomach has become fully compliant; it accepts its maximum volume almost as fast as the balloon can inflate (K approaches values of 2.0–3.9 min^{-1}). Even after prolonged rest (several hours in some fish), when the stomach regains its ability to perform large contractions during phase 2, the relaxation rate remains rather high (K=0.4–0.6 min^{-1}). Three fish were subjected to vagotomy, but this operation did not affect the responses to gastric distension *in vivo*.

These results closely resemble those found previously for the rainbow trout. The main differences were that the volume accepted on first distension (V_{res}) during phase 1 was smaller in the cod (approximately 14–22 % of maximum stomach capacity, cf. 30 % in trout) and the cardiac stomach volume relative to body mass was higher (6–8 ml per 100 g, cf. 2–3 ml per 100 g in trout).

In vitro *studies*

The body masses of the 39 cod used in this part of the study ranged between 285 and 900 g. The mass of the stomach, and the volume it would accept at 0.5 kPa, varied unpredictably – as found *in vivo*. In general, the stomach, with its appendages and fat stores, formed approximately 3.3 % of the body mass. Eighteen of these fish had maximum cardiac volumes comparable to those found *in vivo* (i.e. 5.5–9 ml per 100 g), but the remaining fish had probably not been feeding prior to testing and their stomachs accepted smaller volumes, ranging from only 2.5 to 4.9 ml per 100 g.

Fig. 2A shows the response of the isolated, perfused cardiac stomach of the cod to distension. As expected, the previously undistended organ initially resisted the inflow of water. However, several changes were immediately clear when the *in vitro* preparations were compared with the *in vivo* preparations. Phase 1 was effectively abolished (V_{res} was close to zero). Subsequent relaxation during phase 2 was usually somewhat faster than found *in vivo* (K=0.1–0.2 min^{-1}). Furthermore, a delay of only 30 min between a first and second test revealed little sign that compliance had developed.

This preparation was then subjected to repeated distension with short, but varying, periods of rest interposed, during which the stomach was deflated (Fig. 2B). After previous inflation and a rest of only a few minutes (and unlike the response to the first inflation *in vitro*), phase 1 developed; up to 60 % of the maximum volume was accepted before reflex contractions limited inflow. Phase 2 is characterized by rapid, low-amplitude contractions (approximately 2–4 min^{-1}). With the passage of time, up to 15 min between deflation and re-inflation, the stomach quickly regained its ability to contract reflexly. It was clear in this and three other preparations (see, for example, Fig. 2C) that the *isolated* cod stomach retained its compliance after a previous distension for a much shorter period than was found in the cod *in vivo* or in the trout *in vivo* or *in vitro* (see Fig. 4 in Grove and Holmgren, 1992).

The development of compliance in the isolated cod stomach primarily involved a short-term suppression of reflex contractions. The large, up to ten-fold, increase

