Motor control of the stomach

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Abstract. — Gastric motility is controlled at various levels including the enteric nervous system (ENS). The gastric ENS is involved in the regulation of accommodation reflexes as well as of the peristaltic waves which are responsible for grinding and emptying. Polarised projections consisting of ascending cholinergic and descending nitric muscle motor neurons make up the hard wired circuits for control of muscle activity. In an isolated flat sheet preparation of the gastric corpus we investigated stretch evoked responses. The responses at the site of the distension as well as proximal and distal to the distension consisted of a cholinergic excitation whereas a nitric inhibition was only observed at the site of the distension stimulus. At all sites the responses were significantly reduced by the neurotoxin tetrodotoxin suggesting a neural component. In addition the nicotinic blocker hexamethonium reduced the responses at all sites to the same degree as tetrodotoxin which indicated the strong contribution of ascending and descending cholinergic interneurons. The reflexes of isolated gastric corpus preparations to distension are dominated by excitatory responses. Only the muscle response at the site of distension exhibited an inhibitory response which is usually dominated by the cholinergic excitatory response.

Key Words: Stomach, Motility, Enteric nervous system, Gastric reflexes.

Gastric Motor Patterns and the Gastric Enteric Nervous System

Neural reflexes, together with endocrine and paracrine controls, serve to coordinate and control gastric motor function. Gastric motor function can be divided into two distinct activities which can be differentiated on a regional basis. The proximal stomach consisting of corpus and fundus serves as a reservoir and has a remarkable capacity to accommodate large meals for subsequent delivery to the intestine. The smooth muscle cells in this region generate myogenic tone which can be actively inhibited to bring about gastric relaxation. This occurs during receptive as well as adaptive relaxation. The relaxation involves parasympathetic innervation via the vagus nerve and, in particular for the adaptive relaxation, the enteric nervous system (ENS). Gastric tone is also influenced by the sympathetic innervation leading to inhibition of gastric motility.

The antrum, in contrast, functions as a peristaltic pump to mix, grind and propel gastric contents through the pylorus into the duodenum. These phasic, rather than tonic, contractions originate in a pacemaker region in the middle or orad corpus, and contractions propagate towards the pylorus. During gastric emptying of a meal the corpus and antrum together with the pylorus and duodenum must act in a coordinated fashion to optimize delivery of chyme to the duodenum. Reflexes between corpus and antrum are responsible for the fine tuning of this coordinated activity.

The motor programs for controlling gastric tone and peristaltic contractions rely on feedback from sensory receptors positioned both within and outside the gastric wall. These monitor the prevailing conditions and trigger the appropriate response in the motor supply to the gastric smooth muscle. This reflex activity is organised at a number of different levels, all of which interact in an integrative way. A major local control system is the ENS. Reflexes are triggered by local sensory inputs with the integrative ability of the ENS allowing the assimilation of information from the muscle and mucosa to determine the appropriate motor response. The gastric ENS in turn is influenced by the extrinsic elements of the autonomic nervous system through the parasympathetic and sympathetic nerves, which modulate activity in the ENS. Motor neurons in the ENS, be they excitatory or inhibitory, therefore provide the final pathway for both intrinsic and extrinsic reflexes.
The ENS in the stomach differs from other regions of the gut in that it consists of only one ganglionated plexus. The intrinsic innervation of the stomach is primarily located in the myenteric plexus since a ganglionated submucosal plexus is either very sparsely present or completely absent\(^{3-7}\). Therefore, one must conclude that the myenteric plexus contains neurons involved in the regulation of both muscle and mucosal functions.

**Electrophysiology of Gastric Enteric Neurons**

The electrical and synaptic properties of gastric myenteric neurons differ from those in the small and large intestine in a number of ways. The most striking difference is the total lack of AH (after-hyperpolarization) cells in the corpus region\(^8\). A few neurones with properties similar to AH cells have been described in one study on antral myenteric neurons\(^9\). The majority of gastric myenteric neurons behave like phasic or tonic S (spontaneously firing) cells and they have been termed Gastric I and Gastric II neurons, respectively\(^8\). A small population of neurons (less than 5%) which received fast synaptic input but were otherwise inexcitable were called Gastric III neurons\(^8\).

