J. Physiol. (1984), **354**, *pp.* 477–495
With 11 *text-figures*
Printed in Great Britain

THE RESPONSES OF DUODENAL TENSION RECEPTORS IN SHEEP TO PENTAGASTRIN, CHOLECYSTOKININ AND SOME OTHER DRUGS

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(*Received 20 September 1983*)

SUMMARY

1. The proposal that some post-prandially released alimentary hormones modify ingestive behaviour and gastric emptying by altering impulse activity in alimentary enteroceptors has been tested using a number of gastrointestinal peptide hormone analogues. These and other drugs were applied to single-unit afferent preparations of duodenal tension receptors in chloralose-anaesthetized sheep. In separate experiments the effect of pentagastrin and cholecystokinin on duodenal motor activity was recorded without unitary afferent activity measurements.

2. Local intra-arterial bolus injections of pentagastrin, cholecystokinin, insulin, prostaglandin, acetylcholine, phenylbiguanide, veratrine, 5-hydroxytryptamine and bradykinin aroused or enhanced activity in tension receptors. With the exception of a short-latency effect of insulin B.P., these responses occurred together with local increases in tension and electromyographic activity of the duodenum. Combinations of atropine and hexamethonium reduced duodenal motor activity and abolished most drug-evoked afferent responses. Intracarotid bolus injections of pentagastrin at first increased, then reduced duodenal tension, electromyographic activity and impulse activity of tension receptors. Cholecystokinin (CCK-8) injected by this route caused similar alterations of these parameters, and the response was characterized by periods of reduced activity followed by a prolonged excitation of duodenal motility.

3. From the responses to bolus injections of humoral agents it is concluded that some alimentary hormones released after a meal may have a peripheral excitatory action on the tension receptor environment which causes increased afferent activity. The mechanism probably involves both an alteration in duodenal motility and a sensitization of the receptor ending. In addition, the peptide hormones gastrin and cholecystokinin may act centrally to alter duodenal motor control and thus may influence gastric emptying and post-prandial satiety mechanisms.

INTRODUCTION

Gastrointestinal peptides exist in a variety of locations including mucosal cells (Nilsson, Yalow & Berson, 1973; Buffa, Solcia & Go, 1976; Bunnett & Harrison, 1979), mesenteric ganglia (Larsson & Rehfeld, 1979), pancreatic cells (Grube, Maier, Raptis & Schlegel, 1978), central nervous tissue (Dockray, Gregory, Hutchison, Harris &

Runswick, 1978) and afferent axons in vagus and splanchnic nerves (Lundberg, Hokfelt, Nilsson, Terenius, Rehfeld, Elde & Said, 1978; Dockray, Gregory, Tracy & Zhu, 1981). They are released post-prandially during the digestive process and, in addition to their role in secretory responses, may play a role as hormonal factors in regulating gastrointestinal motility. In the periphery gastrin may regulate the frequency of occurrence of intestinal contraction by directly affecting the smooth muscle membrane (Szurszewski, 1975); gastrin and cholecystokinin (CCK)-like peptides may release acetylcholine from the intramural plexus (Vizi, Bertaccini, Impiccatore & Knoll, 1973) which may determine the strength of contraction (Szurszewski, 1975). Peptides may act as alimentary neurotransmitters (Hokfelt, Johansson, Ljungdahl, Lundberg & Schultzberg, 1980) and may be involved in sensory transduction processes (Lundberg *et al.* 1978; Burnstock, 1979).

These peptides may also have central actions. Pentagastrin reduces the rate of reticular contraction in sheep by a central mechanism (Chapman, Grovum & Newhook, 1979; Grovum & Chapman, 1982; Grovum & Leek, 1982; Nicholson, 1982). Exogenously administered peptides cholecystokinin and pentagastrin depress food intake in most species studied. CCK depresses food intake in rats (Gibbs, Young & Smith, 1973*a*, *b*; Anika, Houpt & Houpt, 1977; Maddison, 1977; Gibbs & Smith, 1977; Kraly, Carty, Resnick & Smith, 1978; Nemeroff, Osbahr, Bissette, Jahnke, Lipton & Prange, 1978), monkeys (Gibbs & Smith, 1977) and sheep (Symons, 1978; Della Fera & Baile, 1979; Grovum, 1981). In man, CCK has either no effect (Glick, Thomas & Mayer, 1971; Greenway & Bray, 1977) or either increases or decreases appetite in a dose-dependent way (Sturdevant & Goetz, 1976). In sheep, pentagastrin depresses food intake (Grovum, Brobeck & Baile, 1979; Grovum, 1981).

The experiments reported here were designed to investigate the effect of exogenously administered gastrointestinal peptide analogues, and some other drugs, on the behaviour of duodenal tension receptors in sheep. The activity of tension receptors was examined together with tension measurements and electromyographs of the duodenal muscularis externa.

METHODS

Adult sheep weighing 40–60 kg were anaesthetized with 1% chloralose. The sheep duodenal preparation (Cottrell & Iggo, 1984*a*) with both open- and closed-loop duodenal preparations was used. The cerebral blood supply was kept intact and the femoral artery was used for monitoring systemic blood pressure. Bolus injections of drugs were made through catheters located in the following vessels: the right gastro-epiploic artery, a small artery joining the left carotid artery and the saphenous femoral vein. During the administration of drugs the following parameters were recorded: afferent activity, muscle tension in both longitudinal and circular (tangential) directions and local electromyographs. The rate of contraction, the amplitude of tension changes and the tone (defined as a maintained change in the base-line tension lasting more than 30 s) in the longitudinal and circular (tangential) direction were measured.

Drugs

Drugs were prepared in solutions of normal saline. Bolus injections were given at rates of 0·1 ml s^{-1} of 2–5 ml volumes. Drugs were tested at concentrations of $\times 1$, $\times 2$, $\times \frac{1}{2}$, $\times \frac{1}{4}$ and $\times 4$ with control flushing with normal saline between trials. They were selected from those known to cause changes in enteroceptor activity (Paintal, 1964; Daniel, 1968) and which alter alimentary motor activity (Bennet, 1972) and include some of the gastrointestinal hormone analogues which modify muscle tonus (Grossman and others, 1974). The following drugs were used on sheep of average weight 50 kg:

pentagastrin ($2{\cdot}5 \times 10^{-3}$–1 μg kg^{-1}, Peptavlon, ICI), cholecystokinin (CCK-8, 5×10^{-3}–5 μg, Squibb and Son Ltd.; CCK-33, 0·05–5 μg; CCK-39, 0·05–5 μg), insulin B.P. (0·1–5·0 u.), prostaglandins E_2 and $F_{2\alpha}$ (50 μg kg^{-1}), noradrenaline acid tartrate (1–50 μg), adrenaline (0·25 μg), acetylcholine chloride (1–50 μg), 5-hydroxytryptamine creatinine sulphate (20–50 μg), bradykinin acetate (1–25 μg; Cambridge Research Biochemicals Ltd.), phenylbiguanide (0·1–2 μg kg^{-1}, 94% by wt., Aldrich Chemical Co., U.S.A.), veratrine (1–100 μg, BDH), atropine sulphate (10–200 μg), hexamethonium bromide (10–100 μg).

