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TENSION RECEPTORS WITH VAGAL AFFERENT FIBRES IN THE PROXIMAL DUODENUM AND PYLORIC SPHINCTER OF SHEEP

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SUMMARY

1. Single-unit afferent activity was recorded from the hepatic–duodenal branch of the vagus nerve of chloralose-anaesthetized sheep during acute electrophysiological experiments.

2. The impulse activity of sixty-seven slowly adapting mechanoreceptors situated in the muscularis externa of the proximal duodenum and pyloric sphincter was synchronous with alterations in electromyographic and tension records. Afferent units were excited during passive distension, compression and drug-induced increases in muscle tension, thus satisfying the criteria for 'in series' tension receptors (Iggo, 1955). From the responses to compression some evidence was found for the existence of separate populations of tension receptors with different mechanical thresholds. Two fibre populations were found: non-myelinated ($0{\cdot}70 \pm 0{\cdot}26$ S.D. m s^{-1}) and myelinated ($7{\cdot}6 \pm 1{\cdot}6$ S.D. m s^{-1}).

3. Mucosal application of solutions of hydrochloric acid, volatile fatty acids, alkali and amino acids, and mucosal probing modified the activity of most units. These changes were reduced by anaesthesia of the mucosa.

4. It is concluded that tension receptors in the sheep duodenum occupy a position 'in series' with longitudinal muscle, and that their activity can be modified by the particulate and chemical composition of chyme by a mechanism involving local nerve plexuses.

5. The activity of tension receptors is compared with that of two other mechanoreceptor classes located serosally (five units) and in the lesser omentum (eleven units). Receptors in neither of these two classes were directly excited by active contraction of the duodenum.

INTRODUCTION

The concept that mechanoreceptors in the muscularis externa of hollow viscera behave as 'in series' tension receptors (Iggo, 1955; Leek, 1969) is based on indirect evidence that alterations in afferent activity occur during local tension changes. Without direct evidence it is premature to conclude either that tension receptors have different mechanical thresholds (Davison & Clarke, 1977) or that separate populations have different reflex effects (Iggo & Leek, 1967; Grundy & Davison, 1981). Circular and longitudinal muscles contract independently during peristalsis and two separate

mechanoreceptor populations may exist in different locations. Mechanoreceptors in circular muscle excited during peristalsis *in vitro* have a higher distension threshold than those in longitudinal muscle (Kosterlitz, Pirie & Robinson, 1956; Kosterlitz & Lees, 1964).

These concepts have been tested in an *in vitro* preparation in which tension measurements and electromyographs have been recorded concurrently with single-unit afferent activity of duodenal mechanoreceptors. The results support the 'in series' nature of alimentary tension receptors and suggest that, in the sheep duodenum, they are probably located in longitudinal muscle. In addition, a smaller number of mechanoreceptors was located in the serosa and omentum and their functional significance is discussed. Pharmacological aspects of the tension receptors are reported separately (Cottrell & Iggo, 1984*a*).

METHODS

Sixty-three sheep of various Scottish breeds, of either sex and approximately 35 kg body weight were anaesthetized with 1 % chloralose (70 mg kg^{-1}) after induction with either intravenous sodium pentobarbitone (24 mg kg^{-1}) or inhaled fluothane (3·5 % in 50:50 nitrous oxide and oxygen). Chloralose was supplemented at intervals of approximately 2 h with one-third the induction dose. After 8 h further chloralose was usually ineffective and pentobarbitone was then used as necessary.

The surgical preparation

A rumenotomy was performed and reticulo-ruminal contents evacuated and the wound sutured. The oesophagus was ligatured in the neck. With the sheep in left lateral recumbency a flat incision was made following the right costo-chondral arch and through which the pylorus could be exteriorized. The abomasum was ligated between the fundus and antrum, as also was the duodenum oral to the entrance of the bile duct. These procedures prevented reflux of chyme and bile into the preparation. After sections through the superficial sheet of the greater and lesser omentum the preparation comprising antrum, pylorus and the first 8 cm of the proximal duodenum could be firmly secured in a polyvinyl chloride organ bath (Fig. 1).

The long pyloric (hepatic–duodenal) branch of the ventral abdominal vagus (Duncan, 1953; Habel, 1956) together with veins, lymphatics, arteries and a prominent fibrous bundle were laid across a dissection pool. This was made paraffin-tight with polyacetyl drawbridges, cotton-wool and Vaseline. The dissection pool was filled with liquid paraffin (B.P.) maintained at 37 °C. The pylorus and proximal duodenum were laid across the cork dissection board of the larger compartment. Two preparations could be made: either (a) the duodenum was left intact, 'closed preparation', or (b) a longitudinal incision was made to expose the mucous membrane of the torus pyloricus, pylorus and duodenum, 'open preparation'. To prevent heat loss by evaporation, and to reduce condensation on to the lens of the dissecting microscope, the abdominal cavity was packed with sterile foam rubber.

Mechanical stimulation and recording

In closed preparations, small perfusion catheters together with recording catheters with either open-tipped ends or balloons (expanded volume 3 ml) attached to Stathem pressure transducers, were passed into the lumen through an antral incision. In both preparations the receptive fields were explored with glass probes, cotton-wool buds and von Frey hairs. The tip diameter of hairs was measured with a microscope and an eyepiece micrometer. The hairs were calibrated against isometric force transducers (Devices Instruments Ltd.) and gram weights. The receptive field could be stretched with blunt forceps attached at a distance from the receptor and also compressed against the cork base with a probe attached to an isometric force transducer. The muscularis externa was made to contract by the transmural application of electric currents, by repetitive electrical excitation of a branch of the ventral vagus (Fig. 1; the stimulus parameters were 1–10 V; 0·1–1·0 ms; 10–50 Hz) and by the administration of drugs intravenously or close intra-arterially. The tension developed in the muscularis externa was recorded in two directions with isometric force transducers