In addition to differences in electrical behaviour, there are also differences in the synaptic properties of gastric and intestinal enteric neurons. Synaptic transmission to gastric myenteric neurons, whether originating from intrinsic myenteric or extrinsic vagal fibers, appears to rely mainly on fast excitatory postsynaptic potentials (fEPSPs). In contrast to intestinal enteric neurons, all gastric myenteric neurons receive fEPSPs\(^{10,11}\). The transmitter for these fEPSPs is acetylcholine which activates postsynaptic neurons via nicotinic receptors. Unlike in the small intestine, where some fEPSPs are mediated by non-cholinergic transmitters, the fEPSPs in the stomach are blocked by hexamethonium and are purely cholinergic\(^{10,12}\). Electrical stimulation of either vagal or intrinsic fibers at moderate frequencies (<20Hz) produced only occasionally slow EPSPs which are very likely mediated by acetylcholine or tachykinins\(^{10,11,13}\). Slow IPSPs are very rare events in the gastric myenteric plexus, do not occur in the corpus and have only been observed in about 1% of antral neurons\(^{10,11}\).

Although slow EPSPs are relatively rarely induced, a long lasting excitation of gastric myenteric neurons may be achieved by prolonged stimulation of cholinergic synapses. This is possible because the fEPSPs in the stomach do not show the run-down phenomenon. Recurrent activation and positive feedback regulation involving release of acetylcholine are also mechanisms to raise the excitation level over extended periods of time\(^{10,14}\). Spontaneous fEPSPs in a minority of gastric myenteric neurons is evidence for ongoing release of acetylcholine which coincides with basal cholinergic tone present in isolated stomach preparations\(^{15,16}\).

The majority of gastric myenteric neurons receive vagal input as demonstrated directly by electrophysiological recordings\(^13\). Electrical stimulation of the vagus evokes cholinergic fEPSPs mediated via nicotinic receptors. Thus both intrinsic and vagal extrinsic transmission is dependent on cholinergic nicotinic synapses. Cholinergic and nitric motor neurons, as well as interneurons, are synaptically activated by vagal fibers.

**Neurochemistry of Gastric Enteric Neurons**

The two enzymes choline acetyltransferase and nitric oxide (NO) synthase are markers for cholinergic and nitric myenteric neurons in the stomach, respectively\(^17\). The most detailed information on the neurochemical coding of myenteric neurons in the stomach has been obtained for the guinea pig. The proportion of cholinergic versus nitricergic neurons is about 70% to 30% both in the fundus and corpus. However, the cholinergic and nitricergic populations may be further subdivided according to the additional presence of other antigens and in this respect more than ten neuronal subpopulations have been described in the guinea pig stomach\(^17\). Cholinergic subpopulations (at least 8) outnumber the nitricergic subpopulations (at least 3).

The relative lack of immunoreactivity to the calcium binding protein calbindin in the stomach initially reported\(^17,19\) is very likely due to the antibodies used. Whereas most antibodies stain enteric neurons in the small and large intestine, only a few of them are able to detect calbindin-positive neurons in the stomach\(^20\). Interestingly, there is an increase in the density of calbindin-positive...
neurons from fundus to antrum (12% in the fundus, 12% in the corpus and 25% in the antrum). Unlike in the small intestine, gastric calbindin neurons do not appear to project to the mucosa but rather to other myenteric ganglia20,21. In the small intestine, one population of calbindin-positive neurons are candidates for sensory neurons22,23. Either the stomach contains only very few intrinsic primary afferent neurons, which is unlikely, or some other cell type serves this role. It remains to be studied whether mechanosensitive interneurons which have been recently described in the colon are also operative in the stomach24. The distension-evoked reflexes in isolated stomach preparations may suggest this (see below).