Chemicals

The responses to intravascular perfusion with selected chemical solutions was also tested in vehicle volumes of 5 and 10 ml, rate 0·2 ml s^{-1}. These included glucose saline (50–1500 mM), lactic acid (50 mM), acetic acid (50 mM) and ammonium chloride (28–37 mM).

RESULTS

Sixty-three tension receptors were tested whose impulse activity changed to a wide range of selected drugs. The character of the drug-evoked change in impulse activity was either of short duration (phasic) or recurred intermittently (periodic) for durations of up to 10 min. After close intra-arterial injection some units were excited within 2 s. Other units had a delayed excitation whose latency exceeded 10 s. When intravenous injections were used the latency of the onset of excitation could usually be accounted for by the circulation time required for the drugs to reach the receptive field. Occasionally prolonged desensitization of a unit to a previously effective stimulus occurred. Intra-carotid injections of CCK and pentagastrin were used in an attempt to detect whether these peptides were centrally or peripherally active. The possible site of action may be deduced from measurements of the latency of the response after administration by different routes as well as from altered sensitivity of the response to different doses.

Pentagastrin

A bolus intravenous injection of pentagastrin (0·05–1·0 μg kg^{-1}) caused an increase in discharge frequency in ten of ten spontaneously active units, or caused a *de novo* discharge in six of six previously silent tension units. The peak frequencies were in the range 55–71 impulses s^{-1}. Quantities below 0·05 μg kg^{-1} gave inconsistent responses by this route. These increases in impulse activity occurred together with enhanced electromyogram (e.m.g.) and tension activity of the duodenal loop which commenced 13–25 s after the intravenous injection.

Similar doses close intra-arterially were effective in exciting units in six cases in eight trials with shorter latency (5 s). Increases in contraction amplitude were less vigorous than intravenously evoked responses and peak frequencies were reduced by 50%. Following intravenous and close intra-arterial routes of administration the receptor discharge, e.m.g. and the movement of the duodenum was depressed for 4–30 min following the initial period of excitation. Intracarotid injection in seven out of thirteen receptors caused enhanced impulse activity and increased duodenal movement after latencies of more than 10 s. An increased contraction of pyloric muscle occurred with shorter latency than duodenal muscle. After a period of increased duodenal contraction lasting 4–10 min there was a reduction of movement for 2–5 min (Fig. 2).

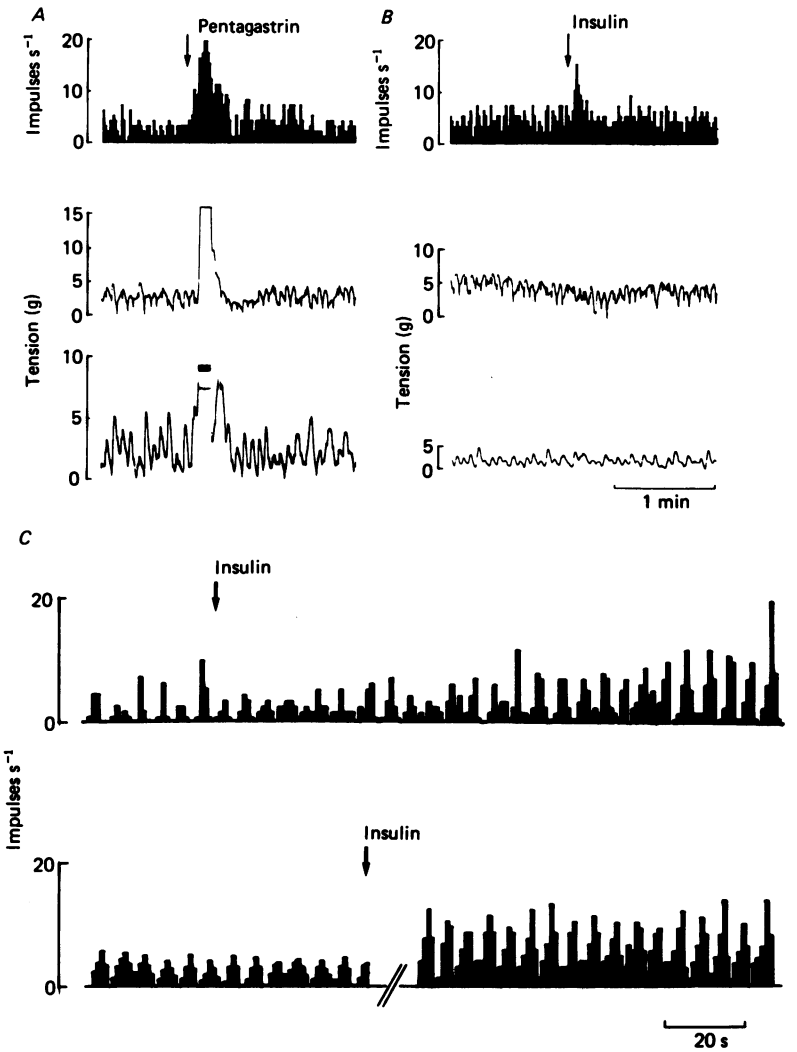

Fig. 1. Comparison of the responses of a sheep duodenal tension receptor to close intra-arterial pentagastrin (0·6 μg kg^{-1}) (*A*) with close intra-arterial insulin B.P. (1 u.) (*B*). There is no change in tension in the circular (middle trace) or longitudinal (lower trace) direction during the response to insulin. *C* illustrates the effect of systemic intravenous injections of insulin B.P. (0·025 u. kg^{-1}) (arrows) on two further tension receptors. In the upper trace there is a gradual increase in impulse activity so that after 2 min the discharge frequency has increased by 100 %. In the lower trace the record was interrupted for 2 min after insulin was injected, during which time there was a gradual increase in frequency. No detectable tension change was recorded in the tissue when control injections of saline solution were injected using the same route and rates.

In one preparation simultaneous recordings were made of two units with bursting discharges characteristic of tension receptors. They each responded differently to pentagastrin administration. Units had asynchronous periodic discharge at frequencies which suggested separate locations. One was discharging at a rate of 10 bursts min^{-1} and the other at 4 bursts min^{-1}. Their compression receptive fields were located at

