THE MYENTERIC PLEXUS IN THE CONTROL AND PHARMACOTHERAPY OF GASTRO-INTESTINAL MOTILITY

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INTRODUCTION

The innervation of the gastro-intestinal tract

Gastro-intestinal functions are controlled by a hierarchy of humoral, neural and myogenic mechanisms. The neural control of the gastro-intestinal tract is provided by three divisions of the autonomic nervous system: the parasympathetic, the sympathetic and enteric divisions. The subdivision is based on the location of the ganglia and the connections with the central nervous system. The parasympathetic and sympathetic nerves supply the extrinsic innervation of the gut. The enteric nervous system (ENS) comprises the intrinsic innervation of the gut.

The structure and function of the enteric nervous system

The ENS is made up of ganglia and interconnected nerve fibres, ranging from the oesophageal body to the internal anal sphincter (Furness and Costa, 1987). The ganglionicated plexuses within the ENS are the myenteric plexus, found between the circular and longitudinal muscle layers, and the submucous plexus in the submucosal connective tissue layer. It is generally accepted that the myenteric plexus is mainly involved in the control of gastro-intestinal motility, while the submucous plexus is mainly involved in the control of secretion, absorption and bloodflow (Furness and Costa, 1987).

During health, two quite distinct functional states of the upper gastrointestinal tract can be recognized; the interdigestive state is observed following a period of fasting, the fed state following a meal. The ENS is involved in the control of both these functional states.

The motor activity of the upper gastrointestinal tract in the interdigestive state

The migrating motor complex (MMC) is a cyclical motor pattern of the stomach and the small intestine in the fasting state in most mammalian species, including man (Szurszewski, 1969; Vantrappen et al., 1977). The MMC consists of four phases of which phase 3, also called the activity front, is the most characteristic one. Phase 1 is a quiescent phase. It is followed by phase 2, a period of persistent but random contractile activity. The contractile activity reaches a maximum in frequency and intensity during phase 3, which is also called the activity front. Phase 4 is a phase of rapidly subsiding contractile activity and merges into phase 1. The duration of one cycle in man is about 100 minutes (Vantrappen et al., 1977). Recording gastro-intestinal motility from several sites simultaneously revealed that the MMC sequence progresses aborally from the stomach or the proximal small intestine to the
terminal ileum (Szurszewski, 1969).

It is now evident that the enteric nervous system is the medium through which the MMC is propagated (Sarna et al., 1981). The control of the initiation of the MMC is incompletely understood. In dog and in man, plasma levels of the hormone motilin are maximal just before and during phase 3 in the distal stomach (Itoh et al., 1975; Vantrappen et al., 1979; Sarna et al., 1983; Bormans et al., 1987), and administration of exogenous motilin induces a premature phase 3 which starts from the stomach (Vantrappen et al., 1979). These observations suggest that motilin has a physiological role in the induction of phase 3 of the MMC. The target for motilin in the gastro-intestinal tract is unknown.

The motor activity of the upper gastro-intestinal tract in the fed state

In the fed state, the principal motor tasks of the stomach are to break up the meal and to deliver it into the intestine at a rate that matches the processing capability of the intestine. The corpus and fundic regions of the stomach act as a reservoir for food storage. The antrum serves as a muscular pump that breaks up food, mixes it with gastric secretions and drives the semisolid chyme in a controlled way through the pyloric sphincter, into the duodenum.

HYPOTHESIS: THE MYENTERIC PLEXUS OF THE STOMACH PLAYS A KEY ROLE IN THE CONTROL OF GASTRIC MOTILITY

The stomach displays specific motor patterns in both of the interdigestive and the fed state. We hypothesized that the myenteric plexus of the stomach is providing the basic neural circuitry that governs these different patterns of contractile activity. In support of this hypothesis, we tried to demonstrate the following steps:

1. The presence of a diversity of myenteric neurones in the stomach becomes apparent using neurochemical electrophysiological morphological and pharmacological studies.
2. In support of a physiological role of the gastric myenteric plexus in the control of the fed state, endogenous substances that are involved in the control of gastric emptying, can be shown to act on myenteric neurones in the stomach.
3. In support of a role of the gastric myenteric plexus in the control of the interdigestive state, an interaction of motilin with myenteric neurones in the stomach can be demonstrated.
4. Pharmacological agents, used in the therapy of upper gastric motility disorders, act on myenteric neurones in the stomach.
5. Pharmacological studies of myenteric neurones in the stomach can identify new agents that will modify gastro-intestinal motility.