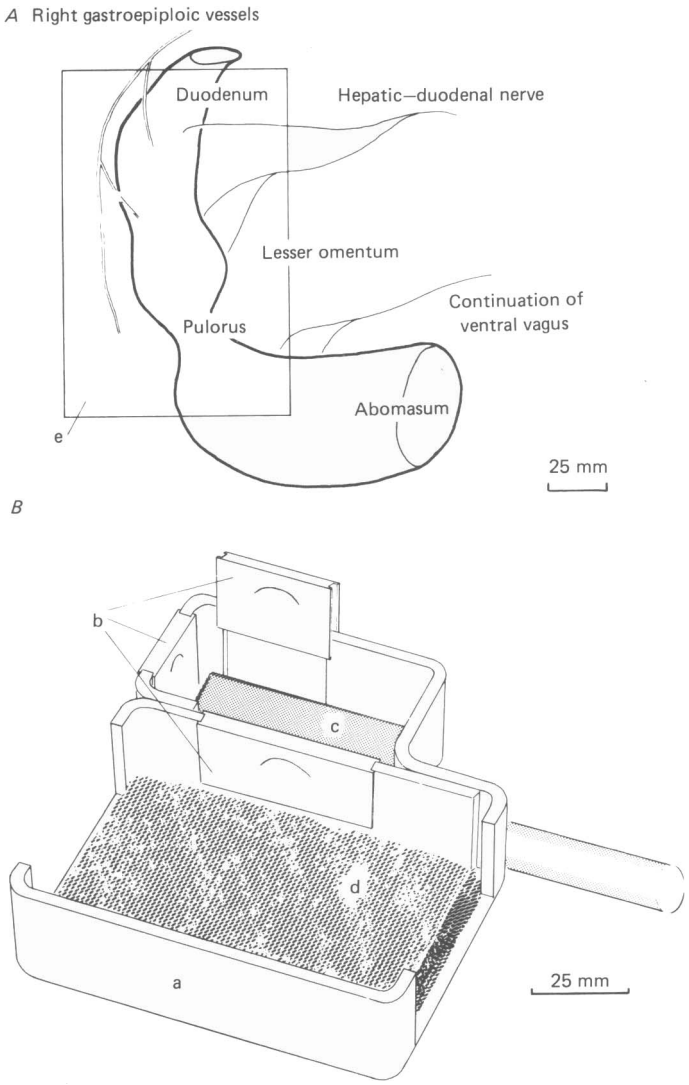

Fig. 1. *A*, schematic drawing of the anatomy of the duodenum, pylorus and abomasal antrum of the sheep with associated nerves, viewed from the right-hand side. Box e is the tissue placed in the dissection compartment (*B*, a). *B*, the sheep duodenal recording bath. a, polyvinyl chloride bath; b, polyacetyl drawbridges; c, nickel-plated brass base of dissection pool; d, cork base of dissection equipment.

attached by thin cotton sutures positioned to monitor longitudinal and circular (tangential) tensions. Tension changes were recorded on an eight-channel recorder (Devices Instruments Ltd.) and magnetic tape.

Chemical solutions

The responses of units were tested to chemical solutions previously used during studies of gastric emptying, the results of which have been interpreted as implying the presence of duodenal sensory receptors (Hunt & Knox, 1962; Bell & Razig, 1973). The following solutions were applied to the mucosa: hydrochloric acid, 10–75 mM; sodium hydroxide, 10–25 mM; acetic acid, 10–100 mM;

butanoic acid, 10–50 mM; sodium bicarbonate, 289–815 mosmol kg^{-1}; tyrosine, 50 mM; tryptophan, 50 mM; bile. Where appropriate the osmolality, measured with an osmometer (Vogel), was adjusted to 300 mosmol kg^{-1} with saline.

Electrical recording

Duodenal nerves were dissected from neurovascular bundles and divided transversely. The perineurium of the distal cut end was peeled off and the exposed nerve was dissected longitudinally using micro-techniques similar to Iggo (1955). Electrical activity was recorded monopolarly with a silver electrode and the second arm of the bipolar electrode was earthed to non-nervous tissue of similar size to the nerve strand under investigation. The preparation was earthed by a large nickel-plated brass plate situated in the dissection pool. The electrical activity was differentially amplified (3160 Digitimer Ltd.), displayed on a storage oscilloscope (Tektronix D13 dual beam) and stored on an FM tape recorder (T.E.A.C. Corporation). Recording conditions were usually very stable and impulse activity was examined for periods lasting between 20 and 60 min. This allowed the following protocol to be used to identify receptor characteristics: spontaneous activity, receptive field size delineated by mechanical probing, response to discrete perpendicular pressure and stretch, response to perfusion fluids of different chemical composition and drug applications. The conduction velocity was measured by the peripheral stimulus technique (Iggo, 1958) over different sections of the nerve. Isolated stimulators were used (Mark IV, Devices Instruments Ltd.) and triggered from a digitimer (Devices Instruments Ltd.). Conduction distances were calculated by accurate measurement of a thin cotton thread placed carefully along the nerve. Electromyographic (e.m.g.) activity was recorded differentially from the outer muscle coats with plastic-covered stainless-steel wires (150 μm diameter, Diamel coated) which were placed with stilettes made from 25 gauge needles. The e.m.g. was stored on magnetic tape and displayed on an eight-channel recorder (Devices Instruments Ltd.).

Data analysis

Spike train analysis was made both on-line and off-line with a spike processor (D130, Digitimer Ltd.) and histograms were stored on heat-sensitive paper together with pH, e.m.g., pressure and tension records (M-8 channel recorder, Devices Instruments Ltd.). More detailed analysis was made using an 'off-line' spike height discriminator (Ramsey, 1975), to transform the nerve impulses into standard voltage pulses, and a PDP-12 computer. Minimum interspike intervals were measured by triggering the oscilloscope from an impulse and, with suitable sweep speeds, measuring the time to the peak of the next impulse. Long spike trains, together with up to two analog channels, were displayed on a 565 dual-beam oscilloscope (Tektronix) and photographed with a continuous recorder camera (Nihon Kohden, Kogyo Co. Ltd.). Interspike intervals were collected in real time and stored on DEC tape as dwell and interval histograms, using a PDP-12 computer and a program called Collect 72 (Young, 1974). Sequential dwell histogram material was viewed and analysed with a program called Data 72 (Young, 1974). Data stored as separate dwell histograms were combined using a program written in FOCAL and stored as interval histograms. Hard copies of the histogram could be made with an X–Y plotter (Complot, Digital Displays Ltd.).

Drugs

The following drug preparations were used: acetylcholine chloride, 50 μg ml^{-1}; insulin B.P., 0·1 u. kg^{-1}; phenylbiguanide, 100–200 μg, 94 % by wt. (Aldrich Chemical Co., U.S.A.); cholecystokinin (CCK-8, 1 μg, Squibb & Son Ltd.; CCK-39, 1 μg, Karolinska Institute, Stockholm); 5-hydroxytryptamine creatinine sulphate, 25 μg ml^{-1} (BDH); veratrine, 1–10 μg ml^{-1} (BDH); noradrenaline acid tartrate, 100 μg ml^{-1}; papaverine, 2×10^{-3} M; atropine sulphate, 10 μg ml^{-1}; hexamethonium bromide, 10 μg ml^{-1}; adrenaline, 0·25 μg; pentagastrin, $2{\cdot}5 \times 10^{-3}$–1 μg kg^{-1} (Peptavlon, ICI); papaverine, 10^{-6} M.