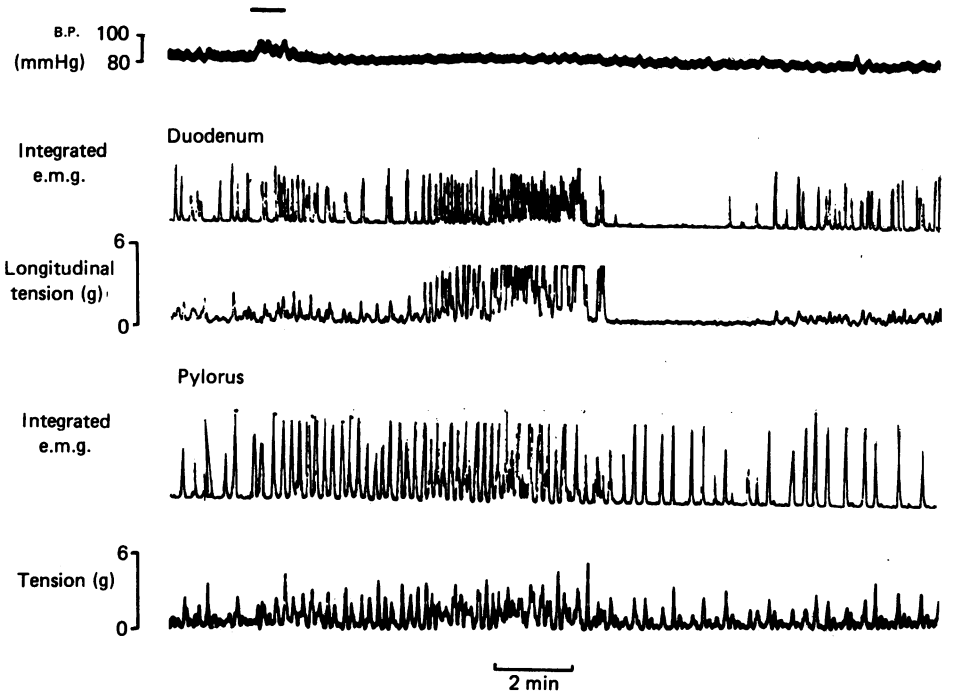

Fig. 2. The effect of intracarotid pentagastrin on tension and e.m.g. activity of duodenal and pyloric muscle. At the bar a 10 μg bolus of pentagastrin was injected. Note the pressor effect, which coincided with the injection (upper trace). There was enhanced contraction of the duodenum after a 5 min delay which lasted for a further 5 min (second trace, integrated e.m.g.; third trace, longitudinal tension). Pyloric contraction was enhanced for a period of 10 min after the injection and was reduced during the 5 min of duodenal inactivity (fourth trace, pyloric e.m.g.; lower trace, pyloric longitudinal tension). The initial enhanced e.m.g. activity in the duodenal record was observed after bolus injections of 10 μg pentagastrin. After doses of 0·1–1·0 μg this period of activity was either unchanged or was replaced by slightly reduced activity for 2 min until the later enhanced response developed. Traces retouched for clarity.

the pylorus. Following the intra-arterial administration of pentagastrin their bursting activity became synchronous after an initial excitation of the slowly bursting unit. During the enhanced activity of this unit the other unit had reduced activity.

Cholecystokinin

A bolus injection of CCK-8 (0·05–5·0 μg) injected close intra-arterially increased the impulse frequency in twenty-four of twenty-four tension receptor units. Bursts became more discrete and the response was sustained for up to 15 min (Fig. 3).

The enhanced activity in twenty-two units was coincident with phasic contractions of the duodenal muscularis externa but was unrelated to detectable differences in resting muscle tone. In two units with little resting activity the response appeared to be related to the enhanced tone of the preparation, which remained increased for 5–6 min following the injection (Fig. 4).

Local intra-arterial injections of CCK-33 (0·05–5 μg) excited seven of eight units

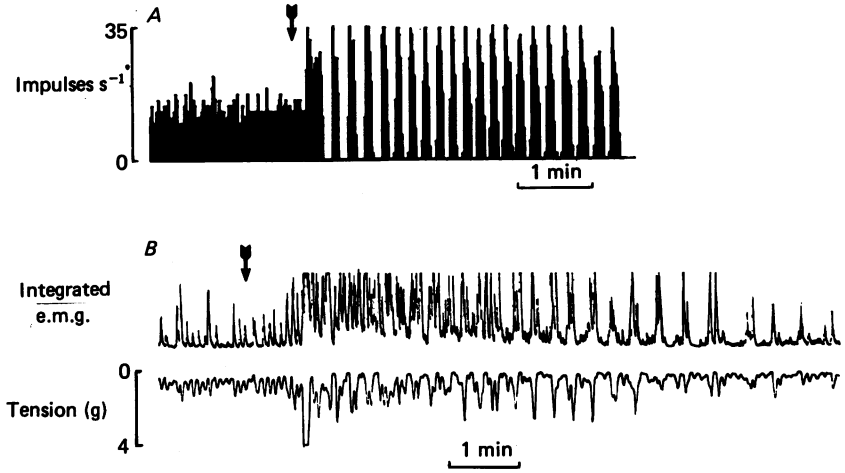

Fig. 3. The effect of close intra-arterial CCK-8 upon the activity of duodenal tension receptors and longitudinal contraction. In *A* a frequency histogram of a duodenal tension receptor shows the onset of discrete bursting activity when 5 μg CCK-8 was injected. The mean impulse frequency increased from 7·8 to 8·5 impulses s^{-1} and was sustained for 15 min after which time activity returned to its original pattern. No tension or e.m.g. records were taken. In *B* measurements of integrated e.m.g. (upper trace) and tension (lower trace) in the duodenum are shown following the application of CCK-8 (0·1 μg) at the arrow. After a latency of 30 s there was enhanced contraction lasting 10 min, during which period the contraction amplitude and rate declined. No impulse activity was recorded on this occasion. Traces retouched for clarity.

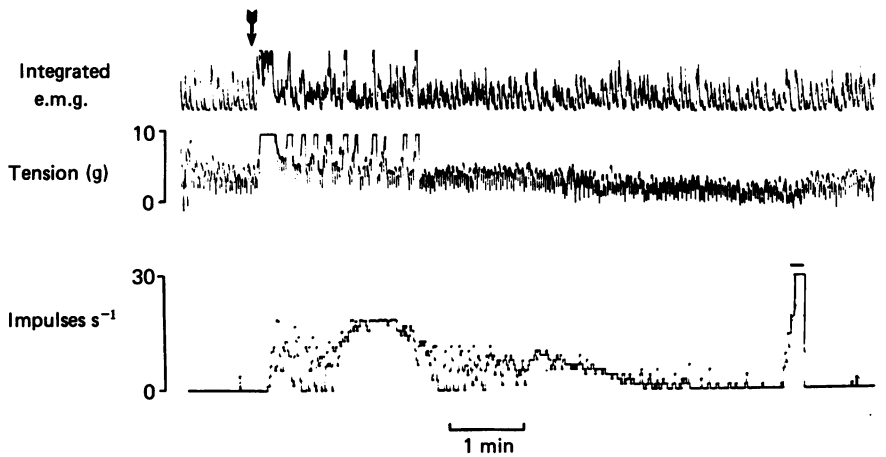

Fig. 4. The response of a previously silent tension receptor in the torus pyloricus to CCK-8 (1 μg = 0·87 nmol). Upper trace, integrated e.m.g.; middle trace, longitudinal tension; lower trace, frequency histogram. There was excitation of the receptor during a period of raised tone in the torus. The phasic activity illustrated in Fig. 3 was not seen. The response to compression is shown at the bar.

and the response was more vigorous than that caused by CCK-39 (0·05–5 μg) which excited five of five units during increased muscle tension (Fig. 5).

Intra-carotid injections of CCK-8 produced a triphasic response in duodenal muscle tension and corresponding changes in impulse activity. At first a period of enhanced contraction amplitude, rate and tone occurred. This phase lasted from 10 to 30 s and

was followed by a prolonged reduction of e.m.g., tension and impulse activity lasting 1–3 min. Thereafter an enhanced contraction amplitude and rate occurred. No inhibition was seen in the tension of pyloric muscle but there was an enhanced frequency of contraction which started within 20 s of CCK-8 application and lasted 5–7 min (Fig. 6).