PHENOTYPIC DIVERSITY OF MYENTERIC NEURONES IN THE STOMACH

In order to control the various motor patterns exhibited by the stomach, several functional types of neurones must be present in the myenteric plexus of the
stomach. The presence of a diversity of myenteric neurones in the stomach becomes apparent using neurochemical electrophysiological, morphological and pharmacological studies.

**Neurochemical studies of the myenteric plexus of the stomach**

Using immunohistochemical techniques, we demonstrated the presence of several neurotransmitters in neurones of the myenteric plexus of the guinea-pig gastric antrum. Immunoreactivity for choline acetyltransferase was present in 62% of all neurones, indicating that the vast majority of neurones in the stomach are cholinergic. This is in agreement with the predominance of cholinergic mechanisms involved in the control of gastric motility. Immunoreactivity for nitric oxide synthase was present in 34% of the neurones, indicating a substantial nitrergic population. This is in agreement with several observations that demonstrate a role for nitric oxide in inhibitory neurotransmission in the stomach. Substance P-immunoreactive neurones (41%) and neuropeptide Y-immunoreactive neurones (33%) formed two distinct subpopulations. A subpopulation of neuropeptide Y-immunoreactive neurones was immunoreactive for nitric oxide synthase. The majority of neurones immunoreactive for substance P and for vasoactive intestinal polypeptide (22%) were separate populations. A subpopulation of nitric oxide synthase-immunoreactive neurones was immunoreactive for nitric oxide synthase. The majority of neurones immunoreactive for substance P and for vasoactive intestinal polypeptide, but a distinct subpopulation of nitric oxide synthase-immunoreactive neurones was vasoactive intestinal polypeptide-negative. These data demonstrate that several neurotransmitters are present in gastric neurones, displaying specific co-localisation patterns. This supports the notion of functional diversity of gastric neurones.

**Electrophysiological studies of the myenteric plexus of the stomach**

We used intracellular recording methods to provide the first electrophysiological studies of antral myenteric neurones. On the basis of their electrophysiological characteristics, antral neurones were classified into four subtypes (Tack and Wood, 1992a). Gastric I neurones were characterized by repetitive spike discharge during intraneuronal injection of depolarising current pulses. Gastric II neurones discharged only one or two spikes at the onset of depolarising current pulses. In both cell types, the action potential was suppressed by TTX, suggesting exclusive involvement of inward Na+ current in the depolarisation phase of the spike. Gastric III neurones never discharged spikes during depolarising current pulses. AH/Type 2 neurones were characterized by spikes which were not abolished by tetrodotoxin due to a calcium component of the inward current. Action potentials in these neurones were associated with a long-lasting hyperpolarising after-potential which began shortly after the positive after-potential of the spike and whose amplitude increased when an increasing number of spikes was fired. Synaptic signals are the way in which enteric neurones communicate with each other. They consist of both excitatory and inhibitory potentials, and have a fast or slow time scale. We used intracellular recording methods and focal electrical stimulation of interganglionic fibre tracts to evoke synaptic potentials, which are likely to reflect the synaptic interactions in the neuronal networks of the gastric antrum (Tack and Wood, 1992b).

Nicotinic cholinergic fast excitatory postsynaptic potentials (EPSPs) could be evoked in every antral neurone. Most neurones received multiple inputs from
axons arriving in several different interganglionic fibre tracts and several neurones received input from multiple axons in individual fibre tracts. Application of acetylcholine (ACh) by micro-ejection mimicked the fast EPSP in all neurones. In about one third of the neurones, this fast nicotinic response to ACh was followed by a long-lasting muscarinic depolarisation.

Slow EPSPs were evoked by repetitive stimulation of the interganglionic connectives. They consisted of a slowly activating depolarisation which persisted for several seconds after the termination of the stimulus. They were observed mainly in AH/Type 2 neurones. Atropine did not inhibit the slow EPSP. The neurotransmitter that mediates the slow EPSP is unknown, but substance P and 5-HT are likely candidates. Elevation of cyclic adenosine 3’,5’-monophosphate by the application of forskolin mimicked the slow EPSP in antral AH/Type 2 neurones, suggesting cyclic AMP may function as an intraneuronal second messenger in the signal transduction process for slow synaptic excitation.

Slow inhibitory postsynaptic potentials (IPSPs) were hyperpolarising potentials evoked by repetitive stimulation of interganglionic fibre tracts. They were only rarely observed. The neurotransmitter mediating slow IPSPs and their functional role are unknown.

