# Vagal influence on the motility of the feline jejunum

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1. The effects of electrical stimulation of the peripheral end of the cervical vagal nerve on jejunal motility were investigated in anaesthetized cats, pretreated with guanethidine, with sectioned splanchnic nerves and ligated adrenal vessels. Motility was monitored as volume changes of an intraluminal balloon.

2. Vagal stimulation elicited frequency-dependent hypermotility with a short latency. Relaxatory events were also observed, which could indicate the presence of a non-adrenergic inhibitory pathway.

3. After atropine treatment, contractions and relaxations could still be elicited. The former were compared to cholinergic contractions and showed a lower maximal amplitude and a longer latency to onset. Moreover, they were antagonized by 80–100% by the opioid receptor antagonist, naloxone.

4. Vagal stimulation after hemicholinium, given in order to deplete the preganglionic acetylcholine content, elicited naloxone-sensitive contractions. This suggests that a subpopulation of the vagal preganglionic fibres is non-cholinergic.

5. Isolation of the balloon-containing segment did not qualitatively alter the responses, indicating that the vagal fibres reach the small intestine via the paravascular mesenteric nerves.

6. It is concluded that cholinergic and non-adrenergic, non-cholinergic (NANC) contractions, as well as relaxations, could be elicited by efferent vagal stimulation. The NANC contractions seem to result from the activation of opioid receptors causing disinhibition of a tonic neurogenic restraint on the gut muscle.

Electrical stimulation of the peripheral end of the cut vagus nerve elicits increased motility of the small intestine, as investigated in anaesthetized animals. This response was entirely antagonized by atropine, according to some authors (Van Harn, 1963; Kewenter, 1965). Others, however, reported an atropine-resistant residual contractile effect (Bayliss & Starling, 1899, 1900; Gray, Hendershot, Whitrock & Seevers, 1955; Nakayama, 1965; Collman, Grundy & Scratcherd, 1983) which was preceded by a relaxation, as noted by Bayliss & Starling (1899, 1900) and Nakayama (1965). Gustafsson & Delbro (1986, 1987) demonstrated in the anaesthetized cat that direct or reflex vagal stimulation, after blockade of noradrenergic and cholinergic neurotransmission, induced contractile motor responses in the jejunum that were antagonized by naloxone. Recently, we presented evidence that these responses are due to (presumably enkephalin-mediated) suppression of tonically active, inhibitory neurons which utilize nitric oxide (NO) as transmitter (Gustafsson & Delbro, 1993*b*). In addition, occasionally at spontaneous motility, but always when tone was raised by various means, vagal stimulation elicited relaxatory responses (Delbro & Gustafsson, 1986; Gustafsson & Delbro, 1988, 1993*a*, *b*). Thus, jejunal motility is partly controlled by non-adrenergic, non-cholinergic (NANC) excitatory, as well as inhibitory, pathways with a vagal input. While the relaxatory effect was abolished by the nicotinic ganglionic receptor blocker, hexamethonium (Delbro & Gustafsson, 1986), the excitatory NANC effect was partly resistant to this compound, possibly indicating the involvement of an 'unconventional' ganglionic transmission step (Delbro & Gustafsson, 1989).

In the present paper the following issues with respect to vagally induced motor responses of the feline jejunum were addressed, which substantially extend the original analyses. (1) A systematic comparison between the excitatory as well as the inhibitory responses before and after atropine, (2) determination of a dose–effect relationship for naloxone on the NANC contractions in order to distinguish the opioid receptor(s) involved,

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(3) further analyses of the non-nicotinic component of the NANC contractions, and (4) clarification of the anatomical pathway for the vagally induced motor responses.