RESULTS

Tension receptors

Sixty-seven units were isolated from the hepatic–duodenal nerve. Background activity occurred in bursts that were coincident with tension changes recorded in the

muscularis externa close to the receptive field. It was less easy to correlate e.m.g. activity with background receptor discharge (Fig. 2). Usually the tension changes were preceded by e.m.g. activity, but this was not invariably the case, and on these latter occasions the e.m.g. electrodes were presumed to record activity from a different muscle layer than that in which the receptor lay. The background activity

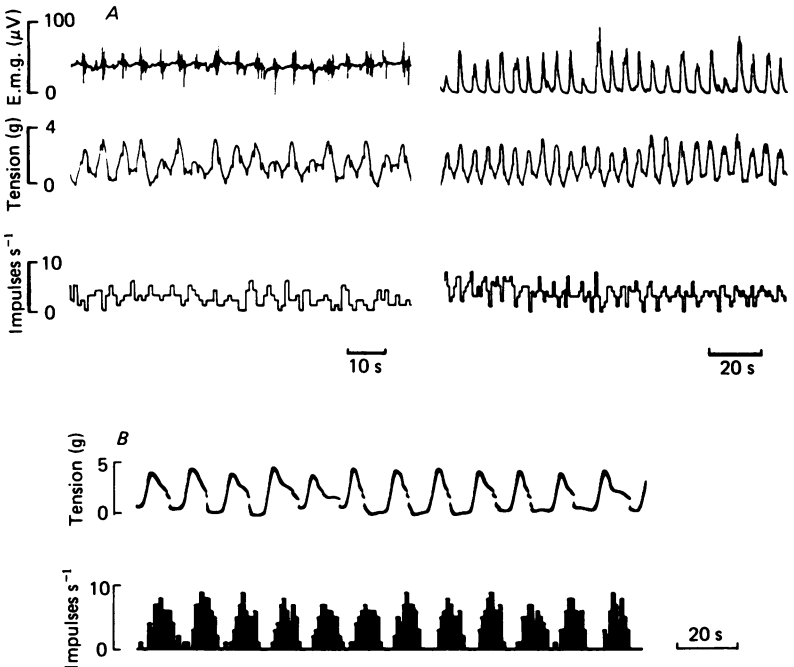

Fig. 2. Records of e.m.g.s, longitudinal tension and impulse activity in two tension receptors. In unit *A* the left records show a close correlation betwen the e.m.g. (upper trace), the longitudinal tension (middle trace) and the frequency histogram (bottom trace). In the right record the e.m.g. has been integrated and the time scale changed. *B*, this unit has a longer interburst interval, and activity was closely related to longitudinal tension (upper trace). The e.m.g. was not measured for this unit. See also Fig. 7.

in the open duodenal preparation was not noticeably different from that in closed preparations except for one unit that had a continuous discharge until a longitudinal incision through the duodenal wall was made when the discharge pattern became bursting. The mean discharge frequency of all units during bursts was $7{\cdot}9 \pm 4{\cdot}1$ S.D. impulses s^{-1} and the minimum interspike interval was $91{\cdot}4 \pm 57{\cdot}8$ S.D. ms. The receptive field size, in response to mucosal probing, was a single discrete area which was ellipsoidal in shape, with a greater axis along the length of the duodenum. The field size varied from 5×15 mm to 2×3 mm (6–75 mm^2). The mean area was $33{\cdot}0 \pm 16{\cdot}5$ S.D. mm^2. The conduction velocity measurements of the units indicated that two fibre populations existed: twenty-one units were non-myelinated (conduction velocity $0{\cdot}70 \pm 0{\cdot}26$ S.D. m s^{-1}) and seven units were myelinated ($7{\cdot}6 \pm 1{\cdot}6$ S.D. m s^{-1}).

Mechanical stimulation

In closed preparations the inflation of a balloon caused discharges with peak frequencies up to 30 impulses s^{-1}. The response was poorly sustained and the discharge declined to silence (Fig. 3*B*). The discharge was followed by a powerful circular constriction which displaced the balloon aborally. Units remained silent for 4–8 s while the circular constriction remained in the receptive field.

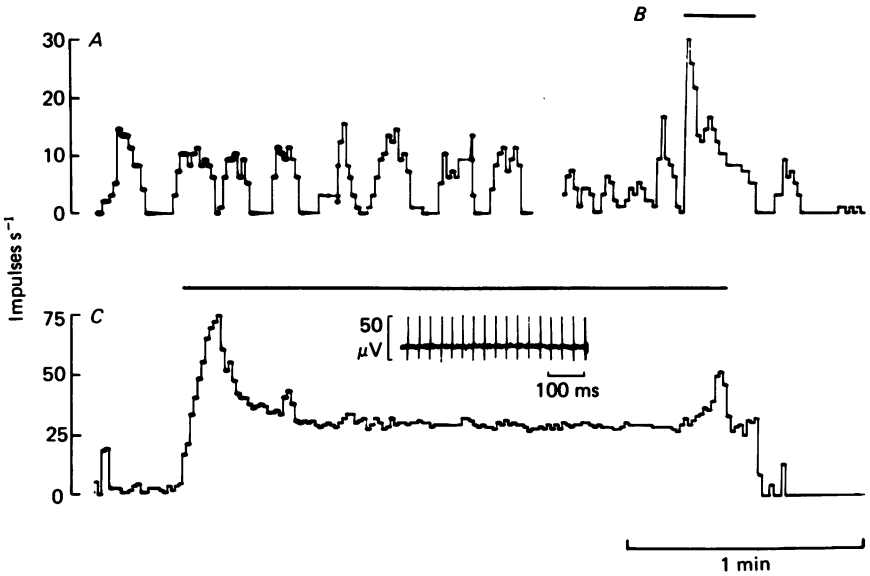

Fig. 3. To illustrate the background activity of a tension receptor and its responses to distension of the duodenal loop with a balloon and to compression of the duodenal wall. *A* is a frequency histogram of the rhythmical activity of the discharge occurring without an applied stimulus. *B* shows that, after placing an undistended balloon in the lumen, the spontaneous activity that was present in *A* was reduced. When the balloon was inflated with 5 ml air (at the bar) there was an increase in impulse activity which decayed exponentially to silence. The unit gave a burst of activity similar to those in *A* after the balloon was deflated. *C*, when the receptive field was compressed at the bar there was an initial phasic response followed by a period of persistence in the discharge. The increase towards the end of the record was due to readjustment of the probe and was not generally seen. The discharge was maintained for 15 s after the stimulus was removed. The inset illustrates the impulse pattern during the period of persistent discharge.

When sustained compression was applied across the wall of the preparation the discharge that was evoked persisted. The mean compression threshold necessary to initiate a few impulses was 6·12 g (±2·93 S.D.). When compressions below 20 g were applied there was an early period of adaptation followed by a stationary discharge (Fig. 3*C*). During the initial 1 s of the response the mean frequency of all units studied was 38·8±17·5 S.D. impulses s^{-1} and the minimum interspike interval was 24·6 ±11·3 S.D. ms. Units with myelinated fibres had higher peak frequencies (up to 90 impulses s^{-1}) and minimum interspike intervals of 9 ms.