**Links Between Neurochemistry, Gastric Enteric Circuits and Stomach Functions**

The chemical coding of gastric myenteric neurons agrees with the differential motor responses evoked by electrical stimulation of the isolated intact stomach or of gastric muscle strip preparations25,26. The response to field stimulation is generally biphasic with an initial transient excitation followed by a longer lasting relaxation of the muscle. The major mediator of the contractile response at the neuroeffector junction is acetylcholine acting at muscarinic receptors. The acetylcholine is probably released from more than one population of cholinergic myenteric neurons along with other transmitters including substance-P. The relaxation is mediated by non-adrenergic non-cholinergic (NANC) nerves. Several NANC transmitters including purines27, vasoactive intestinal polypeptide28, pituitary adenylate cyclase-activating peptide29 or NO30 have been proposed and shown to be released upon field stimulation31. The coordinated release of two or more substances is likely to be involved in the relaxation response which can often reveal multiple components. Blockade of NO release also enhances cholinergic responses evoked by electrical field stimulation and ganglionic stimulation by nicotine or dimethylphenylpiperazinium (DMPP)32,33. Transmural stimulation is reported to evoke stronger contractions, more vigorous peristalsis and a more pronounced relaxation than electrical stimulation of the vagus alone25,26 while DMPP evokes a stronger relaxation than that evoked by stimulation of the vagus34. It appears, therefore, that transmural stimulation activates nerves inaccessible to the vagus, which may be truly “autonomic”25,26. This has been confirmed by electrophysiological studies demonstrating that not all gastric myenteric neurons receive vagal input13.

The cholinergic and nitric pathways within the myenteric plexus are polarized into ascending and descending projections, respectively, in all gastric regions21,35-37. Very few neurons in the stomach, all of them calbindin-positive, have multiple long processes: they have very characteristic features, like an elongated soma shape, and they have about threefold the average size of other neurons and basket-like terminal endings outside the ganglionated plexus20. Although their function is unknown, their projections and morphology would indicate that they are involved in interneuronal and probably sensory functions. In the stomach, as in the small intestine, there is a projection preference of cholinergic and nitric myenteric neurons20,21,36-42. The vast majority of cholinergic motor neurons projects in an ascending direction; in contrast, the vast majority of nitric motor neurons is descending37. This projection pattern applies also to interneurons. Thus, cholinergic interneurons have primarily ascending and nitric interneurons primarily descending projections. In contrast to the transmitter phenotype of descending motor neurons, descending interneurons are relatively more often cholinergic39. This means that in the stomach ascending and substantial parts of descending interneuronal pathways release acetylcholine and are therefore excitatory. The fibers typically run for 0.5-20 mm in the longitudinal direction, with 90% of the fibers projecting within 0.5 to 5 mm. Long projections over several centimeters are extremely rare in all regions of the stomach.

Unlike in the ileum, the gastric longitudinal muscle layer receives a polarized innervation by ascending cholinergic and descending nitric myenteric neurons. Interestingly, the neurochemical coding of the longitudinal muscle motor neurons is not different from that of circular muscle motor neurons43. However, it is clear that different subpopulations of motor neurons supply the different muscle layers because the projection patterns strikingly differ between circular and longitudinal muscle motor neurons. This target specific projection pattern is revealed by the finding that most longitudinal muscle motor neurons have longitudinal projection preferences whereas
circular muscle motor neurons tend to project circumferentially. Thus, the projection of the muscle motor neurons mainly follows the orientation of their target muscle layer\textsuperscript{43}. Functionally, the projection preference of cholinergic and nitrergic motor and interneurons would indicate the presence of a hardwired circuit which upon stimulation could initiate ascending excitation and descending inhibition of the muscle layers. The isolated guinea pig stomach approximately triples its volume and enlarges both in the circular and longitudinal direction in response to distension over a physiological range\textsuperscript{15}. This adaptive relaxation has been attributed to the release of NO\textsuperscript{44}; the final motor pathways involved in this reflex are the nitrergic pathways in the myenteric plexus. Prior to the adaptive relaxation, the isolated guinea pig stomach initially shows an increase in intraluminal pressure in response to distension\textsuperscript{16}. This response is atropine-sensitive and mediated by the release of acetylcholine, which indicates that the cholinergic pathways in the myenteric plexus might be involved in this reflex.