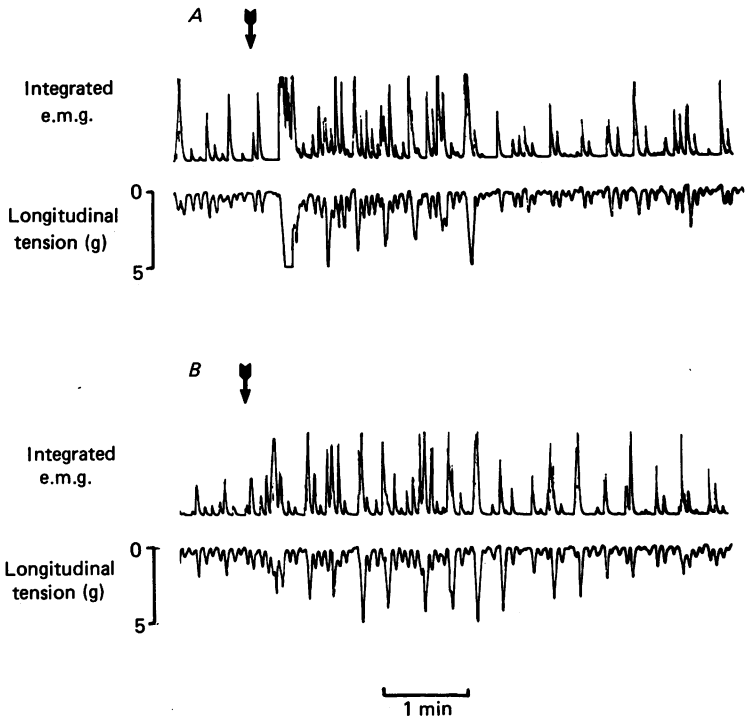

Fig. 5. The effect of close intra-arterial injections of *A*, CCK-33 (1 μg = 0·26 nmol) and *B*, CCK-39 (1 μg = 0·22 nmol) on duodenal mechanical activity. Upper traces, integrated e.m.g.; lower traces, longitudinal tension. Both peptides caused enhanced duodenal contraction. Traces retouched for clarity.

Intra-carotid injections of CCK-33 (1 μg) caused a 50% reduction of duodenal muscle contraction amplitude which lasted for 2 min without causing changes in contraction rate or tone. No change was demonstrated with similar doses of CCK-39 by this route.

Insulin

Two kinds of response were observed following intra-arterial injection of insulin B.P. (0·1–5·0 u.). In twelve of twenty units the response was a brief increase in impulse activity without altering the muscle tension (Fig. 1). The most sensitive unit responded to 0·003 u. kg^{-1} with vigorous discharges (mean frequency 18 impulses s^{-1}), maintained for 4 min. In addition, a delayed excitation by insulin was present in five of twelve units tested by close arterial injection and in five of eight units tested with systemic intravenous injections. Effective doses were in the range 0·03–0·1 u. kg^{-1}. There was a gradual increase in impulse frequency per burst and after 2 min units fired at 100–150% of pre-treatment levels of activity. This rate was sustained for more

than 10 min. The interburst interval was unchanged and no over-all change in duodenal tone was evident. A reduction in impulse activity occurred in two units when 4 u. insulin were given intravenously.

Insulin was administered together with separate 10 ml intravenous injections of the following chemical solutions: glucose (30 mg kg^{-1}); lactic acid (50 mmol kg^{-1}); acetic acid (50 mmol kg^{-1}). These drugs on their own did not alter the activity of the drugs tested nor did these chemicals affect the insulin response.

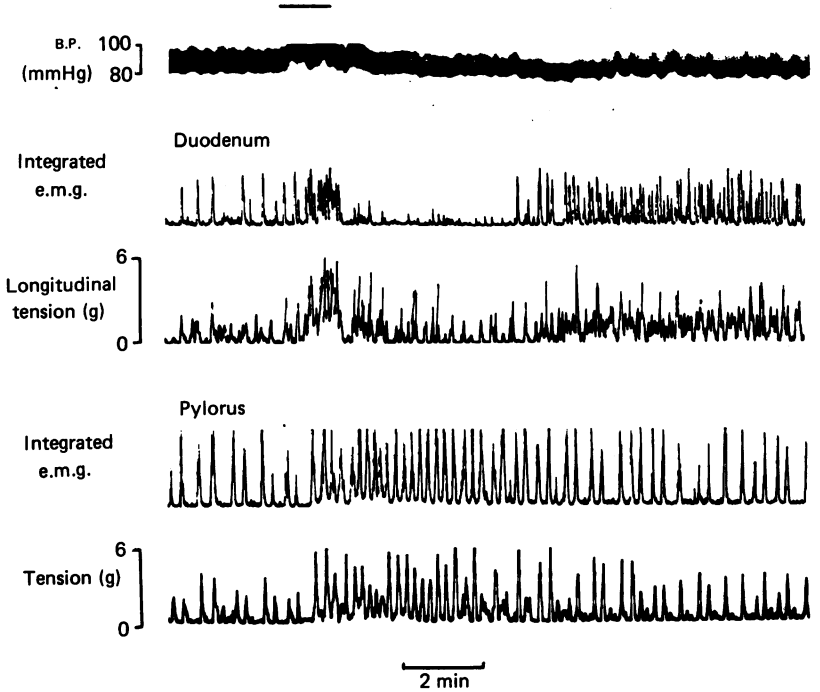

Fig. 6. The effect of intracarotid CCK-8 (0·05 μg) at the bar on the activity of duodenal and pyloric muscle contraction. Upper trace, systemic blood pressure; second trace, integrated e.m.g. duodenum; third trace, longitudinal duodenal tension; fourth trace, integrated e.m.g. pylorus; lower trace, longitudinal pylorus tension. Three phases of duodenal contraction are illustrated: an initial excitation, a period of inhibition, an enhanced rate of contraction. The pyloric contraction rate was enhanced and sustained for 10 min. Traces retouched for clarity.

Prostaglandins

Close intra-arterial injections of prostaglandins E_2 and $F_{2\alpha}$ (50 μg kg^{-1}) caused an enhanced bursting discharge after a latency of 5 s in nineteen of twenty-one units. Discharges at mean rates of 20 impulses s^{-1} were maintained for up to 90 s. The response coincided with increases in contraction amplitude of the duodenum which continued throughout the afferent discharge (up to 15 min). Prostaglandin E_2 caused a maximum impulse frequency of 71 impulses s^{-1}, which was 50% greater than changes produced by prostaglandin $F_{2\alpha}$. In fifteen units tested with both drugs the length of bursts and interburst intervals were different, although the total number of impulses of a 6 min sample were not significantly different. For example, Fig. 7 illustrates a unit with a mean rate of 8 impulses s^{-1}.