Adaptation

Semilogarithmic plots of impulse frequency against sequential time were made of the initial 20 s of the response to determine the time constant (τ) of the decline of impulse frequency ($\tau = 1/k$, where $y = e^{-kt}$, y = variable declining exponentially, t = time for particular value of y, k = regression coefficient, $\log y$ against t). The discharge frequency, to maintained compression, reached a peak and then began to decline exponentially. For the two tension units tested in this way, the decay fitted a single regression line with time constants of 18·17 and 20·16 s for the initial 18–24 s of the response. Thereafter there was a stationary discharge which continued for periods exceeding 5 min. Firm, sustained compression with a finger reduced the rate of adaptation in the unit.

The characteristic stationary discharge pattern during maintained compression had a regular interspike interval, with coefficients of variation in five units of 0·15, 0·18, 0·21, 0·12 and 0·24 (Fig. 4). There was a positive correlation between the compression applied and the frequency of the adapted stationary discharge. The response to compression of two units in the same experiment is illustrated in Fig. 5. One unit was approximately three times more sensitive at all levels of compression. In ten experiments a difference in sensitivity was demonstrated between twelve pairs of tension units. The difference in paired compression thresholds ranged from 1·2 to 3·0 times (range 2–20 g). After a period of compression lasting more than 10 s there was a period of silence lasting 5 s before spontaneous bursting activity resumed. In 20% of compression trials there was an 'after-discharge' which persisted for up to 20 s (Fig. 3*C*).

Units were also excited by both longitudinal and tangential stretch applied by blunt forceps and sutures in the serosa. The greatest response to 80% of units was caused by longitudinal stretch, and this was also the case during longitudinally recorded active changes in tension. The tension developed in the longitudinal direction varied between preparations and the threshold to excite units ranged from 60 to 120 mg. When longitudinal stretch and compression were combined in twelve units the mean discharge frequency during the initial second of the response increased from 40 to 60 impulses s^{-1}.

The action of superficial mucosal probing upon tension receptor discharge

A characteristic feature of 70% of tension receptor responses was the brief inhibition of activity during light mechanical brushing or probing and spraying of solutions on to the mucosa. The inhibition was coincident with the mechanical stimulus and was usually followed by a burst of activity. In Fig. 6, a tension receptor and a mucosal receptor with overlapping fields were found and their discharges were recorded simultaneously. During light mechanical stimulation, sufficient to excite only mucosal receptors directly (Cottrell & Iggo, 1984*b*), the tension receptor discharge was inhibited. The discharge returned with an increased frequency after the mechanical stimulus was withdrawn and the mucosal afferent activity had ceased. No measurable tension change occurred during the mucosal stimulation, although increases in tension always followed with delays of 1–4 s.

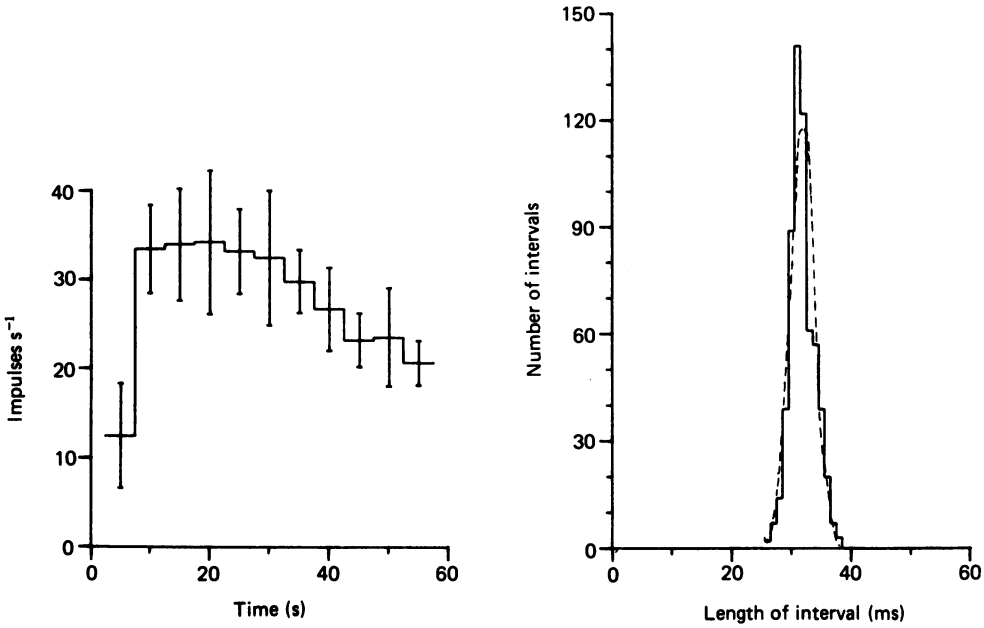

Fig. 4. The analysis of an impulse train of a tension receptor during compression of 25 g on the mucosal surface. The error bars in the frequency histogram (left) represent the S.D. of the frequency converted from the S.D. of the interspike intervals and assume a Gaussian distribution of intervals for each 5 s period. The response during the first 20 s was statistically stationary and an interval histogram (right) was constructed for this period. The distribution of intervals during 20 s was compared with the theoretical Gaussian distribution: $f(x) = 1/(\text{S.D.}/\sqrt{(2\pi)}).\exp^{-\frac{1}{2}}((X-\bar{X})/\text{S.D.})^2$, with $\chi^2 = \Sigma(O-E)^2/E$, and was not found to be normally distributed. The slope of the theoretical curve is indicated by the dotted curve. Mean = 31·28, n = 644, S.D. = 4·619, coefficient of variation = 0·148, χ^2 = 37·16, degrees of freedom = 10.

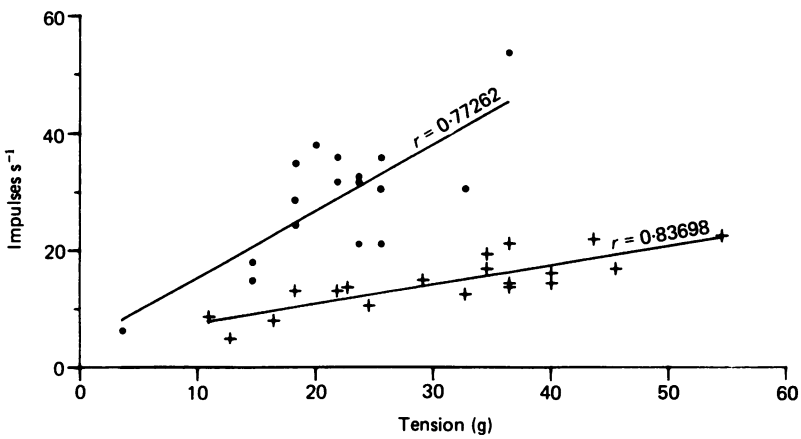

Fig. 5. The stimulus–response relationship of a duodenal tension receptor to compression. The adapted response during the period of persistence (see Fig. 3*C*) from two tension units was recorded during separate trials in the same preparation. Unit + was from the torus pyloricus and had a conduction velocity of 0·72 m s^{-1}. Unit ● was from the middle of the duodenal preparation and had a conduction velocity of 6·0 m s^{-1}. Regression lines were calculated by the method of least squares.