The coordinated behavior of muscle activity might also be relevant for peristalsis in the stomach. The degree of relaxation and the level of contractile activity or tone at adjacent regions is a major determinant of the propulsive force of the peristaltic wave and thereby influences gastric emptying\textsuperscript{45}. A functional descending inhibition is indicated by myotomy experiments designed to sever the intramural pathways between the corpus and antrum\textsuperscript{46}. As a result of this projection the contractile activity aboral to the myotomy increases and leads to constriction of the antrum. Increased tone of the isolated stomach treated with the nitro-L-arginine methyl ester (L-NAME) would indicate that NO is responsible, although not solely, for maintenance of inhibitory tone\textsuperscript{16}.

In vivo studies based on X-ray examination of the stomach indicate the importance of muscle activity and tone just oral and aboral to a peristaltic wave\textsuperscript{45}. The degree of relaxation and the level of contractile activity above and below a peristaltic contraction determines the propulsive force of the peristaltic wave and thereby the net transport of gastric content towards the pylorus\textsuperscript{47}. The spatial specificity and local modulation of neural activity determines the occurrence and timing of lumen occlusion relative to adjacent segments. Since peristaltic activity in the stomach can be observed in the isolated stomach\textsuperscript{25,26,48}, it must be concluded that intact extrinsic reflexes are not required for peristalsis. Moreover, even a muscle preparation where the myenteric plexus has been removed will respond to stretch with contractions indicating that myogenic mechanisms may also be involved\textsuperscript{49}.

However, it appears that the classical peristaltic reflex consisting of a contraction oral and a relaxation aboral to the stimulus\textsuperscript{50} is not readily reproducible in the stomach. Yet there is numerous evidence for intrinsic reflex activity in the isolated stomach of a variety of species, starting with the pioneering work at the end of the 19th century which demonstrated responses to mechanical (touching the serosal surface), chemical (crystals of sodium chloride) and electrical stimuli which sometimes induced peristaltic waves and produced variable motor responses in the stomach at the site of the stimulus as well as at oral and aboral sites\textsuperscript{16,49,51-56}. In most cases atropine-sensitive local contractions and contractions oral to the stimulus were observed. Mechanical or chemical stimuli can also evoke an aboral contraction which sometimes obscures the small relaxations\textsuperscript{49}. These cholinergic influences are readily demonstrable in isolated muscle strips\textsuperscript{15,57} in which phasic activity will cease after removing the myenteric plexus\textsuperscript{49}.

Later it has become apparent that the isolated stomach can orchestrate complex contractile responses to mechanical stimulation of the muscle or mucosa despite the absence of connections to the brain and spinal cord. Distension of the isolated stomach evokes or enhances peristaltic activity\textsuperscript{58,59}. In most studies gastric reflexes occurring close to the site of stimulus have not been carefully distinguished from reflexes occurring at a distance. This is relevant as the projection of ascending and descending neurons in the gastric myenteric plexus is only a few millimeters\textsuperscript{21,36-38}. For initiation of phasic contractions and for the propagation of peristaltic contractions continuity within the myenteric plexus has to be preserved\textsuperscript{49,60-64}. It is likely that a chain of cholinergic neurons is involved in the longitudinal spread of the peristaltic wave because the ganglionic blocker hexamethonium is able to prevent peristalsis in response to transmural stimulation in an isolated stomach preparation\textsuperscript{26,65}. The neural control mechanisms in the stomach can obviously initiate a variety of different reflex patterns depending on whether adjustment more locally or at more distant sites is required\textsuperscript{66}. These reflex pathways have been de-
duced from intracellular muscle recordings in response to distension. Descending inhibitory reflex pathways exist between the fundus and corpus, ascending inhibitory and excitatory reflex pathways between the antrum and corpus and between corpus and fundus.