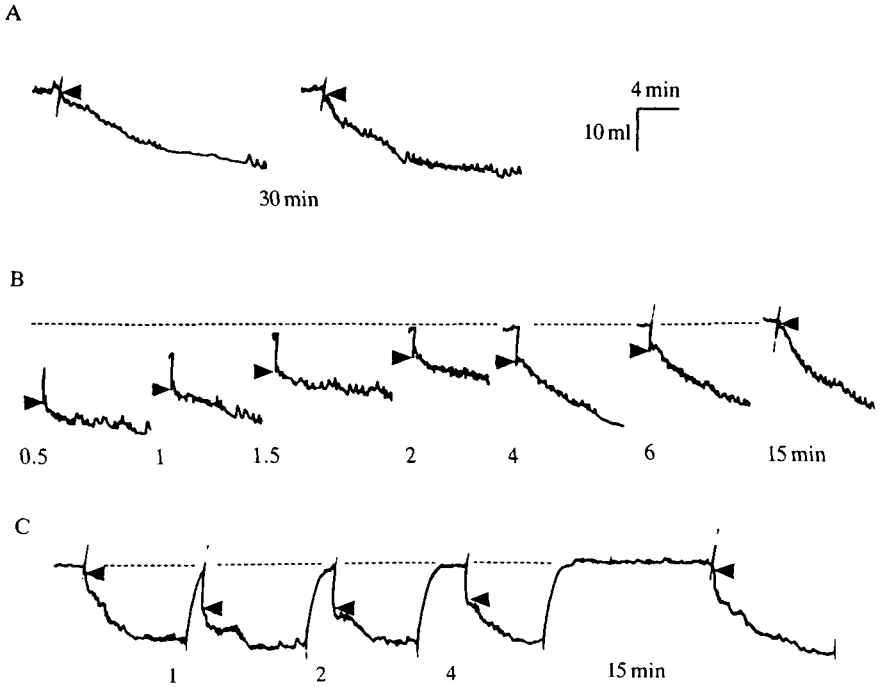

Fig. 2. *In vitro* responses of the perfused cod stomach to distension. (A) Tests made 30 min apart show little sign of induced compliance (cf. Fig. 1). When tests were made at shorter intervals (B), an increase in compliance is clearly seen. Rapid acceptance of fluid occurs (volume V_{res}, arrowhead) at first (phase 1) followed by induction of contractions and relaxation of the stomach (phase 2), especially after repeated distension. Return to the initial conditions (suppression of phase 1) occurs after only 15 min of resting. (C) A similar test in which relaxation rate during phase 2 was unchanged by repeated inflations. Broken lines indicate zero volume (the volume of the stomach on full recovery). Numbers indicate deflation period (min). Distension pressure: A, 1.5 kPa; B,C, 0.5 kPa.

in phase 2 relaxation rate (K) found *in vivo* no longer occurred. K rarely exceeded 0.4 min^{-1}. During the course of a number of our experiments, resistance of the stomach to distension slowly *increased*, a phenomenon never seen in the trout, or in the cod *in vivo*. In such preparations, after approximately 2–3 h, the maximum volume of the cardiac stomach was reduced by 25 %. Preparations showing this trend were not used in the drug tests described below.

Perfusion of the cod stomach (N=2) with tetrodotoxin (10^{-7}–10^{-6} mol l^{-1}) to impair the function of enteric neurones produced consistent but unexpected responses when compared to the trout. The resting stomach partially relaxed as the toxin was infused (Fig. 3), indicating that, prior to blockade with TTX, some excitatory nervous activity leading to raised muscle tone was present. This may explain the rather smaller maximum volume observed in many fish *in vitro* and the tendency for V_{max} to decrease with time in some of the preparations. On distension in the presence of TTX, a small increase (approximately 8–10 %) in

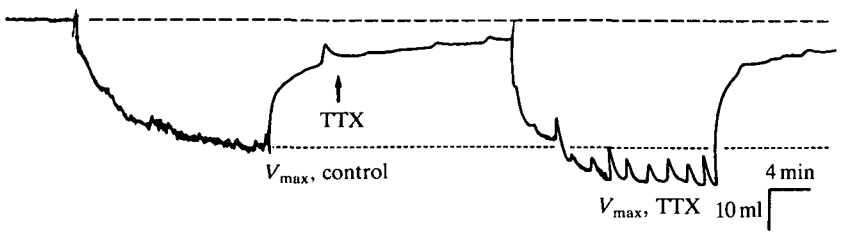

Fig. 3. *In vitro* infusion of tetrodotoxin (TTX, 10^{-6} mol l^{-1}) at the arrow relaxes the resting stomach. Distension now leads to a greater maximum volume (V_{max}, TTX) and some increase in relaxation rate. Low-frequency, higher-amplitude contractions after TTX may be myogenic. Distension pressure 0.5 kPa. The broken line indicates the volume of the undistended stomach; the lower dotted line indicates V_{max} in the untreated stomach.

V_{max} was found which, when added to the relaxation before distension was applied, amounted to a final volume some 25 % above that in the control period. Relaxation rate (K) in phase 2 increased in the TTX-treated preparation to reach values close to 0.7 min^{-1}. Unlike the situation in trout, spontaneous contractions during distension were not abolished. Instead, the original contractions were replaced by larger, low-frequency contractions (0.7–1 min^{-1}). These may represent myogenic contractions similar to those found in *Pleuronectes platessa* after treatment with the same toxin (Stevenson and Grove, 1977, 1978).

The major difference between our *in vivo* and *in vitro* cod preparations is the absence of extrinsic innervation. Distension of the cod stomach was therefore carried out whilst the intestinal branch of the vagus nerve was stimulated electrically. Stimulation with 1 ms pulses (Fig. 4) partially contracted the isolated stomach (raising the recorded baseline) without abolishing spontaneous contractions. The response to distension was somewhat reduced (Fig. 4A) or unaffected (Fig. 4B). We found no evidence for inhibitory tracts running in the vagus, using this type of stimulation, confirming the findings of Nilsson and Fänge (1969). Perfusion with atropine (10^{-6} mol l^{-1}) during stimulation had several effects. The baseline was returned to pre-stimulation levels, spontaneous contractions were abolished (both before and during distension) and relaxation rate (K) increased dramatically (from 0.4 to 1.7 min^{-1}, Fig. 4A). Results from similar preparations suggest that the excitatory fibres running in the vagus are cholinergic. Much of the resistance to distension in the isolated stomach, in the absence of extrinsic vagal stimulation, may also depend on reflex activation of excitatory cholinergic nerves in the gut wall.