In three tension receptors, bursting activity was inhibited by brushing the serosa overlying the receptive field. Whether this stimulus caused a change in tension inside the muscularis externa was not investigated.

Pentagastrin

The rapid intravenous injection of pentagastrin (1 μg kg^{-1}) was found to be one of the most effective ways of increasing the tension in the preparation and also exciting the receptor. Two examples of the response are illustrated in Fig. 7. A characteristic excitation occurred after a latency accounted for by the circulation delay from the injection site to the receptive field. The excitatory effect therefore appeared to be mediated peripherally rather than centrally. A more detailed analysis supporting this conclusion is presented elsewhere (Cottrell & Iggo, 1984*a*).

Fig. 6. The interaction between tension receptor discharge and mucosal probing. The impulse activity of a duodenal tension receptor (small spike) to light mucosal probing with a cotton-wool ball is illustrated. At the bar the probe was placed on the receptive field of a 'silent' mucosal receptor which overlapped the receptive field of the tension receptor. A mucosal receptor discharge was excited (large spike) during which the background activity of the tension receptor was absent. The tension receptor discharge increased after the withdrawal of the probe. The mucosal receptor discharge and the two phases of tension receptor discharge were abolished by mucosal anaesthesia.

Electrical stimulation

Repetitive electrical stimulation applied transmurally to the so-called 'continuation of the ventral vagus' which supplies the abomasal antrum and pyloric region (Habel, 1956) caused a stimulus-related increase in impulse activity. The increased impulse activity always coincided with increased tension, and usually with e.m.g. activity. A silent phase occurred late in the response, which had a duration that varied with the strength of the stimulus applied. For example, a 2 s stimulus produced a silent phase lasting 16 s, and a 4 s stimulus period gave a silent phase which lasted 25 s. During the silent phase the unit could still be excited by compression. Compression during the active phase increased the discharge frequency by 50%.

Chemical sensitivity

Irrigating the mucosa with appropriate solutions at 39 °C caused an increase in the impulse frequency of tension receptors. The following solutions were effective: potassium chloride, 25–150 mM; hydrochloric acid, 25–75 mM; hydrochloric acid, 50 mM in normal saline; acetic acid, 50–100 mM in normal saline; butanoic acid,

22 mM; lactic acid, 100 mM in saline; sodium hydroxide, 25 mM in saline; sodium bicarbonate, 299–815 mosmol kg^{-1} and tyrosine, 50 mM. Normal saline alone caused no change in activity, although saline and also bile reduced the activity caused by acids (Fig. 8*A*). The increased impulse activity started within 5–20 s of application and with continuous exposure persisted for up to 10 min. It was always coincident

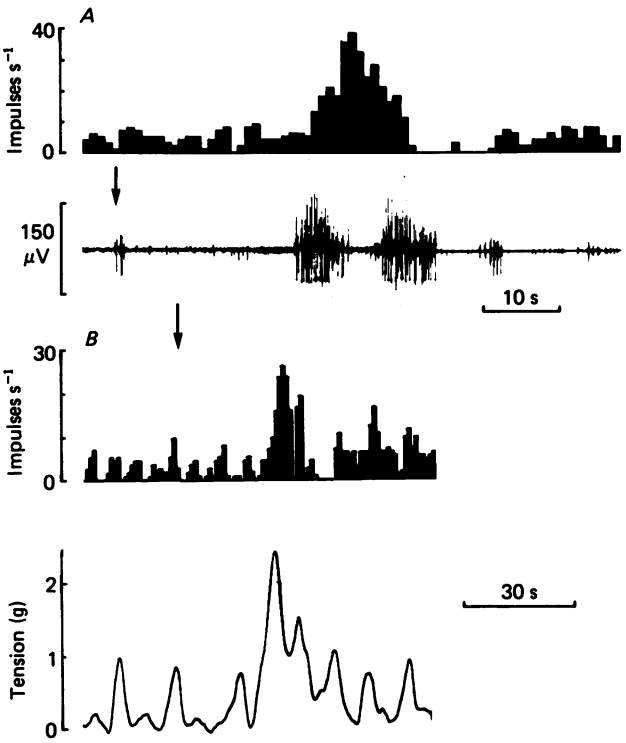

Fig. 7. The effect of intravenous pentagastrin (1 μg kg^{-1}, at arrows) on the discharge of tension receptors (upper trace in *A* and *B*) and on simultaneously recorded e.m.g. (lower trace in *A*) and longitudinal tension (lower trace in *B*). *A*, an increase in the e.m.g. activity precedes the rise in impulse frequency and there is a pause before the background discharge is resumed. *B*, there is a close correlation between the tension developed and the impulse activity. The characteristic pause in the impulse train is also present.

with visible movements, changes in tension and e.m.g. records. Flooding the receptive field with lignocaine (2 mg ml^{-1}) increased the latency by a further 15 s, suggesting that an indirect neural mechanism was involved in the early excitation by chemical solutions. In four of ten units the response was greatest for acetic acid (50 mM in saline, 340 mosmol kg^{-1}) and least for hydrochloric acid (25–50 mM in saline, 346–384 mosmol kg^{-1}) (Fig. 8*B* and *C*). There were smaller pH changes recorded at the mucosal surface with acetic acid (0·8 pH units) than HCl (2·4 pH units).

Serosal stretch receptors

Five mechanosensitive units were located serosally. They were either silent in the absence of applied stimuli, or had a low-frequency discharge (1–3 impulses s^{-1}). The receptive field was associated with a small tortuous artery below the serosal surface