The enhanced bursting discharge usually continued for up to 10 min. Following the return to pre-stimulus levels of activity receptors were more sensitive to mechanical distortion for the remaining period of investigation. The response to compression was enhanced by 20–50% for at least 25 min after initial injection. During this hypersensitive period, close intra-arterial perfusion of ammonium chloride (0·8–1·06 mmol kg^{-1}), which previously had caused no response, but not normal saline, initiated the characteristic bursting activity which lasted for several minutes.

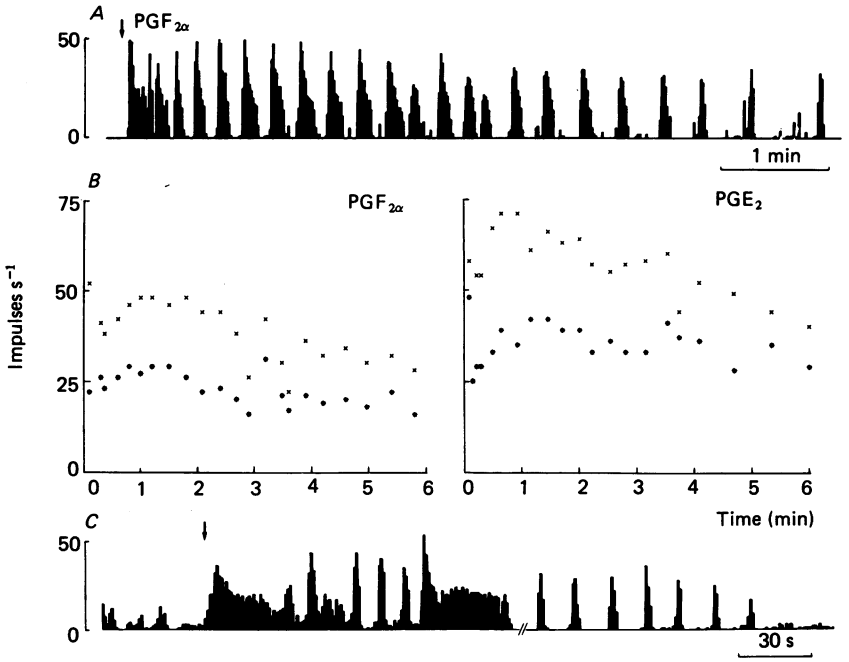

Fig. 7. In *A* and *B* the response is shown of a previously silent tension receptor to close intra-arterial injections of prostaglandins $F_{2\alpha}$ and E_2. In trace *A* the total response to $PGF_{2\alpha}$ (50 μg kg^{-1}) is shown. In *B* this response is expressed as peak frequency per burst (×) and mean frequency per burst (*) and is compared with the response to the same dose of PGE_2. The mean length of the burst for $PGF_{2\alpha}$ was 7·6 s and PGE_2 was 4·3 s. Trace *C* illustrates the effect on a sheep tension receptor of $PGF_{2\alpha}$. Five minutes after the close intra-arterial injection of $PGF_{2\alpha}$ (1 μg kg^{-1}) which caused little change in this relatively inactive unit the mucous membrane was lightly stroked (arrow). A prolonged rhythmical activity developed and persisted following this brief mechanical stimulus. The break in the record was of 1 min duration. Because tension measurements were not made for this unit it is not known whether the response was due to receptor sensitization or altered local contractions.

Bradykinin

Nine units were excited by close intra-arterial bradykinin injections. The response consisted of three phases which were to some extent dose-dependent. Doses between 10 and 25 μg produced an initial receptor excitation, which coincided with increased contraction amplitude and tone of the duodenum lasting up to 20 s. This phase was followed by a 80 s period of inhibition and later by enhanced impulse activity for

4 min. Doses of 1–5 μg caused a shorter period of initial excitation, a longer period of inhibition lasting up to 4 min, and later enhanced receptor activity (Fig. 8).

In five units the latency of the response was greater than 10 s (Fig. 9).

5-Hydroxytryptamine, acetylcholine, noradrenaline and adrenaline

Close intra-arterial injections of 5-hydroxytryptamine (20–50 μg) produced excitation in eight of ten duodenal tension receptors (Fig. 10).

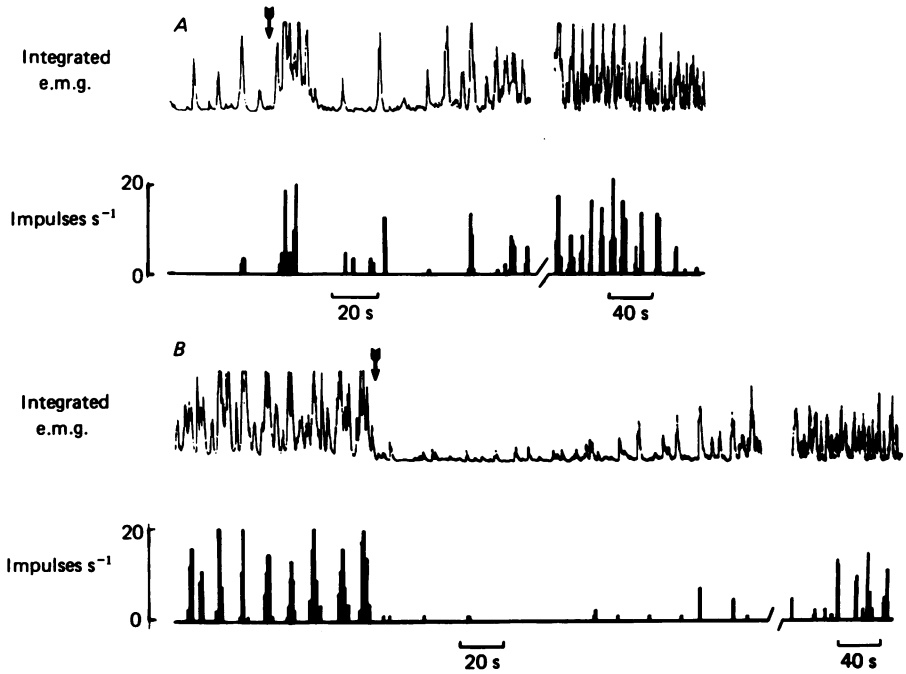

Fig. 8. The response of a duodenal tension receptor to two different doses of bradykinin administered close intra-arterially. *A*, 25 μg bradykinin produced an initial receptor excitation (lower trace, frequency histogram). The discharge coincided with active muscle contraction (integrated e.m.g., upper trace). The unit was less active during the period of atony which followed. Impulse activity was enhanced later when muscle contraction occurred. *B*, lower doses (5 μg) produced a prolonged inhibition which lasted for 4 min before returning with enhanced impulse and e.m.g. activity. The breaks in each record represent an alteration in chart speed.

The response to 5-hydroxytryptamine was characteristically an early burst with peak frequencies up to 44 impulses s^{-1}, and a total period of excitation lasting up to 90 s. In the remaining two units there was a reduction of impulse activity which coincided with increases in contraction amplitude. Acetylcholine caused an initial excitation followed by periods of bursting activity in five of six units which occurred during evoked peristalsis, increased e.m.g. activity and increased contraction amplitude.