To examine this hypothesis further, selected antagonistic drugs were perfused into the stomach vasculature in micromolar quantities to test their effects on the responses to distension. The adrenergic antagonist phentolamine (N=6) sometimes increased resistance to distension (Fig. 5A,D), usually had little effect (Fig. 5B,C) and occasionally contracted the relaxed stomach to raise the baseline

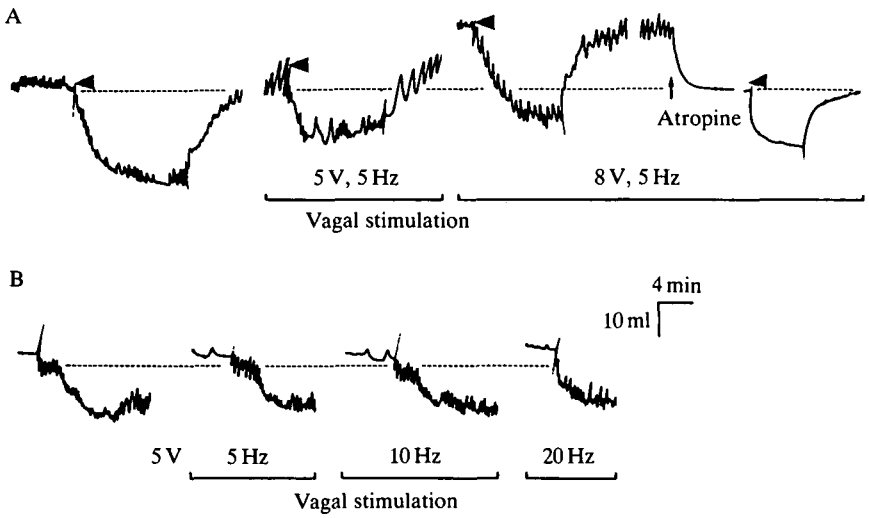

Fig. 4. Effects of *in vitro* electrical stimulation of the intestinal vagus nerve (1 ms pulses). (A) Stimulation at 5–8 V and 5 Hz increased resting muscle tone but did not affect spontaneous contractions. Distension following inflation was reduced. Atropine (10^{-6} mol l^{-1}) abolished both the increase in tone and all contractions. There was no evidence for increased compliance during vagal stimulation. (B) Stimulation at 5 V and 5, 10 or 20 Hz raised muscle tone but had no effect on compliance or spontaneous contractions. Arrowheads indicate stomach volume before distension, broken lines indicate volume before distension in the unstimulated stomach, distension pressure 0.5 kPa.

(Fig. 5D). The basis for these variable effects are not known. The serotonergic blocker methysergide (N=7), unlike its effects in the trout, where it relaxed the stomach, had little or no effect on the response to distension in the cod (Figs 5B, 6A,B). Atropine (N=7), however, had clear and consistent effects. It abolished increased gastric tone when this was raised by phentolamine (Fig. 5D). It typically increased relaxation rate during phase 2 of distension (Figs 5C, 6A–D), although in one preparation (Fig. 5D) it failed to overcome the effects of phentolamine. In addition, atropine abolished spontaneous contractions, immediately raised distended stomach volume to its maximum (V_{max}) and dramatically increased relaxation rate (K) to values (1–2 min^{-1}) comparable to those found in the fully compliant stomach *in vivo*.

These results, especially those following methysergide, strengthen our belief that gastric tone and reflex contractions in the cod are maintained primarily by activity in intramural cholinergic excitatory nerves alone. Unlike the situation in trout, serotonergic nerves play an insignificant part in maintaining the tone and resistance to stretch of the cardiac stomach smooth muscle. Serotonergic intramural neurones have been reported from the cod intestine (Jensen and Holmgren, 1985), but studies on the cod stomach have not been made previously. Immuno-