**Distension-Evoked Mechanical Responses in the Guinea Pig Stomach**

We performed a series of experiments to test the mechanical response of an *in vitro* preparation of the guinea pig stomach corpus to stretch distension. Male and female guinea pigs (weight 459.6±10.4 g, range 257-700 g) were killed by cervical dislocation. All experimental procedures were approved by the Ethical Committee of the Technische Universität München. The stomach was taken out, placed in cold (4°C) Krebs solution (in mM: 117 NaCl, 4.7 KCl, 1.2 MgCl₂, 6H₂O, 1.2 NaH₂PO₄, 25 NaHCO₃, 2.5 CaCl₂, 2H₂O and 11 glucose; all chemicals from Sigma, Steinheim, Germany) and opened along the greater curvature. For all experiments a region of the parietal side of the corpus of 5 cm (longitudinal) x 2 cm (circular) was cut away and placed in a plastic dish with a Sylgard (Dow Corning, Wiesbaden, Germany) bottom, containing fresh, cold Krebs solution (Figure 1). The mucosa was left intact in the preparations; only in the experiments with atropine and L-NAME we removed the mucosa with fine forceps and scissors. To apply a stretch stimulus a small plate (polypropylene) of 0.523 x 0.375 cm was placed under the central part of the tissue. A fine thread (Braun-Silkam, Braun, Germany) was tied to the plate and fed through the tissue with a needle. The tissue was then pinned flat under light stretch with the mucosal side facing up. The tissue around the plate was additionally pinned to the bottom of the dish. Thus it was ensured that the stretch stimulus was applied only to a limited area of the preparation. The mechanical response of the circular muscle was recorded with force transducers (FSG-01) at the site of stimulation (central), 1 cm proximal (proximal) and 1 cm distal (distal) to the site of distension (Figure 1). Thus it was possible to record the functional output of local (central recording site), ascending (proximal recording site) and descending (distal recording site) enteric pathways. All force transducers were connected to a bridge amplifier (Octal bridge, ADInstruments, Spechbach, Germany) and signals were recorded with a PowerLab 8sp (ADInstruments) and Chart software (Chart 4.12, ADInstruments). During the experiments the tissue was continuously superfused with 37°C Krebs solution.

After an equilibration period of 45 min the tissue maintained a stable baseline muscle tone, with mucosa (mean±SEM): proximal 19.0±0.9 mN, central 17.4±1.2 mN, distal 20.7±1.1 mN, n=20; without mucosa proximal: 26.2±1.0 mN, central 28.1±1.1 mN, distal 34.6±1.6 mN (n=20). The basal tone was superimposed by regular contractions. Their frequency was with mucosa (mean±SEM): proximal 5.2±0.4 contractions/minute (cpm), central 4.5±0.1 cpm, distal 4.0±0.2 cpm; without mucosa: proximal 5.1±0.1 cpm, central 4.2±0.1 cpm, distal 4.3±0.1 cpm (n=20). The contraction amplitude was with mucosa: proximal 3.9±0.4 mN, central 4.7±0.4 mN, distal 11.2±0.8 mN; without mucosa: proximal 5.3±0.3 mN, central 5.4±0.5 mN, distal 10.4±0.9 mN (n=20). The experimental protocol was as follows. Initially, a
A series of random stretch stimuli (10, 25, 50, 75, 100 mN corresponding to 0.51, 1.26, 2.55, 3.83, 5.1 kPa, respectively) was applied to the tissue by elevating the plastic plate with the aid of the motorized manipulator. The stretch was released after 60 s and the responses of the three muscle strips (distal, central and proximal) were recorded simultaneously (Figure 2). The response to these stimuli served as controls. Drugs (see below) were added to the superfusing solution. After an equilibration period the same stimuli as above were applied. Finally, we perfused papaverine (10 µM) to relax the muscle completely and reapplied the stretch stimuli to record the passive response of the tissue to the applied stretch. For analysis, we calculated the mean of all tissue responses to a specific stimulus and subtracted the mean of the response in the presence of papaverine. Thus, the data presented in the following reflect only the active (myogenic and neurogenic) responses of the tissue to stretch stimuli. Data were compared with t-test or paired t-test with a significance limit of p<0.05.