Noradrenaline (1–50 μg) administered close intra-arterially in four of eight cases, and adrenaline (0·25 μg) in six of six cases, caused a reduction of impulse activity which coincided with decreased duodenal tone and contraction amplitude lasting up

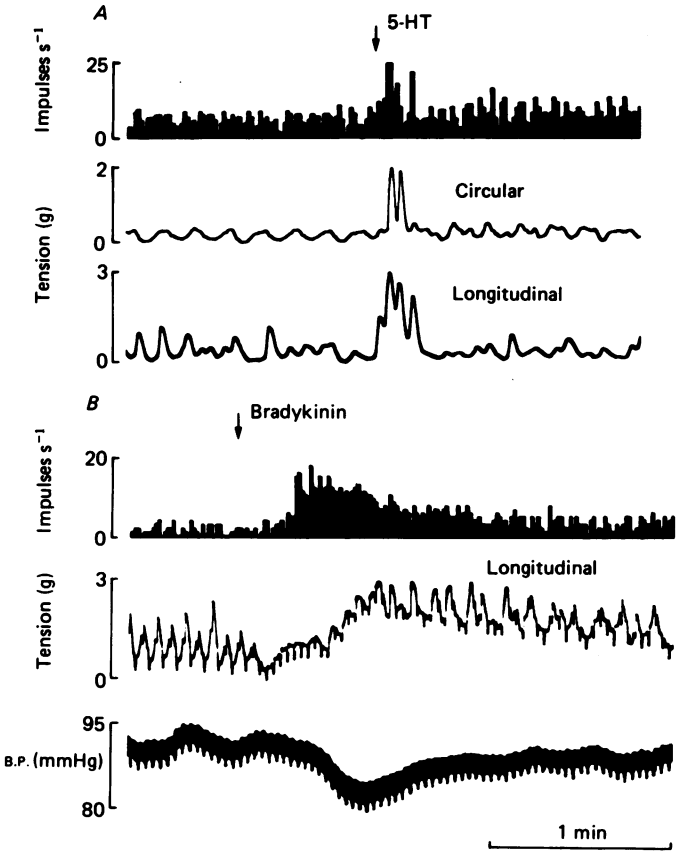

Fig. 9. The response of separate tension receptors to close intra-arterial injections of 5-hydroxytryptamine (5-HT) and bradykinin. *A*, 5-HT (25 μg) caused contraction in both the longitudinal and circular directions. The impulse activity increased during the early and late phases of the longitudinal contraction. *B*, in this receptor, bradykinin (25 μg) excited the receptor after a long delay (> 10 s). The impulse frequency increased during a period of raised longitudinal muscle tone (middle trace) which developed during depression of systemic blood pressure (lower trace).

to 20 min. During this atonic phase there was no response to compression or to previously effective close intra-arterial injections of phenylbiguanide (50 μg), 5-hydroxytryptamine (20–50 μg) and pentagastrin (1 μg kg^{-1}). Four of eight units were excited by noradrenaline during a period of increased tone (Fig. 11). The phasic excitation produced was continued throughout the systemic arterial pressor response caused by this drug, and was followed by enhanced periodic activity.

Veratrine and phenylbiguanide

Close intra-arterial injections of veratrine (1–100 μg) excited five of eleven receptors which had impulse frequencies up to 54 impulses s^{-1} during a period of increased contraction amplitude. This was followed by insensitivity to compression lasting 10–12 min. These effects were not seen with veratrine administered intravenously at dose levels of 20 μg kg^{-1}. Phenylbiguanide (5–90 μg) given close intra-arterially

caused an increase in tone and amplitude of contraction usually in the longitudinal direction and an increase in impulse activity in thirteen of twenty units (Fig. 11).

During the period of increased tone the discrete bursting pattern of the resting discharge was absent and the mean discharge frequency increased by 80–200 %. The excitation by phenylguanide was abolished by intra-arterial injections of noradrenaline (1 μg kg^{-1}) and combinations of atropine (4 μg kg^{-1}) with hexamethonium (2 μg kg^{-1}).

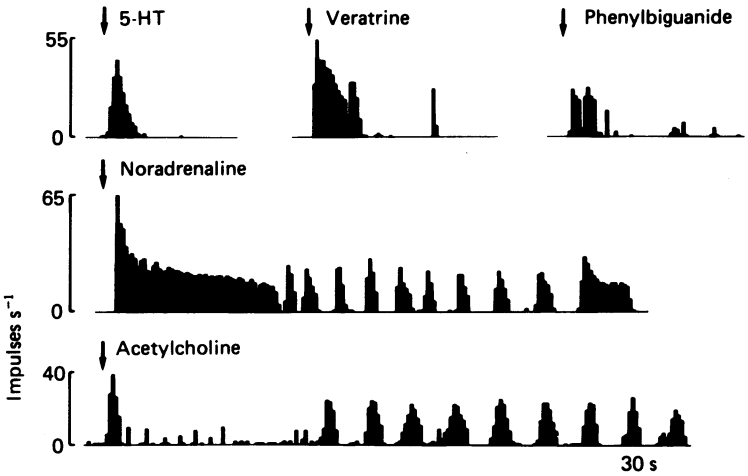

Fig. 10. The responses of a single tension receptor to close intra-arterial injections of 50 μg of the drugs indicated. The total responses are shown. A 10 min recovery period was allowed between drug applications. The bursting activity in the responses to noradrenaline and acetylcholine coincided with observed movements of the duodenum.

Atropine and hexamethonium

Close intra-arterial injections of atropine (4 μg kg^{-1}) caused inhibition of the periodic response of acetylcholine, and reduced the total response to the prostaglandins by 40 %. Intra-arterial injections of atropine (2 μg kg^{-1}) caused two of eleven units to become excited and there was a later periodic discharge. In ten units combinations of hexamethonium (2 μg kg^{-1}) and atropine (2 μg kg^{-1}) reduced the tone of the preparation and prevented the responses to acetylcholine and abolished all other drug-evoked responses which had previously characterized the receptor. Although atropine and hexamethonium together caused a reduction of gut tone, in most cases phasic motor activity was maintained at a reduced level and tension receptor discharges usually continued. Close intra-arterial injections of hexamethonium (0·2–2 μg kg^{-1}) caused one of five units to fire in a characteristic way. This response was an initial excitation lasting 12 s, followed by an inhibition for 12 s and then a return to pre-stimulus levels of activity. The response was consistently present over six trials. No detectable change in afferent activity was demonstrated in four of five units treated in this way.

DISCUSSION

From the responses of sheep duodenal tension receptors described in this paper several possible mechanisms of tension receptor excitation may be proposed. In general, drugs may act in a variety of ways: by directly altering the sensitivity of

receptor endings; by indirectly modulating neurotransmitter release in the enteric plexus which changes gut motility or receptor sensitivity; by acting directly on the smooth muscle membrane and affecting the rate of oscillation of membrane potential, or in more than one of these ways.