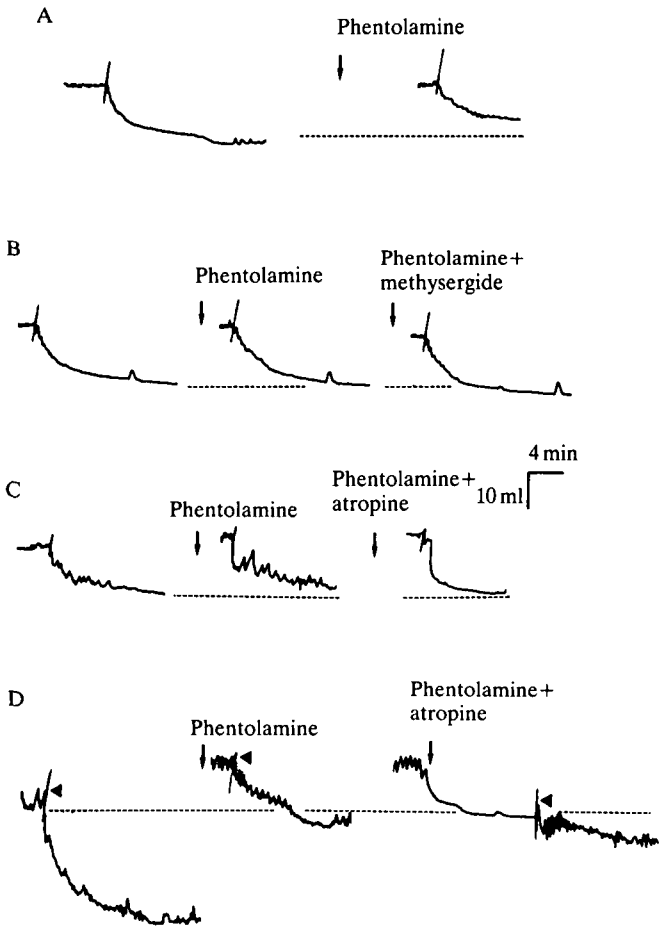

Fig. 5. Phentolamine (10^{-6} mol l^{-1}, Ph) decreased compliance (A), was without effect (B,C) or raised tone as well as decreasing compliance (D). Methysergide (10^{-6} mol l^{-1}) was ineffective (B). Atropine (10^{-6} mol l^{-1}) usually abolished spontaneous contractions and increased relaxation rate (C), except in one case following phentolamine treatment (D). *In vitro*, distension pressure 0.5 kPa. Arrowheads indicate V_{res}, broken lines indicate V_{max} on first distension (A,B,C) or volume before first distension (D).

histochemical examination of the cardiac stomach of the cod (which is the part recorded from in the present study) failed to reveal the presence of serotonergic neurones, while a few varicose fibres were observed in the submucosa of the pyloric part. However, fibres frequently occurred in the intestine, particularly in the submucosa and the myenteric plexus, confirming previous results (Jensen and Holmgren, 1985). This finding is consistent with our conclusions from drug treatment, that serotonergic nerves are not involved in the control of the smooth muscle of the cardiac stomach of the cod. Accordingly, the great increase in compliance that occurs after distension *in vivo* in the cod (Fig. 1) requires the intervention of an agent that suppresses cholinergic activity.

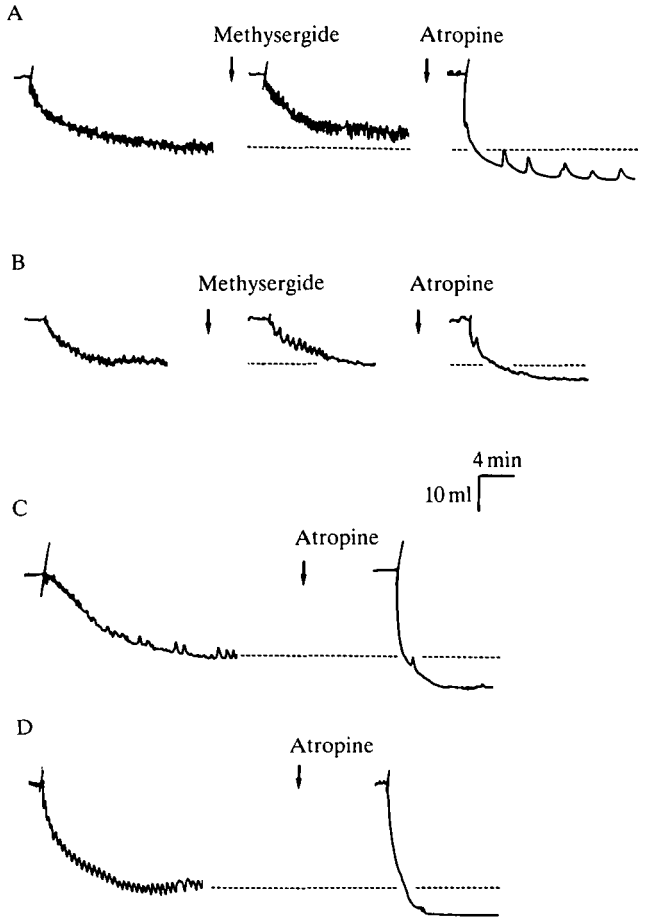

Fig. 6. Methysergide (10^{-6} mol l^{-1}) was ineffective (A,B) but atropine (10^{-6} mol l^{-1}) regularly increased compliance (A–D), seen as an increase in both relaxation rate and V_{max}, to approach levels seen in compliant stomachs *in vivo* (cf. Fig. 1). In A, contractions similar to those following TTX treatment (Fig. 3) are unmasked. *In vitro*, distension pressure 0.5 kPa, broken lines indicate V_{max} on first distension.