**The Mucosa Influences Stimulus Evoked Responses of the Stomach**

The control responses to mechanical stimulations differ between preparations with and devoid of mucosa (Figure 3). In the preparations with mucosa we always observed contractile responses and they were in most of the cases larger in amplitude than the control responses in preparations without mucosa. Our study does not provide a final explanation for this discrepancy but there are several possibilities. Firstly, the differences could be merely due to the fact that the presence of the mucosa alters the mechanical properties of the preparation. Secondly, cutting all axonal connections to the mucosa may alter the properties of the enteric neurons, in particular those with mucosal projections, which could affect responsiveness to mechanical stimulation. Thirdly, sensitivity of the reflexes may depend on tonic release of mediators from the mucosa.

**The Response to Mechanical Stimuli is Region Specific**

The stretch distension stimulus applied to the central corpus always caused a contractile response in the central, the proximal and the distal recording site. The preparations never showed a clear relaxation response to the control stimuli. A similar behavior has been found by Hennig et al. using a whole stomach preparation in which a guinea pig stomach was gradually filled. In this setup, the stomach always showed an increase in pressure with increasing infusion volume. The reason for these findings might be that the stomach must maintain a certain shape during filling in order to ensure proper mixing of its contents.

![Figure 2](image-url). Responses of the distal, central and proximal region of the guinea pig stomach to a stretch distension (75 mN) of the central stomach under control conditions (black line), in the presence of the neuronal blocker TTX (0.5 µM, dark gray line) and in the presence of TTX and the smooth muscle relaxant papaverine (10 µM, light gray line). The sketches below the traces indicate the beginning of the stretch stimulus (ramp with arrow pointing upwards), the 60 s period during which the stimulus was maintained (horizontal part) and the release of the stretch (ramp with arrow facing downwards). For the analysis we calculated the difference between the response in the presence of papaverine (showing the passive tissue properties) and the other responses. The control response shows regional specificity: the response in the distal part is weaker than the response in the central and proximal corpus. TTX reveals that the responses in the distal and proximal corpus depend nearly completely on neuronal transmission while the response in the central part is of mixed neurogenic and myogenic origin.
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Figure 3. Responses to stretch stimuli of the proximal (left column), central (middle column) and distal (right column) guinea pig gastric corpus. The strength of the stimulus (10-100 mN) is indicated on the x-axis while the response amplitude is indicated on the y-axis. White bars denote the response under control conditions while the black bars denote the response in the presence of the various pharmacological agents. All data are presented as mean ± standard error. Significant differences are indicated by the black stars ($p<0.05$). The experiments with TTX and hexamethonium were done with preparations with attached mucosa while the other experiments were done with preparations in which the mucosa was removed. For discussion of the results see text.
In our experiments, the amplitudes of the contractile responses in the proximal and central corpus were always larger than the responses at the distal recording site. For a whole stomach this would be equal to an antrally directed transport. The presence of a weaker contraction at the distal site instead of a relaxation could indicate that the stomach has to maintain its shape or that there is a relative lack of descending excitatory muscle motor neurons.

The Response to Mechanical Stimuli is of Mixed Neurogenic and Myogenic Origin

Perfusion of the neuronal blocker tetrodotoxin (TTX, 0.5 µM, n=12) significantly increased basal tone in the central and proximal corpus region while the distal region was not significantly affected (mean±SEM; distal 22.0±2.1 vs. 21.7±2.2 mN, p=0.82, central 19.9±24.7 vs. 24.7±2.7 mN, p=0.05, proximal 23.8±2.5 vs. 29.6±3.3 mN, p=0.02). Similar small range specializations have so far been described in the circumferential direction of the stomach (13;36). TTX also significantly decreased the amplitude of spontaneous contractions in the central and proximal corpus regions (mean±SEM; distal 4.3±1.0 vs. 2.6±0.4 mN, p=0.14, central 3.8±0.6 vs. 2.3±0.4 mN, p=0.01, proximal 7.7±1.4 vs. 3.2±0.7 mN, p=0.01). This is consistent with the concept that ongoing contractions are generated by slow waves originating from interstitial cells of Cajal and amplified by neuronal activity67. The response to stretch stimuli was significantly reduced by TTX in all corpus regions (Figure 2, top row). While the responses were nearly abolished in the proximal and distal corpus, there are still relatively strong responses in the central region which is at the site of the distension stimulus. This indicates that the distension stimulus evoked locally a myogenic response. It is noteworthy that this response is also an excitatory one. Thus, the corpus regions of the stomach react to distension with an increase in tone and contractile activity, with myogenic and neural components at the site of distension while responses at closely adjacent sites are purely neurogenic. The presence of an intact myenteric plexus is absolutely necessary for a coordinated response of larger parts of the stomach. Interneurons must be involved here because the projection length for muscle motor neurons is usually shorter than the distance between the recording sites in the present study68,69.