## METHODS

### Animals and general surgery

The experiments were conducted with thirty-five cats of either sex (2–5 kg), which were deprived of food for 24 h (water *ad libitum*). The study design was approved by the Ethics Committee of the University of Göteborg. Anaesthesia was induced with ether, after which a tracheal cannula was inserted for artificial ventilation. A femoral vein and artery were catheterized and anaesthesia was maintained with α-chloralose (0·16 mmol $kg^{-1}$ I.V.; supplementary, smaller doses were injected in the course of the experiment). An infusion (0·1 ml $min^{-1}$) of glucose and sodium bicarbonate (0·28 M; 0·1 M) in distilled water was administered via the femoral artery to counteract the acidosis caused by the surgical trauma. In order to prevent reflex adrenoceptor-mediated inhibition of gastrointestinal motility (cf. Furness & Costa, 1974), the adrenergic neuron blocker, guanethidine (6 μmol $kg^{-1}$ I.V.), was injected and, after mid-line laparotomy, the adrenal vessels were ligated to counteract the release of catecholamines to the circulation. Corticosteroid substitution (hydrocortisone, 9–30 μmol $kg^{-1}$ I.M.) was administered to compensate for the elimination of the adrenocortical secretion. Furthermore, in most cats the greater and lesser splanchnic nerves were sectioned bilaterally.

#### Recording of effector responses

Small intestinal motility was recorded by means of a thin rubber balloon (length, 5 cm) tied onto a tube (i.d. 3·5 mm) and introduced into the jejunum via an antimesenteric incision about 20 cm anal to the ligament of Treitz. The tip of the balloon was directed 10 cm orally. In addition, a drainage tube for bile and pancreatic secretions was placed in the duodenum via a separate incision and emptied outside the animal. The incisions were closed with purse-string sutures, without interrupting the continuity of the gut. The system was filled with body-warm water and connected to a container (diameter, 3 cm) placed on a force–displacement transducer (Grass FT 10C, Grass Instrument Co., Quincy, MA, USA). The level of intraluminal pressure was set by adjusting the height of the container above the abdominal cavity and was kept constant throughout the experiments (5–12 $cmH_2O$). In this way, jejunal motility could be monitored as volume changes at a fairly constant transmural pressure. In seven cats, the balloon-containing jejunal loop was isolated by ligatures and the rest of the gut was excised from the duodenum to the rectum by peripheral division of the mesentery. These experiments are referred to as 'pedicle' and the remainder 'intact gut'. In some cats, a branch of the superior mesenteric artery was cannulated in the retrograde direction for the close administration of certain drugs to the balloon-containing segment. The data obtained by this procedure have previously been presented (Delbro & Gustafsson, 1989; Gustafsson & Delbro, 1990) and will not be repeated in this report. Blood pressure was recorded by a transducer (Statham P23 AC, Statham Laboratories, Inc., Hato Rey, Puerto Rico) connected to the femoral artery and heart rate by a tachograph unit triggered by the arterial pulse wave. All recordings were made on a polygraph (Grass 7D). During surgery, the abdominal organs were covered with swabs moistened with body-warm isotonic saline. After completion of surgery and throughout the experiments, the abdomen was held closed with clips. Body temperature was thermostatically maintained at 38 °C by radiant heat and a heating pad.

At the end of the experiments, the animals were killed by an overdose of pentobarbitone and the hearts were excised.

#### Nerve stimulations

The cervical vagal nerves were carefully dissected and cut. Either the right or left nerve was arranged for electrical stimulation of the peripheral end by means of a bipolar silver-ring electrode, which was insulated with silicone grease to impede spreading of electrical current to the surrounding musculature.

#### Evaluation of the results and statistical analyses

A 'minimal response' was defined as a change of volume equivalent to 0·1 ml, taking place within 1 min after the completion of a nerve stimulation. The 'latency' to onset was the time from the beginning of stimulation to the appearance of the resulting effect. 'Duration' was estimated as the time between the onset of the response and return to the basal level of motility. The contractions or relaxations were further evaluated with respect to their 'peak amplitude'. In one series of experiments, the contractile responses to vagal stimulation were expressed as the product of peak amplitude and duration (motility index; MI). The data are presented as median values and ranges, or means ± S.E.M. Statistical analyses were performed with the Mann–Whitney $U$ test, Wilcoxon signed rank test or Spearman rank-order correlation coefficient test, when relevant. A $P$ value of $< 0·05$ was considered significant.