In the trout, vasoactive intestinal polypeptide (VIP) proved to be a powerful stomach relaxant, but its action appeared to be directed primarily towards suppression of the effect of intramural, serotonergic nerve activity, which maintains gastric muscle tone. In the cod stomach, where serotonergic mechanisms seem to be weak or absent, perfusion with VIP (10^{-9}–10^{-7} mol l^{-1}, N=3) had little detectable effect on the response to distension (Fig. 7A). Negative results were also obtained following perfusion with met-enkephalin (10^{-9}–10^{-7} mol l^{-1}, N=3, Fig. 7B) or neurotensin (10^{-11}–10^{-7} mol l^{-1}, N=4, Fig. 7C). These two peptides are known to have inhibitory effects on gastric motility in mammals (Hellström *et al.* 1982; Garzon *et al.* 1987; Okamoto *et al.* 1988).

Cholinergic intramural neurones in the trout were primarily associated with the

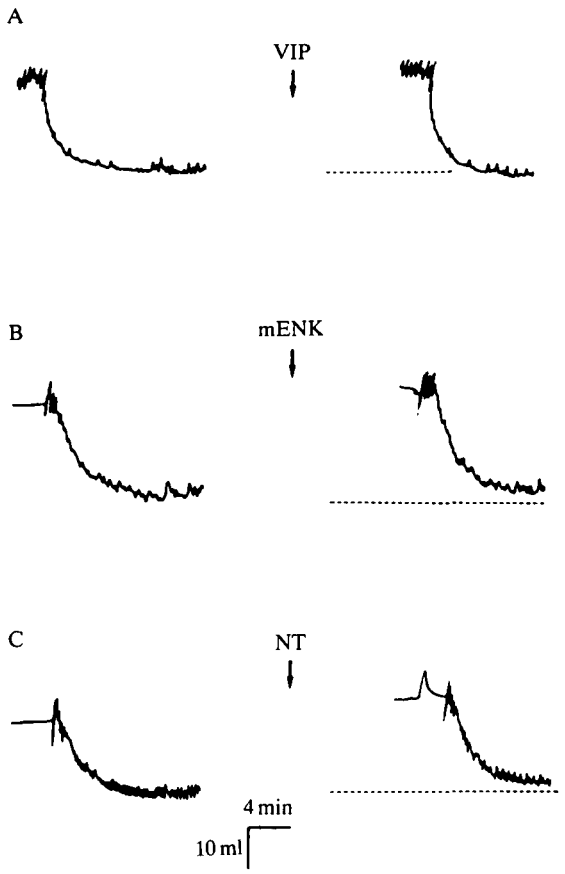

Fig. 7. *In vitro* perfusion with vasoactive intestinal polypeptide (10^{-7} mol l^{-1}, VIP), met-enkephalin (10^{-7} mol l^{-1}, mENK) or neurotensin (10^{-7} mol l^{-1}, NT) failed to affect the response to distension. Distension pressure 0.5 kPa, broken lines indicate V_{max} before treatment.

control of reflex contractions following distension, even as gastric tone fell. This reflex recovers relatively quickly after distension but was readily suppressed by somatostatin (SST). Similar reflexes occur in the cod (Fig. 2) so the effects of this peptide (10^{-9}–10^{-7} mol l^{-1}, N=3) were also tested (Fig. 8). Somatostatin (10^{-7} mol l^{-1}) abolished spontaneous contractions in the resting stomach and decreased muscular tone (cf. the effect of tetrodotoxin). It did not, however, abolish spontaneous contractions following distension or increase compliance.

In general, the studies on isolated cod stomachs suggest that removal from extrinsic control (whether nervous or endocrine) prevents the long-term relaxation/inhibition that follows distension.

Other fish

To test whether other species, besides rainbow trout and cod, also show increased compliance after distension, we made a preliminary examination of the

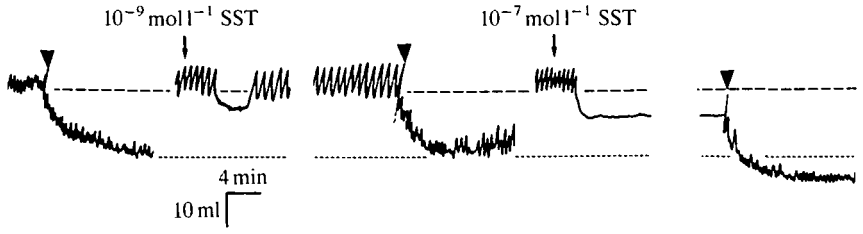

Fig. 8. *In vitro* perfusion with somatostatin (10^{-9} or 10^{-7} mol l^{-1}, SST) abolished spontaneous contractions of the resting, perfused cod stomach and, at higher doses, reduced gastric tone (cf. TTX, Fig. 3). It had no effect on the pattern following distension. The upper broken line indicates the volume of the resting stomach, the lower dashed line indicates V_{max} on the first distension. Distension pressure 0.5 kPa. Arrowheads indicate start of distension.