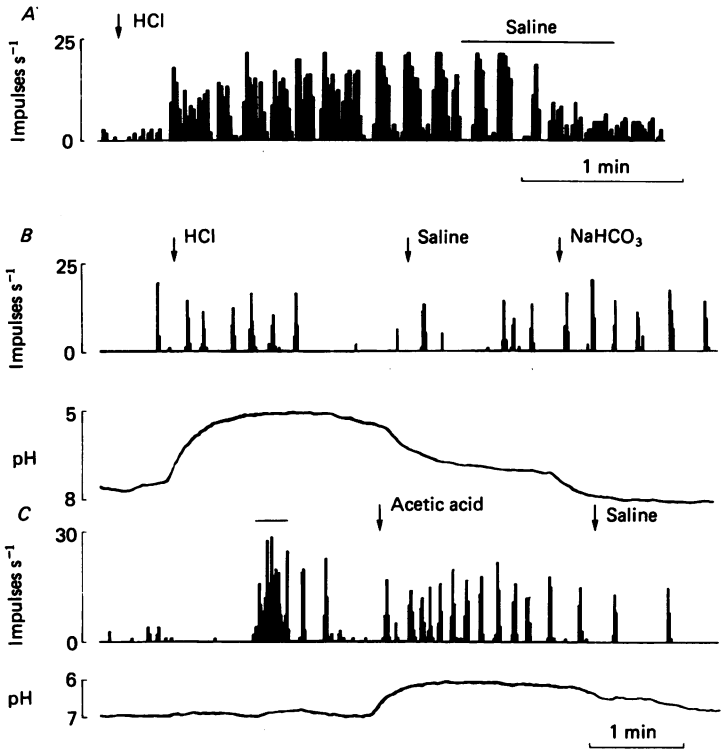

Fig. 8. *A*, a histogram of the response of a duodenal tension receptor to mucosal irrigation with acid (25 mM-hydrochloric acid in saline, 346 mosmol kg^{-1}). The latency of the response could be delayed further by antecedent mucosal application of 2 % lignocaine to the receptive field. The activity could be inhibited by removal of the test solution and washing with saline. In *B* and *C*, the upper traces are histograms of the afferent discharge and the lower traces show the pH recorded at the mucosal surface. The time scales for *B* and *C* are the same. *B*, at the first arrow 25 mM-hydrochloric acid was applied. At the second arrow the mucosa was washed with saline, and at the third arrow it was flooded with sodium bicarbonate (297 mosmol kg^{-1}). *C*, digital compression was applied at the bar followed by 50 mM-acetic acid in saline (321 mosmol kg^{-1}) and then washed with saline. Note that the pH changes in *C* were less than *B* and that more vigorous activity was provoked by acetic acid than by hydrochloric acid. All solutions were at 39 °C.

but impulse activity was unrelated to arterial vascular rhythms or peristaltic activity. Serosal probing with von Frey hair thresholds above 0·132 g caused the receptor to discharge a few spikes. The receptive field was, in four cases, a single spot 4–8 mm^2 in area. In the remaining unit two spots 8 mm apart were found in the receptive field. When a solid probe filled the duodenum and the serosa was compressed against it, the units gave discharges with maximum impulse frequencies for the initial second of the response of 29 impulses s^{-1}. Tangential and longitudinal stretching of the serosa with blunt forceps and passive distension with intraluminal balloons caused sustained activity. These lasted throughout the stimulus with maximum frequencies of 23–43 impulses s^{-1} during the initial second of the response. Discrete probing from the mucosal surface was ineffective. Repetitive electrical excitation of the duodenal wall and local nerves caused a poorly sustained increase in impulse activity. The mean conduction velocity of three units was $1{\cdot}8 \pm 0{\cdot}5$ S.D. m s^{-1}.

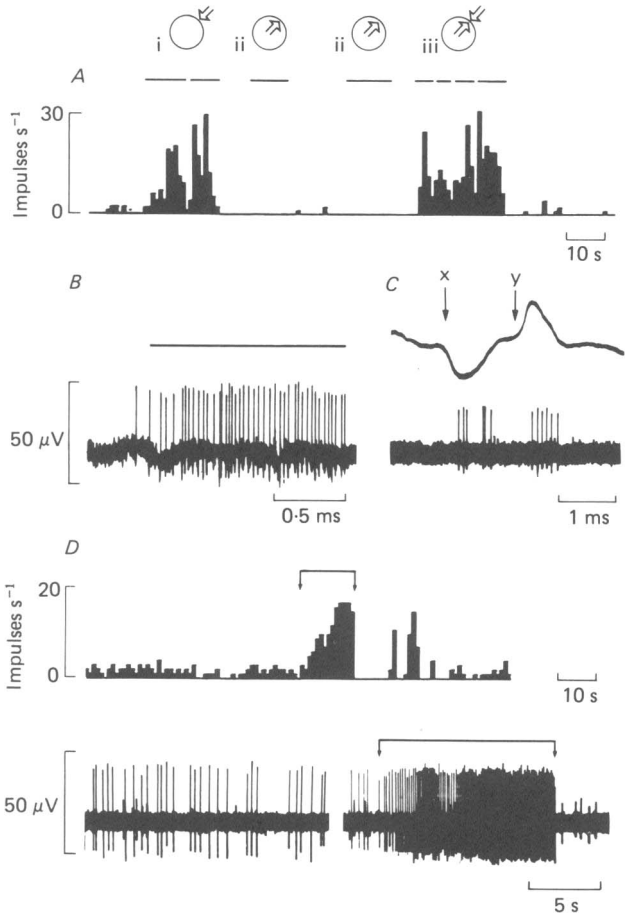

Fig. 9. The responses of serosal and omasal receptors to mechanical stimulation. *A*, serosal receptor. The effect of probing (i) the serosa, (ii) the mucosa and (iii) both on the discharge of a serosal receptor are illustrated as frequency histograms. *B*, the spike train caused by sustained serosal probing at the bar. *C*, a rapidly adapting discharge was caused by brushing the same serosal unit with a fine paint brush attached to a force transducer. Movement is denoted by the change in force recorded at arrows x and y. *D*, omental receptor. The histogram illustrates the background activity of a receptor located in the lesser omentum. Between the arrows the lesser omentum was manually twisted and a peak frequency of 17 m s^{-1} (minimum interspike interval 50 ms) was initiated. The discharge frequency built up in crescendo and abruptly stopped when the twist was released. A period of background activity and the twisting response are illustrated below.

Effect of drugs on the serosal units

All impulse activity was abolished when a single drop of procaine HCl was applied to the receptive field. Topical application of veratrine (one drop 100 μg ml^{-1}) caused a bursting discharge which lasted up to 16 min. Bursts occurred at a rate of 5·4 per minute and the discharge frequency was 20 impulses s^{-1} during bursts. Veratrine caused no change in the mechanical sensitivity to compression. Cholecystokinin (CCK-8, CCK-39, one drop 1 μg ml^{-1}) applied topically caused a 50 % increase in the background activity in two units. Potassium chloride solutions (298 mosmol kg^{-1}),

atropine, sulphate and hexamethonium applied topically (5–10 μg) each excited units. The evoked activity increased to peak frequencies of 7–11 impulses s^{-1} and decayed to silence after 30–60 s. The latency of the response was less than 1 s for potassium chloride, 4 s for atropine and 10 s for hexamethonium (Fig. 10). There was no response to topical serosal applications of acetylcholine (50 μg); adrenaline

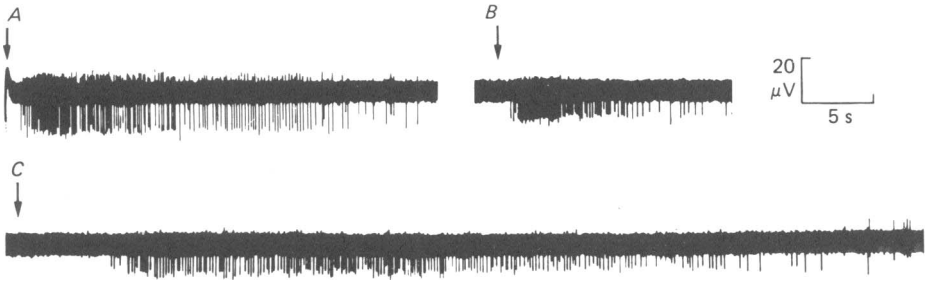

Fig. 10. The effect of topical application at the arrows of selected drugs on the discharge of serosal receptors. The total responses are shown. *A*, atropine (one drop of 200 μg ml^{-1}) caused a prolonged discharge reaching frequencies of 12 impulses s^{-1}. *B*, isotonic potassium chloride (150 mM) caused a shorter discharge at peak frequencies of 12 impulses s^{-1}. *C*, hexamethonium (one drop of 200 μg ml^{-1}) caused a more prolonged discharge after a longer latency, reaching frequencies of 6 impulses s^{-1}.