Morphological studies of the myenteric plexus of the stomach

Intracellular dye injection during electrophysiological studies of gastric neurones allows to study their morphological characteristics. The size of the neuronal cell bodies is variable. Most gastric neurones are unipolar, with one long axon. This axon can project in oral or anal directions. The dendrites can be short, broad and club-like (Dogiel I neurones), or fine, tapering and filamentous (Filamentous neurones). These data demonstrate that several morphological types of neurones are present in the stomach. This supports the notion of functional diversity of gastric neurones.

ROLE OF THE MYENTERIC PLEXUS IN THE STOMACH GASTRIC EMPTYING

We hypothesized that the myenteric plexus in the gastric antrum plays a key role in the coordination of gastric emptying of solids. Therefore, we studied the interaction of endogenous and exogenous substances, that are known to modulate gastric emptying, with neurones in the gastric antrum.

Norepinephrine

Norepinephrine has a well established role as a neurotransmitter in the gastro-intestinal tract (Furness and Costa, 1987). Stimulation of adrenergic nerves inhibits gastric tone and antral motility (Guimarães, 1969). We used electrophysiological methods to directly study the actions of norepinephrine on antral myenteric neurons, in order to elucidate the mechanism by which norepinephrine inhibits gastric motility. Our study revealed two different actions of norepinephrine on myenteric neurons (Tack and Wood, 1992c). Both mechanisms may contribute to the neurally mediated inhibitory action of norepinephrine on gastric contractility.

One action was presynaptic inhibition of fast and slow excitatory synaptic potentials: norepinephrine interacts with presynaptic α1-receptors to inhibit the release of ACh and the (presently un-
known) non-cholinergic mediator for the slow EPSP. By this mechanism, norepinephrine may decrease the probability of suprathreshold responses in the excitatory motor neurons and may directly or indirectly decrease the amount of excitatory transmitters that reaches the gastric smooth muscle.

The second action of norepinephrine was a postsynaptic depolarisation with increased input resistance and enhanced excitability, which is mediated by an α1 adrenoceptor. In the stomach, these receptors are probably located on non-cholinergic inhibitory neurons (Hillsley et al., 1991) and by directly stimulating these inhibitory motor neurons, norepinephrine may increase the amount of inhibitory neurotransmitter that is released and may consequently also contribute to the neurally mediated inhibition of gastric motility by norepinephrine.

5-Hydroxytryptamine

A great deal of evidence has accumulated to support the hypothesis that serotonin, or 5-hydroxytryptamine (5-HT), is a neurotransmitter in the ENS (Furness and Costa, 1987). In the stomach in vitro, 5-HT causes a relaxation (Van Nueten and Janssen, 1980), but nerve-mediated excitatory actions of 5-HT in the stomach were also described (Yamaguchi, 1972). The purpose of our study was to directly study the actions of 5-hydroxytryptamine on the electrical and synaptic properties of antral myenteric neurons and to characterize the receptors involved. We found that 5-HT has multiple actions on myenteric neurons in the gastric antrum (Tack et al., 1992b). 5-HT evoked both fast and slow depolarising responses in antral myenteric neurons. Furthermore, 5-HT inhibited the stimulus-evoked release of acetylcholine and non-cholinergic neurotransmitters. Finally, 5-HT caused a slow hyperpolarising response in a small subgroup of antral myenteric neurons. Specific 5-HT receptor subtypes mediate the different responses to 5-HT. The fast depolarising response is mediated by a 5-HT3 receptor. The slow depolarising response is mediated by a 5-HT1P receptor. The hyperpolarising response and the presynaptic inhibition of neurotransmitter release are both mediated by a 5-HT1A receptor.

It has been suggested that the 5-HT1P receptor plays a role in the control of gastric emptying (Mawe et al., 1989). Recent studies by our group have shown that 5-HT3 receptors are involved in the control of gastric activity fronts (Wilmer et al., 1993). Enteric 5-HT1A receptors may be involved in 5-HT-mediated gastric relaxation (Meulemans et al., 1991).

Cholecystokinin

Cholecystokinin (CCK) stimulates antral contractile activity in the guinea-pig stomach in vitro by acting on intrinsic cholinergic neurons (Gerner and Haffner, 1977). We used electrophysiological recordings to directly study the actions of CCK on antral myenteric neurons. Antral myenteric neurons responded to CCK in one or more of the following ways: a brief depolarisation, a prolonged hyperpolarising response or a prolonged depolarisation (Tack et al., 1992c). All responses seemed to be mediated by CCK-A receptors.