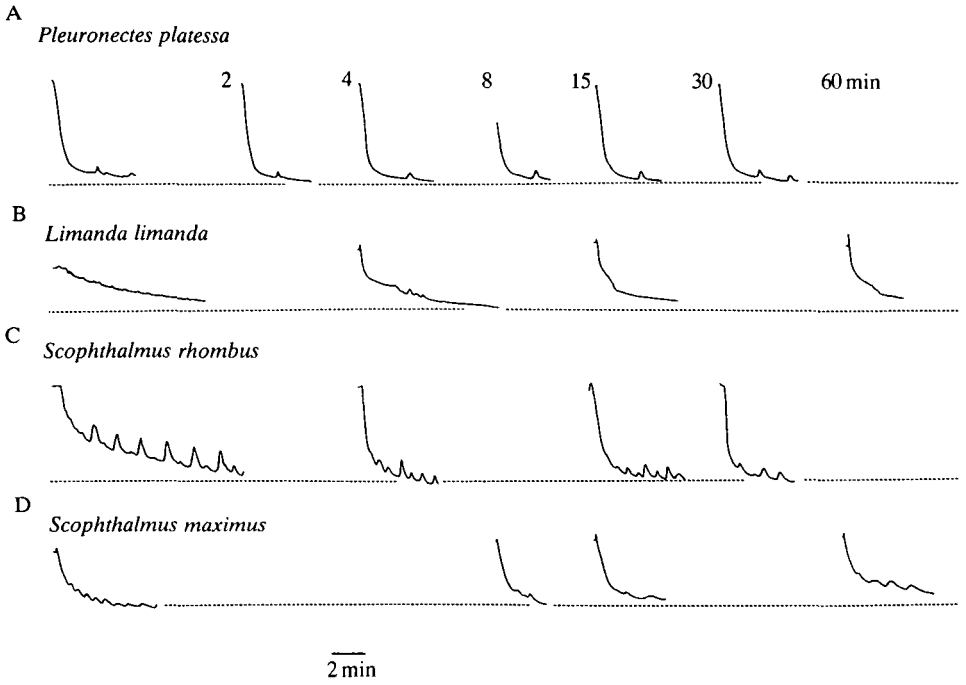

Fig. 9. *In vivo* responses of flatfish to gastric distension. Horizontal broken lines indicate V_{max} in fully compliant preparations. Numbers indicate deflation time (min) between distensions, distension pressure 0.5 kPa, 14°C.

in vivo responses of four marine teleosts to gastric distension. All were flatfish, but they differed to some extent in feeding habits and in the relative size of their stomachs. The plaice (*Pleuronectes platessa*) has a relatively small stomach (approximately 3–4 ml per 100 g body mass). Its natural diet primarily includes small molluscs and polychaete worms, although larger shrimps are taken as its size reaches 1.5 kg. The records show (Fig. 9A) that, when distended at pressures of

0.5 kPa, regardless of any previous stimulation, the stomach always distends rapidly and with little resistance (K=2.5–3.5 min^{-1}). The results resemble those from fully compliant stomachs of other species. The plaice, however, is known to have a reduced stomach, part of an evolutionary trend that has led to loss of the stomach in some of the other Pleuronectidae (Grove and Campbell, 1979). Its close relative *Limanda limanda* has a larger stomach (approximately 8 ml per 100 g body mass) and ingests a wider range of forage species, including shrimps, crabs, molluscs, worms and brittle-stars. Fig. 9B clearly shows that, after resistance during the initial distension, compliance develops and lasts for at least 1 h (K increases from 0.3 to 1.5 min^{-1}). Two members of the Bothidae, *Scophthalmus rhombus* and *S. maximus*, were similarly tested. These fish consume fish as a major part of their diet and have even larger stomachs (8–10 ml per 100 g body mass). In both species (Fig. 9C,D), repeated stimulation increased relaxation rate from initial values of approximately 0.6 min^{-1} to rates usually three times higher; these persisted for at least 30 min. It appears that the responses to increased gastric compliance following distension in cod and trout are also found in other fish that possess well-developed stomachs.