Cholinergic Interneurons Mediate Signal Transmission in the Gastric Corpus

Nicotinic receptors are the main receptors for ascending and descending fast synaptic transmission in the guinea pig stomach (10,70). We therefore used the nicotinic receptor antagonist hexamethonium (200 µM, n=8) to block interneuronal transmission. This reduced basal tone and spontaneous contraction amplitude in the proximal part of the preparation, while these parameters remained unchanged in the distal and central part. Hexamethonium also significantly reduced the stretch response in all regions of the corpus to a similar degree as TTX (Figure 3, second row). Interneurons are therefore necessary for the transmission of information in the longitudinal direction of the stomach. This is consistent with findings from tracing studies that showed a longitudinal projection preference for interneurons in the guinea pig gastric corpus68. Additionally, even the local response is dependent on interneuronal signal transmission.

Distension Evoked Activation of Muscle Motor Neurons

Perfusion of the muscarinic antagonist atropine (1 µM, n=10) had no effect on basal tone in all corpus regions and reduced the amplitudes of spontaneous contractions significantly in the central and proximal part of the corpus. Atropine also reduced the contractile response to stretch stimuli in all corpus regions (Figure 3, third row). In some experiments the tissue showed a small relaxation under the influence of atropine. It can be concluded that the main excitatory transmitter that mediated the distension-evoked contractile responses is acetylcholine acting on muscarinic receptors. Other excitatory transmitters (such as substance P) that are colocalized in cholinergic muscle motor neurons seem to be of lesser importance, at least with the experimental parameters we have used69.

The perfusion of the NO synthase inhibitor L-NAME (100 µM, n=10) had no effect on basal tone or ongoing contractions in all parts of the corpus. It enhanced the response to stretch stimulation only in the central part of the stomach and at stronger stimulation intensities (Figure 3, bottom row). This shows that the local response is mediated by a simultaneous activation of excitatory and inhibitory pathways. On the other hand, the lack of effect of L-NAME in the distal and proximal regions was unexpected because NO is the main inhibitory neurotransmitter in the
guinea pig stomach that is responsible for the adaptive relaxation\textsuperscript{16,44}. It may be that the distended area was too small or the stimulus was not sufficient to elicit a relaxant response. The latter seems unlikely as our distension levels were in the same range as those used in whole stomach preparations\textsuperscript{6,35,44,71,72}. Relaxations can be evoked by electrical stimulation in all parts of the stomach and nitrergic motor neurons also are not confined to specific regions. It remains to be studied whether distension of larger areas may evoke a relaxation more readily.

### References


50) Bayliss WM, Starling EH. The movement and innervation of the small intestine. J Physiol (Lond) 1899; 24: 99-143.


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59) **SCHÜTZ E.** Über die Einwirkung von Arzneistoffen auf die Magenbewegungen. Arch exp Pathol Pharmacol 1886; 27: 337-341.


61) **POOS F.** Zur Differenzierung der Magenfunktion hinsichtlich Reizbildung, Erregungsleitung und Tonus. Pflügers Arch 1923; 83-100.


63) **CHAPLIN SB, DUKE GE, HUNT H, DEGERNES LA.** Chemical denervation of the myenteric plexus of the muscular stomach of turkeys. Comp Biochem Physiol C 1987; 88: 201-207.


67) **SANDERS KM.** A case for interstitial cells of Cajal as pacemakers and mediators of neurotransmission in the gastrointestinal tract. Gastroenterology 1996; 111: 492-515.


69) **MICHEL K, REICHE D, SCHEMANN M.** Projections and neurochemical coding of motor neurones to the circular and longitudinal muscle of the guinea pig gastric corpus. Pflugers Arch 2000; 440: 393-408.