Prokinetic substituted benzamides

Substituted benzamides, such as cisapride, have clinical application in the treatment of gastroparesis. It is generally accepted that they act on myenteric neurons to stimulate gastric motility, possibly by acting on 5-HT3 receptors. We conducted the first study of the effects of cisapride at its target: the myenteric neurons in the gastric antrum. We found that cisapride inhibits both 5-
HT₃ and 5-HT₁P receptor-mediated responses to 5-HT. However, cisapride also directly depolarized a subpopulation of antral neurons, through a non-5-HT mechanism (Tack et al., 1992d). We found no evidence for an interaction with 5-HT₄ receptors. Through a similar mechanism of action, cisapride also enhances cholinergic neurotransmission between antral myenteric neurons (Tack et al., 1992d). Our hypothesis that this non-5-HT-related action of cisapride is responsible for its gastrokinetic actions is supported by recent in vivo studies (de Ridder and Schuurkes, 1993).

Sumatriptan
Recently, we demonstrated that sumatriptan, a 5-HT₁D agonist at cerebral arteries, clinically used in the treatment of migraine, is an agonist at the 5-HT₁P receptor (Vanden Berghe et al., 1995). Thus, sumatriptan provides us with a tool to study the effect of 5-HT₁P receptor activation on gastro-intestinal functions in vivo. In the interdigestive state in man, administration of sumatriptan induces a premature activity front with jejunal onset and suppresses phase 3 motor activity in the stomach (Tack et al., 1995a). The observed effects on interdigestive motility are not accompanied by changes in the cyclical pattern of motilin plasma levels. Furthermore, sumatriptan inhibits somatostatin release. Gastric phase 3 activity induced by the motilin agonist erythromycin is blocked by sumatriptan (Coulie, 1996).

Sumatriptan increases the amplitude and the duration of oesophageal contractions (Houghton et al., 1994). In addition, administration of sumatriptan in man results in an immediate and profound relaxation of the gastric fundus (Tack et al., 1995b). This relaxation allows larger volumes to be accommodated before the thresholds for perception and discomfort are reached during gastric distention (Tack et al., 1996). Studies in cats suggest that activation of a nitrergic pathway underlies the relaxatory effect of sumatriptan on the gastric fundus (Coulie, 1996).

In the postprandial state, sumatriptan causes a significant increase in the gastric half emptying of both solids and liquids, and it increases the lag phase for the gastric emptying of solids (Coulie et al., 1997). Unlike other agents that delay gastric emptying, sumatriptan induces a prolonged lag phase for the gastric emptying of liquids.

ROLE OF THE MYENTERIC PLEXUS IN THE CONTROL of INTERDIGESTIVE GASTRO-INTESTINAL MOTILITY

The target for motilin in the gastrointestinal tract is unknown. In the rabbit and in man, the existence of motilin receptors on smooth muscle cells has been demonstrated in vitro (Louie and Owyang, 1984). In the dog however, no smooth muscle receptors for motilin could be demonstrated, although motilin is able to induce a premature gastric phase 3 in this species (Itoh et al., 1975). Moreover, the complicated and highly organized motility pattern of the MMC seems to suggest an underlying neural control mechanism. Therefore, we hypothesized that, besides a direct effect on intestinal smooth muscle, motilin also has a direct effect on intrinsic neural elements.

We used intracellular recordings to study the actions of motilin on the electrical behaviour of myenteric neurons of the guinea-pig gastric antrum in vitro. In a subpopulation of antral neurons, motilin evoked a long-lasting depolari-
sation of the cell membrane associated with enhanced excitability, thus mimicking slow synaptic excitation (Tack, 1992). The effects of motilin appeared to be directly on the neurons from which the recordings were made because they still occurred during synaptic blockade with tetrodotoxin or removal of Ca²⁺. Dose dependency of the membrane depolarising action of motilin suggests that it is receptor mediated.

**Erythromycin.**

The motilin agonist erythromycin has been shown to stimulate gastrointestinal motility and to accelerate gastric emptying in man (Peeters et al., 1989; Tack et al., 1992a; Janssens et al., 1990). Indirect observations suggest that erythromycin exerts its prokinetic effect by acting on motilin receptors on presynaptic enteric neurons (Sarna et al., 1991). We demonstrated that erythromycin directly depolarizes a subpopulation of myenteric neurons in the gastric antrum. Moreover, the same subpopulation is depolarized by motilin and motilin and erythromycin cause mutual desensitisation of each others effect (Tack et al., 1991). We therefore conclude that erythromycin is an agonist for the motilin receptor on myenteric neurons, and that this mechanism is involved in its prokinetic actions.

**LITERATURE**


Mawe, G., Brancheck, T.A. and M.D. Ger­shon.: Blockade of 5-HT-mediated enteric slow EPSPs by BRL-24924: Gastrokinetic