(1/20000); phenylbiguanide (100 μg); pentagastrin (250 μg) or insulin (2 u.). One unit was excited by topically applied phenylbiguanide after a 2 s latency. Four units were inhibited by close intra-arterial injections of 5-hydroxytryptamine (25 μg). One unit was excited by close intra-arterial 5-hydroxytryptamine after a 5 s latency. The response coincided with visible movements of the receptive field. Local intra-arterial injections of CCK-8 (1 μg) and phenylbiguanide caused no change in impulse activity. No change in discharge pattern was seen with intravenous insulin (0·1 u. kg^{-1}).

Omental receptors

Eleven units were located in the lesser omentum. They had a resting discharge at frequencies of 2–3 impulses s^{-1} which was abolished by off-loading the omentum. The receptive field of a unit was usually a single spot (16–100 mm^2) situated at the bifurcation of the arterial, lymphatic and venous vessels. One unit had two discrete receptive fields, each 10 mm^2 in size and 1·2 cm apart. Probing caused a brief response of a few spikes only. Stretching at thresholds above 200 mg caused a persistent adapting response at peak frequencies of 25 impulses s^{-1} which was maintained for 60 s with the probe in place. The impulse activity was unchanged by the removal of the parietal sheet of the omentum in the region of the receptive field. Units were easily excited by weak electrical currents applied at the receptive field. Four units had a mean conduction velocity of $0{\cdot}75 \pm 0{\cdot}18$ S.D. m s^{-1} and a further unit had a conduction velocity of 7·3 m s^{-1}.

Effect of drugs on omental receptors

With the exception of atropine, hexamethonium and potassium chloride solutions there was no excitation of units by drugs used unless distortion of the omentum resulted. Atropine (10 μg), hexamethonium (10 μg) and potassium chloride

(298 mosmol kg^{-1}) excited units for periods up to 5 s at peak frequencies of 4–6 impulses s^{-1} when they were applied topically. The following drugs were without effect on the activity of units: pentagastrin, 250 μg topically, 1 μg kg^{-1} intracarotid and intra-arterially; noradrenaline, 1/10000 topically; phenylbiguanide, 200 μg intravenously; papaverine, 2×10^{-4} M topically; veratrine, 200 μg intravenously.

DISCUSSION

Duodenal and pyloric slowly adapting mechanoreceptors are present in the cat (Mei, 1970; Bitar, Mei & Michelucci, 1975) which are excited by distension and electrically evoked muscle contractions. These and the majority of the mechanoreceptors situated in the muscularis externa of the sheep duodenum therefore have characteristics which fit them into the proposed concept of 'in series' tension receptors (Iggo, 1955; Leek, 1969). The evidence in this paper indirectly suggests that those in the sheep duodenum are associated with longitudinal muscle. Although the tension measurements in the muscularis externa were not precise enough to demonstrate whether individual muscles were involved in the development of tension, usually receptors were excited during increases of longitudinal tension which suggested that they were situated in longitudinal muscle. Longitudinal duodenotomy, a procedure causing complete section of circular muscle, usually did not alter the discharge pattern. Further support for this interpretation is that receptive fields which responded to perpendicular probing had an elliptical longitudinal axis. The activity of an 'in series' receptor in the longitudinal muscle would be expected to be inhibited during reflex contraction of circular muscle during peristalsis and also following coaxial electrical stimulation (Kottegoda, 1970). When peristalsis was provoked by electrical excitation the increased impulse activity coincided with the earliest mechanical and e.m.g. changes, and the variable period of silence which followed excitation may correspond with relaxation of tension in the longitudinal muscle during circular muscle contraction. In addition, when the circular muscle was not contracting the discharge would be expected to persist throughout the stimulus (Kottegoda, 1970). A persistent discharge was, in fact, demonstrated during local compression. The poorly sustained discharge present during propulsive movements of an intraluminally placed balloon, when an annular constriction developed behind the balloon, may also have coincided with a reduced tension in the longitudinal muscle. The general conclusion reached, therefore, is that the tension receptors reported here do function as 'in series' tension receptors, lying in the longitudinal muscle layers.

Matthews (1933) demonstrated that some mechanoreceptors are functionally in parallel with the contractile elements of skeletal muscle and that such receptors are off-loaded when active contraction occurs, and excited when stretch is applied. 'In parallel' receptors respond therefore to changes in length rather than other movement parameters. This concept has been applied to hollow viscera: units with 'in parallel' characteristics have been described in the dog gastric antrum (Takeshima, 1974), and the cat oesophagus (Harding & Titchen, 1975), and Todd (1964) found units in the anal sphincter of the cat which were silenced during contraction and which may belong to this category. Sheep serosal units were functionally distinct from 'in series'

tension units because they did not have periodic discharges, were superficially located in the serosa and had different sensitivities to the drugs applied. They may function as 'contact' receptors (Leitner & Perl, 1964) because of their sensitivity to touch. Their persistent discharge pattern distinguishes them from 'movement detectors' (Bessou & Perl, 1966). They resemble closely the receptors found by Morrison and colleagues (Floyd & Morrison, 1974*a*, *b*; Floyd, Hick & Morrison, 1976*a*, *b*; Floyd, Hick, Koley & Morrison, 1977). Mesenteric and omental units (Iggo, 1957; Morrison, 1973, 1977) cannot be included in the 'in parallel' concept because they are not functionally involved in the contractile apparatus. As was apparent here, receptors in the omentum are excited by distortion of the omentum and, depending upon their relative position, they may behave as tension or stretch monitors of the omentum only. The mechanoreceptors first described by Bower (1966) in the broad ligament of the uterus may also function in this way.