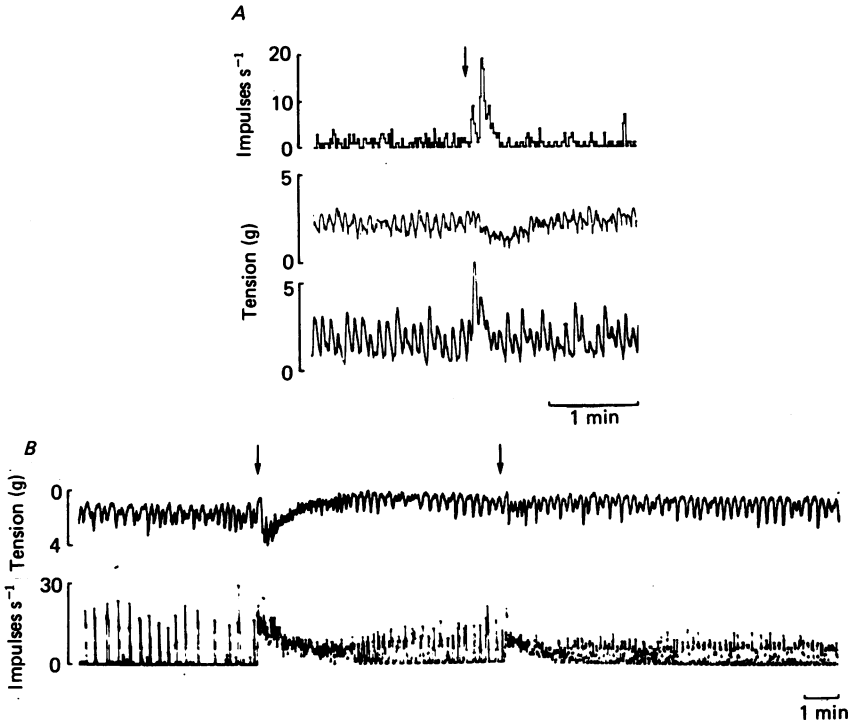

Fig. 11. The response of tension receptors to phenylbiguanide. *A*, 20 μg injected close intra-arterially at the arrow. During an increase of impulse activity there is concurrent relaxation of the circular muscle tension (middle trace) and increased longitudinal tension (lower trace). *B*, in a different unit 100 μg was injected close intra-arterially at the first arrow and 5 μg at the second arrow. There was an increase in tone of circular muscle (base line upper trace) and the mean discharge frequency increased, but the phasic pattern of discharge was reduced until the tone returned to control levels.

The mechanism of excitation of tension receptors by bolus injections of pentagastrin and CCK appears to be similar. Intravenous and intra-arterial injections of pentagastrin produced phasic increases in impulse activity, followed by extended periods of inhibition. CCK enhanced periodic activity which lasted longer than pentagastrin-induced responses. In both cases excitation was associated with an increased muscle contraction. It is known that pentagastrin and CCK-like peptides may release acetylcholine from the intramural plexus (Vizi *et al.* 1973), which determines the strength of muscle contraction (Szurszewski, 1975). Such a mechanism in the sheep duodenum may have caused an enhanced contraction amplitude which in turn led to the receptor responding more vigorously. These peptides also increased the frequency of bursts. Pentagastrin may regulate the frequency of occurrence of contraction by directly affecting the muscle membrane (Szurszewski, 1975). The results do not, however, establish whether the mechanism of receptor excitation by

peptides involves direct or indirect mechanisms. Because the response to pentagastrin was inhibited by atropine and hexamethonium it is probable that an acetylcholine release mechanism is necessary for receptor excitation, which is inhibited by noradrenaline and adrenaline.

Grovum & Leek (1982) have found that slow perfusions of pentagastrin may directly cause reticulo-rumen muscle to contract, excite gastric tension receptors and initiate reflex excitatory effects. This response is followed by a depression of gastric centre activity (Grovum & Leek, 1982). The relaxation of the abomasum in sheep by pentagastrin (Ruckebusch, 1971) may be a reflex effect of altering the tonic discharge of gastric receptors caused by pentagastrin (Bell, Titchen & Watson, 1977). No explanation is offered for the observation that the same injection of pentagastrin given locally was less effective than systemic intravenous ones at exciting duodenal tension receptors. This effect was not observed by Grovum & Leek (1982), who report a dose-dependent effect of pentagastrin on reticular tension receptors. These authors found an increased afferent discharge when low dose rates were administered by slow perfusion. The disparity in results may be accounted for by the differences between bolus and slow perfusion administration of the drug.

Intracarotid injections of both CCK and pentagastrin caused periods of inhibition of duodenal movement and reduced tension receptor discharges. The effect of pentagastrin by this route is therefore similar to the findings of Grovum & Leek (1982), who demonstrated that slow intracarotid perfusion in conscious sheep depresses reticular contractions and efferent discharges presumed to supply the reticulo-rumen. The inhibition of duodenal movement produced by CCK injections may be reflex in nature. The effect may be due to gastro-duodenal reflexes evoked by the increased mechanical activity of the antral and pyloric area by CCK (Daniel & Sarna, 1975). Alternatively the mechanism may involve a central inhibitory effect. The relative sensitivity of the duodenum to CCK-8 and the insensitivity to CCK-33 and CCK-39 may be explained by the permeability characteristics of the blood–brain barrier to higher molecular weight peptides (Mutt, 1980).

The demonstration of synchronization of impulse activity in two tension receptors with different bursting patterns by pentagastrin may indicate an alteration in muscle contraction patterns at the pylorus which might operate post-prandially. Because this co-ordination only occurred following intracarotid injection this implies that gastrin may play a role in the modulation of central integrative mechanisms controlling gastric emptying. These mechanisms presumably involve activation of specific motor nerves controlling muscle contraction at the antral–duodenal junction.

Paintal (1953) demonstrated an increased activity in feline gastric tension receptors when perfused intra-arterially with glucose. Paintal (1953, 1954*b*) was the first investigator to attempt to correlate known single-unit afferent activity with alimentary sensation, and he suggested that the activity of vagal afferent units from gastric stretch receptors might be inversely related to 'hunger pangs'. Although Paintal's assumption that reduced afferent activity occurred during contraction was later disproved by Iggo (1957*a*) his demonstration that tension receptors are glucose sensitive suggested the basis for a mechanism of peripheral interaction between energy status and afferent activity of alimentary mechanoreceptors. For this reason, the excitatory response to insulin in sheep duodenal receptors was particularly

interesting. The excitation of alimentary tension receptors by insulin has not been demonstrated before. Mei (1978) has shown that the activity of glucose receptors in the cat duodenum increases after intravenous insulin. Elsewhere, in the taste system, the electrophysiological threshold is unchanged by insulin (Pfaffman & Hagstom, 1955). The results obtained in the sheep duodenum demonstrate that tension receptor activity increased together with tension and e.m.g. activity in the duodenum, and indicate that e.m.g. records of the muscularis externa of the gastrointestinal tract can now be interpreted to indicate the level of afferent activity of tension receptors. Bueno & Ruckebusch (1976) have shown that increased e.m.g. activity in the dog jejunum occurs with intravenous glucose treatment, and in the sheep intestine after the intravenous injection of volatile fatty acids. In ruminant animals volatile fatty acids release insulin (Bueno & Ruckebusch, 1975). Bueno & Ruckebusch (1976) show that the e.m.g. pattern of sheep jejunum becomes a continuously spiking one, similar to fed dogs, when insulin, D-glucose and amino acids (L-leucine, L-arginine) are administered and when insulin and volatile fatty acids are given to sheep. They concluded that endogenous insulin levels are important in the control of jejunal motor profiles. Whether the effect of insulin is peripherally or centrally mediated is not known. The present report could suggest that enhanced duodenal tension receptor activity by insulin in the absence of measurable tension and e.m.g. changes may involve direct receptor sensitization, although this point is extremely difficult to test. The variability of the response may be partly explained by the endogenous insulin, volatile fatty acids and glucose levels, which were not measured.