Discussion

The cod is an active predator in the sea, consuming a variety of relatively large organisms, including crustaceans, molluscs and fish (Daan, 1973). Compared with the rainbow trout, it has a relatively large stomach, eats larger organisms and can feed to satiation within a few minutes, rather than over a period of approximately 1 h, when suitable food is available. Stomach distension *in vivo*, to mimic the arrival of food, leads to a profound and long-lasting increase in compliance similar to that found in rainbow trout.

However, several major differences have been established between these two species as a result of our studies. The trout depends on intramural serotonergic neurones to maintain gastric tone, whilst both serotonergic and cholinergic nerves are involved in rhythmic, reflex contractions induced by distension. In isolated stomachs, VIP opposes serotonergic nerve effects whilst SST suppresses cholinergic activity. The cod lacks the serotonergic mechanism and VIP has no effect on the isolated stomach. Instead, responses to stretch mainly depend on the reflex activation of cholinergic nerves and (after prolonged distension *in vivo*) the suppression of their activity. SST failed to suppress contractile activity following distension, although spontaneous contractions of the undistended stomach were abolished and its tone reduced.

It is important to bear in mind that the cod and trout are widely separated in teleost taxonomy. Failure of the cod to react to a mammalian peptide (such as VIP) might mean that its equivalent peptide is different in primary structure from that infused, and that its receptors reflect such changes. However, the structure of cod VIP has been described (Thwaites *et al.* 1989) and it shows little difference from mammalian and avian VIP molecules in a variety of assay systems.

The isolated, perfused trout stomach reacts in a similar fashion to recordings made *in vivo*, under anaesthesia. However, the isolated cod stomach, unlike the *in vivo* preparation, loses compliance rapidly when allowed to deflate. This suggests that factors extrinsic to the cod stomach are essential to promote prolonged receptive relaxation. These factors may be humoral or may involve the splanchnic nerves.

In neither the anaesthetized cod nor trout were we able to demonstrate changes in gastric reactions following vagotomy. It may be inappropriate to conclude that extrinsic nervous reflexes, similar to those found in mammals (Cannon and Lieb, 1911; Abrahamsson, 1973; Abrahamsson and Jansson, 1973), play no part in gastric receptive relaxation or accommodation in these fish. Instead, clarification of possible extrinsic reflexes, involving vagal and splanchnic pathways, should involve studies of gastric motility in conscious, actively feeding fish (e.g. Edwards, 1973) rather than tests in the presence of anaesthetic. There is currently a rapid expansion in the number of neurocrine and endocrine substances known to be present in fish and that are likely to affect gastrointestinal muscles. Full understanding of the coordination of stomach motility, and receptive relaxation, during feeding and digestion will depend on such developments. The factor that promotes prolonged gastric relaxation in the intact cod (Fig. 1) remains to be found.

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References

ABRAHAMSSON, H. (1973). Studies on the inhibitory nervous control of gastric motility. *Acta Physiol. Scand.* (Suppl.) **390**, 1–38.

ABRAHAMSSON, H. AND JANSSON, G. (1973). Vago-vagal gastro-gastric relaxation in the cat. *Acta physiol. scand.* **88**, 289–295.

CANNON, W. B. AND LIEB, C. W. (1911). The receptive relaxation of the stomach. *Am. J. Physiol.* **29**, 267–273.

COSTA, M., BUFFA, R., FURNESS, J. B. AND SOLCIA, E. (1980). Immunohistochemical localization of polypeptides in peripheral autonomic nerves using whole mount preparations. *Histochemistry* **65**, 157–169.

DAAN, N. (1973). A quantitative analysis of the food intake of North Sea cod (*Gadus morhua*). *Neth. J. Sea Res.* **6**, 479–517.

EDWARDS, D. J. (1973). The effect of drugs and nerve sectioning on the rate of passage of food through the gut of the plaice, *Pleuronectes platessa* L. *J. Fish Biol.* **5**, 441–446.

FLOWERDEW, M. AND GROVE, D. J. (1979). Some observations of the effects of body weight, temperature, meal size and quality on gastric emptying time in the turbot, *Scophthalmus maximus* (L). *J. Fish Biol.* **14**, 229–238.

GARZON, J., HOLLT, V. AND HERZ, A. (1987). Cholecystokinin octapeptide activates an opioid mechanism in the guinea pig ileum: a possible role for substance P. *Eur. J. Pharmac.* **136**, 361–370.

GROVE, D. J. AND CAMPBELL, C. (1979). The role of extrinsic and intrinsic nerves in the coordination of gut motility in the stomachless flatfish *Rhombosolea tapirina* and *Ammotretis rostrata* Guenther. *Comp. Biochem. Physiol.* **63**C, 143–159.

GROVE, D. J. AND HOLMGREN, S. (1992). Intrinsic mechanisms controlling cardiac stomach

volume of the rainbow trout (*Oncorhynchus mykiss*) following gastric distension. *J. exp. Biol.* **163**, 33–48.