The three mechanoreceptor classes found in the sheep duodenum may not be fundamentally different receptors, for their responses may be determined by their different location. It has previously been suggested that the same receptor type in the ferret stomach may well respond to tension in one location and stretch in another (Andrews, Grundy & Scratcherd, 1980). In other locations, visceral mechanoreceptors respond to transmural compression, and others to tangential stretching. Some tension receptors in the reticulo-rumen are insensitive to transmural compression (Leek, 1969). Tangential stretching is important in the isolated intestine of the guinea-pig as a stimulus for peristalsis (Kosterlitz, 1968) and the carotid sinus baroreceptor reflex (Hauss, Kreuziger & Asteroth, 1949), although the actual locations of the relevant receptors are unknown. Aortic and atrial baroreceptors respond to transmural pressure (Goetz, Hermreck, Slick & Starke, 1970; Angell James, 1971). Both these studies are based on the observation of reflex responses and the receptor mechanisms are not known. Iggo (1955) demonstrated the sensitivity of alimentary tension receptors to compression in the oesophageal groove of goats, and this characteristic response was utilized in the present experiments to provide responses for quantitative analysis. This stimulus presumably reduced the effect of the elastic and compressive properties of underlying tissues, which thus must contribute significantly to adaptation. However, because of differences between individual preparations it was only possible to compare the sensitivities of units found in the same animal, and there were often marked differences in mechanical threshold in units in similarly located receptive fields. This result gives some support to the concept of separate populations of tension receptors with different mechanical thresholds (Iggo & Leek, 1967; Grundy & Davison, 1981). Because sheep duodenal tension receptors responded to longitudinal increases in tension evoked by drugs with greater peak frequencies at lower tensions (Cottrell & Iggo, 1984*a*), compression is apparently not the most effective stimulus. The impulse frequency was greatest when compression occurred together with natural contraction or electrically provoked activity. However, it seems probable that transmural pressures have an important influence on the sensitivity of the tension receptor and that hydrostatic pressure changes, in different parts of the abdomen, probably influence their behaviour. Thus compression may play an important sensitizing role in reflexes which modify food intake, for example during pregnancy or following accumulation of excess abdominal fat (Forbes, 1980).

Together with the pharmacological responses reported elsewhere (Cottrell & Iggo, 1984*a*), these results support the idea that 'in series' mechanoreceptors function differently from serosal and omental receptors. In summary, 'in series' receptors are noticeably excited by intra-arterial injections of cholecystokinin, 5-hydroxytryptamine and acetylcholine. These three drugs enhance contraction of the muscularis externa. Most serosal units either did not respond to these drugs or were inhibited by them. This agrees with the different drug sensitivity of omental units and tension receptors examined elsewhere (Paintal, 1957, 1964). The insensitivity of omental units to phenylbiguanide found in the cat (Iggo, 1957) has now been observed in the sheep. A summary diagram of the three receptor classes is given in Fig. 11.

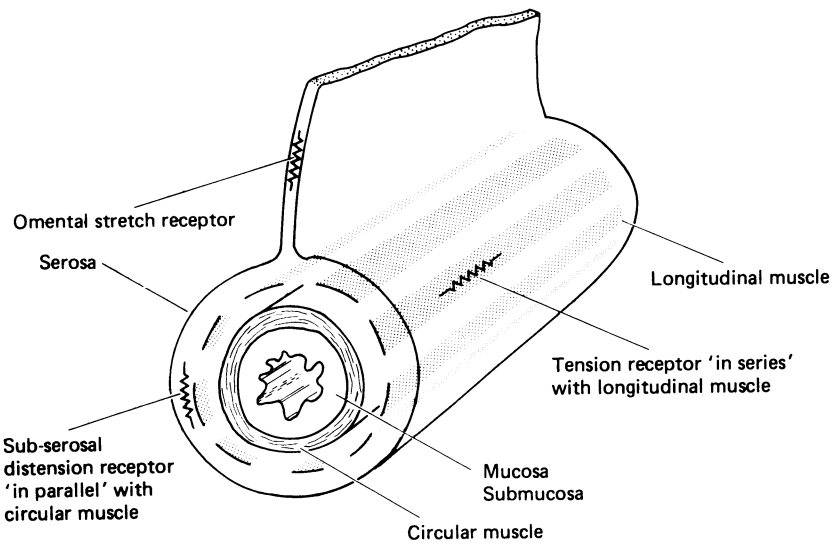

Fig. 11. Summary diagram of non-mucosal mechanoreceptors of sheep duodenum. The results suggest that tension receptors are located in series with longitudinal muscle, serosal receptors respond to stretch in the tangential direction, and omental receptors respond to stretching of the omentum only. -⋀⋀⋀- = mechanoreceptor.

The excitation of tension receptors by chemical solutions, particularly by acids, was considered to be a consequence of intramural tension changes caused by intrinsic reflex mechanisms. This conclusion was reached because responses were delayed by mucosal anaesthetics which did not otherwise affect the mechanical responses of tension receptors. There is indirect support for this proposed mechanism because Gregory (1979) has found that acid infusion abolishes gastric motility in chronically bivagotomized sheep, a preparation in which local mechanisms are active. The long latency of the response to chemical solutions in the presence of anaesthetic does not exclude the possibility that local hormone release may also be involved. No excitatory response to chemical solutions has been seen in preparations of gastric tension receptors in the sheep (Iggo, 1957; Harding & Leek, 1972) and rat (Clarke & Davison, 1975). One explanation may be that volatile fatty acids are known to inhibit reticulo-rumen motility in sheep (Gregory, 1979). However, there is evidence that acid will enhance motility in intestinal circular muscle (Summers, 1978), cause initial

excitation of duodenal bulb motility in sheep (Gregory, 1982*b*) and cause excitation of a mixed afferent population from the intestine (Zamiatina, 1957).

The interaction between discharges of tension and mucosal receptors during probing of the mucosa suggests that an intrinsic mechanism, initiated by the contents of the duodenum, can alter the motor profile of the viscus. Thomas & Baldwin (1968) and Davison (1972) have found, during studies of reflex responses, that a duodenal catheter will cause brief inhibition of gastric peristalsis. The results of single-unit afferent activity presented here show that catheters may also affect the activity of the local tension receptors in the duodenum, probably by an intrinsic reflex mechanism which may first inhibit and then excite them. Presumably the mechanism acts by altering muscle tension rather than by directly influencing the receptor itself. This mechanism may explain the ambiguous sensation resulting from the afferent input from gastric tension receptors. Thus, during active contraction of the empty stomach there is a feeling of 'hunger pangs' and emptiness, whereas with a distended stomach, with presumably vigorous activity in tension receptors, there is a sensation of 'fullness'. This conflict may be resolved centrally (Leek, 1972). The evidence presented here suggests alternative mechanisms: excitation of sensitive mucosal receptors may initiate inhibitory mechanisms, not necessarily utilizing long extrinsic reflexes and 'high-threshold' nervous pathways, but involving peripheral interactive mechanisms in the intrinsic plexus. In addition, extrinsic reflex mechanisms may be involved in the sensation of fullness because both serosal and omental receptors are probably excited by gastric distension. Thus the medullary centres controlling gastric function may receive sensory information from separate populations of modality-specific receptors located in the duodenum and its vicinity.

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