The prostaglandins used enhanced the peristaltic reflex and excited receptor activity during increased tension changes in the external muscle coat. The mechanism probably involved enteric neurones because the interburst interval, which was unaltered, probably depends on myogenic events (Sarna, 1975). Prostaglandins also caused the receptor to discharge when intravenous ammonium chloride solutions were perfused. This solution causes a pressor response in urethane-anaesthetized cats when intestinal blood vessels are perfused, an observation which allowed Poliakova (1959) to conclude that there were chemoreceptors in the intestinal wall. The present findings suggest that the reflex response in the cat may have involved sensitized mechanoreceptors, although once again the position of the receptor and the recording devices may not be in exact agreement.

During smooth-muscle atony produced by noradrenaline, tension receptors were insensitive to phenylbiguanide, 5-hydroxytryptamine and pentagastrin which otherwise excited them. This suggests that these drugs do not act directly on tension receptors, independently of changes in muscle tone. The effect of noradrenaline-induced atony on the administration of insulin and prostaglandin was not tested. When noradrenaline itself excited afferent activity this was accompanied by tension changes. This excitatory effect of noradrenaline on tension receptors in the region of the sheep pyloric sphincter is consistent with the findings in the goat that adrenaline causes contraction of the pyloric sphincter (see Bell & Watson, 1975, p. 342). Tension receptors are also excited by noradrenaline in the cat stomach (Paintal, 1953, 1954*b*, *c*; Iggo, 1957*a*, *b*) and intestine (Leitner & Perl, 1964).

The motor response and receptor excitation caused by 5-hydroxytryptamine suggest that its effect is an indirect one and that, in sheep duodenum,

5-hydroxytryptamine does not act directly on tension receptor membranes. The insensitivity of the receptors to 5-hydroxytryptamine during atony induced by noradrenaline supports this suggestion. Splanchnic-projecting mechanoreceptors in cats are excited by intra-arterial bradykinin (doses 1–10 μg) (Floyd, Hick, Koley & Morrison, 1977*b*) by a mechanism of reflex excitation involving autonomic nerves in the gut (Floyd, Hick, Koley & Morrison, 1977*a*). Duodenal tension receptors in the sheep were also excited by close intra-arterial injections of bradykinin. The latency in some cases was over 10 s and is consistent with a possible indirect reflex mechanism similar to that found in the cat.

The results do not support the suggestion that 5-hydroxytryptamine and phenylbiguanide act directly on the receptor membrane, as is suggested elsewhere (Daniel, 1968). Nor do they support the suggestion that phenylbiguanide directly excites receptor endings of C-fibres (Paintal, 1954*a*, 1964), because most of the units tested had conduction velocities below 2 m s^{-1} (Cottrell & Iggo, 1984*a*). Also, sheep omental receptors have non-myelinated axons and they do not change their activity after close intra-arterial application of phenylbiguanide (10 μg kg^{-1}) (Cottrell & Iggo, 1984*a*).

In conclusion, the results reported here support the hypothesis that peptide hormones may act reflexly by altering sensory receptor activity as well as acting directly on the effector tissues. Evidence of other investigators suggests that peptides play a role in the sensory mechanism: ligation and transport-blocking techniques demonstrate the accumulation of substance P, somatostatin, vasoactive intestinal polypeptide and CCK-like peptides on the central side of the ligature in vagus and splanchnic nerves (Lundberg *et al.* 1978; Dockray *et al.* 1981). This is interesting because CCK and pentagastrin interrupt feeding patterns and induce satiety behaviour in a variety of species studied (for references see Introduction). What is not known is whether the levels of circulating exogenous hormones, applied via bolus or continuous perfusion, are similar to endogenous levels or whether exogenously applied hormone analogues act at specific receptor sites normally activated by endogenous hormones. During the experiments reported here only bolus injections were used, and this was partly due to the difficulty of holding afferent units for long periods of time. This method of drug administration may not be ideal for demonstrating physiological processes for which continuous perfusion techniques are preferred (Grossman, 1973). The doses used are comparable with those used elsewhere in sheep: CCK-8, levels between 0·001 and 4·2 μg kg^{-1} h^{-1} (Della Fera & Baile, 1979) and 1·5 and 3·0 μg kg^{-1} h^{-1} (Grovum, 1981) are used; for pentagastrin, levels of 0·075–11·75 μg kg^{-1} h^{-1} (Della Fera & Baile, 1979), 1–27 μg kg^{-1} h^{-1} (Grovum, 1981) and bolus injections of 2–32 μg kg^{-1} (Grovum *et al.* 1974) are used. Exogenously applied peptides may act in one of three ways: (a) peripherally by exciting enteroceptor activity, for which this report provides evidence. Duodenal mucosal receptors are not excited or sensitized by local intra-arterial injections of insulin, CCK-8 or pentagastrin (Cottrell & Iggo, 1984*b*); (b) centrally by mimicking the effect of increased alimentary enteroceptor activity and inducing reflex behavioural responses by modulating gastrointestinal control mechanisms and behavioural processes; or (c) central inhibition. Grovum and co-workers (Grovum & Chapman, 1982; Grovum & Leek, 1982) and Nicholson (1982) provide evidence for a central depressing effect of pentagastrin. The effect of CCK on food intake suppression may be mediated elsewhere, in the lungs (Grovum, 1982).

The evidence here supports a partially central acting inhibitory mechanism of duodenal motility for both CCK and pentagastrin. The central role of CCK, first identified in the brain stem by Dockray *et al.* (1978) remains speculative. Genetically obese mice have been found to have low CCK-8 levels in the brain (Straus & Yalow, 1979), although these observations were unconfirmed by Schneider, Monahan & Hirsch (1979). Lateral ventricular injections of pentagastrin are more effective than intravenous injections at inducing satiety behaviour in sheep (Grovum *et al.* 1974).

These results of unitary afferent activity indicate that gastrointestinal hormones released after a meal may cause duodenal tension receptors to alter their afferent activity. Peripheral mechanisms may involve both an increased receptor sensitivity (insulin, prostaglandins) as well as increased duodenal muscle contraction (gastrin, CCK). Peptides may act centrally, where they have an inhibitory role. Post-prandially released hormones may therefore play an important modulating role in the reflex mechanisms of short-term appetite control and the neural regulation of gastric emptying.

Financial assistance for the investigation was provided by the Agricultural Research Council, Grants AG 15/174 and AE 245. Technical assistance was by Mrs Marion MacDowell and Ms Jane Anderson. The Wellcome Animal Unit provided animal care and accommodation. The authors would like to thank Squibb and Son Ltd. for the gift of CCK-8.

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