HELLSTRÖM, M. P. M., NYLANDER, G. AND ROSELL, S. (1982). Effects of neurotensin on the transit of gastrointestinal contents in the rat. *Acta physiol. scand.* **115**, 239–243.

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*. *Neurosci* **14**, 683–693.

HOLMGREN, S. AND JÖNSSON, A. C. (1988). Occurrence and effects on motility of bombesin-related peptides in the gastrointestinal tract of the Atlantic cod, *Gadus morhua*. *Comp. Biochem. Physiol.* **89**C, 249–256.

HOLMGREN, S. AND NILSSON, S. (1974). Drug effects on isolated artery strips from two teleosts, *Gadus morhua* and *Salmo gairdneri*. *Acta physiol. scand.* **90**, 431–437.

HOLSTEIN, B. (1979). Gastric acid secretion and water balance in the marine teleost *Gadus morhua*. *Acta physiol. scand.* **105**, 93–107.

HOLSTEIN, B. (1982). Inhibition of gastric acid secretion in the Atlantic cod, *Gadus morhua*, by sulphated and desulphated gastrin, caerulein, and CCK-octapeptide. *Acta physiol. scand.* **114**, 453–459.

HOLSTEIN, B. (1983). Effect of vasoactive intestinal polypeptide on gastric acid secretion and mucosal blood flow in the Atlantic cod, *Gadus morhua*. *Gen. comp. Endocr.* **52**, 471–473.

HOLSTEIN, B. AND CEDERBERG, C. (1984). Effects of 5-HT on basal and stimulated secretions of acid and pepsin and on gastric volume outflow in the *in vivo* gastrically and intestinally perfused cod, *Gadus morhua*. *Agents Actions* **15**, 290–305.

HOLSTEIN, B. AND CEDERBERG, C. (1986). Effects of tachykinins on gastric acid and pepsin secretion and on gastric outflow in the Atlantic cod, *Gadus morhua*. *Am. J. Physiol.* **250**, G309–G315.

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**, 361–373.

JENSEN, J. AND HOLMGREN, S. (1985). Neurotransmitters in the intestine of the Atlantic cod, *Gadus morhua*. *Comp. Biochem. Physiol.* **82**C, 81–89.

JENSEN, J. AND HOLMGREN, S. (1991). Release of substance P-like immunoreactive material from the stomach of the rainbow trout. *J. comp. Physiol.* (in press).

JENSEN, J., HOLMGREN, S. AND JÖNSSON, A. C. (1987). Substance P-like immunoreactivity and the effects of tachykinins in the intestine of the Atlantic cod, *Gadus morhua*. *J. Autonomic Nervous System* **20**, 25–33.

JÖNSSON, A. C., HOLMGREN, A. C. AND HOLSTEIN, B. (1987). Gastrin/CCK-like immunoreactivity in endocrine cells and nerves in the gastrointestinal tract of the cod, *Gadus morhua*, and the effect of peptides of the gastrin/CCK family on cod gastrointestinal smooth muscle. *Gen. comp. Endocr.* **66**, 190–202.

LARSSON, L.-I. AND REHFELD, J. F. (1977). Evidence for a common evolutionary origin of gastrin and cholecystokinin. *Nature* **269**, 335–338.

NILSSON, S. AND FÄNGE, R. (1969). Adrenergic and cholinergic vagal effects on the stomach of a teleost (*Gadus morhua*). *Comp. Biochem. Physiol.* **30**, 691–694.

OKAMOTO, T., KURAHASHI, K. AND FUJIWARA, M. (1988). Effects of naloxone and opioid agonists on gastric excitatory responses to stimulation of the vagus nerve in cats. *Br. J. Pharmac.* **95**, 329–334.

STEVENSON, S. V. AND GROVE, D. J. (1977). The extrinsic innervation of the stomach of the plaice, *Pleuronectes platessa* L. I. The vagal nerve supply. *Comp. Biochem. Physiol.* **58**C, 143–151.

STEVENSON, S. V. AND GROVE, D. J. (1978). The extrinsic innervation of the stomach of the plaice, *Pleuronectes platessa* L. II. The splanchnic nerve supply. *Comp. Biochem. Physiol.* **60**C, 45–50.

THWAITES, D. T., YOUNG, J., THORNDYKE, M. C. AND DIMALINE, R. (1989). The isolation and chemical characterization of a novel vasoactive intestinal peptide-related peptide from a teleost fish, the cod, *Gadus morhua*. *Biochim. biophys. Acta* **999**, 217–220.

TREASURER, J. W. (1988). Measurement of regurgitation in feeding studies of predatory fishes. *J. Fish Biol.* **33**, 267–271.