#### Drugs

The following drugs were used: atropine sulphate (Sigma Chemical Co., St Louis, MO, USA), α-D(+)-gluco-chloralose (Merck, Darmstadt, Germany), guanethidine sulphate (Ismelin®, Ciba-Geigy AG, Basel, Switzerland), hemicholinium-3 (Sigma), hydrocortisone 21-sodium succinate (Solu-Cortef®, Upjohn S.A., Puurs, Belgium) and naloxone hydrochloride (Narcanti®, Du Pont Pharmaceuticals, Wilmington, DE, USA or Sigma). The solid drugs were dissolved in isotonic saline or distilled water, except for chloralose, which was dissolved in 0·13 M $Na_2B_4O_7$ (Borax).

## RESULTS

### General

At the completion of surgery, the animals were allowed an equilibration period of at least 1·5 h, to permit the gut motility to recover from the effects of surgery. Thereafter, spontaneous motor activity was either totally absent (9

out of 35 animals) or present at a greater or lesser degree in the remainder. The results were qualitatively similar for the pedicle and intact gut experiments.

## Vagally induced jejunal motility prior to atropine administration

The peripheral end of the right or left vagus was stimulated electrically, which generally elicited jejunal contractile responses in a frequency-dependent fashion (see also below) concomitant with bradycardia and hypotension. With respect to the gut motor responses obtained, no difference was noted between the two vagal nerves.

In ten of the intact gut experiments, a systematic analysis of the vagally induced contractile responses before and after atropine administration was undertaken. The series began with an estimate of the voltage needed to produce a maximal contractile effect at a frequency of 10 Hz and a pulse duration of 5 ms (10–30 V; hereafter denoted 'optimal voltage'). Stimulation was then performed (1, 2, 4, 8, 16 and 32 Hz; 5 ms; optimal voltage) for 30 s at 5 min intervals. The responses were evaluated with respect to their maximal amplitude as well as the latency to onset and duration of these contractions. The results were compared to the corresponding data obtained after atropine treatment (see below). The frequency–effect relationships with regard to the peak amplitudes are shown in Fig. 1.

The augmented motility elicited by frequencies $\leqslant 8$ Hz quickly returned to control level at the discontinuation of the stimulation (when performed for 30–60 s; Fig. 2*A*). At 16 ($n = 7$) and/or 32 Hz ($n = 10$), however, a prolonged contractile effect (2–7 min duration) was observed in fourteen cats (Fig. 2*B* and *C*). Since this latter response was qualitatively similar to the naloxone-sensitive contraction which is elicited by stimulation after atropine administration (see below), we investigated four cats to see whether the long-lasting component was NANC. When atropine (1·4 $\mu$mol kg$^{-1}$ I.V., $n = 3$) was administered at the peak of such a response, there was an immediate return to, or even below, control level. Naloxone (1·25 $\mu$mol kg$^{-1}$ I.V., $n = 1$), on the other hand, had no effect, which thus refutes an opioidergic involvement.

In all seven cats in which spontaneous motor activity was present prior to stimulation, an *inhibition* of jejunal tone (of up to 30 s duration), preceding the contractile response, occurred by vagal activation for 15–60 s (Fig. 2*C*). The inhibition could be observed at low (1–4 Hz) and/or high (8–32 Hz) stimulation frequencies. The contractile phase appeared either during the stimulation or as a post-stimulatory rebound. The maximal amplitude of the inhibition ranged between 0·25 and 0·5 ml, amounting to 10–50% of the ensuing contraction. Furthermore, biphasic, or sometimes, purely relaxatory responses could be demonstrated on a stimulation-induced jejunal tone ($n = 4$). Subsequent to the contraction, a relaxation (1–2 min duration) was noted in twelve cats (Fig. 2*D*). This effect occurred at low and/or high frequencies, and its maximal amplitude was 0·1–0·6 ml, amounting to 5–67% of the preceding contraction. In twelve animals, the contractile response faded during the 30 s stimulation period, either slowly or as a sudden fall in tone (Fig. 2*D*). Such a fading could usually be observed at high frequencies. After cessation of the stimulation, the motor response either returned to the control level directly, or after a rebound contraction. At 32 Hz, an excitatory 'breakthrough' following the fading occurred during the stimulation (Fig. 2*D*).

## Spontaneous and vagally induced jejunal motility after atropine administration

At the completion of the vagal stimulations, spontaneous contractile activity was very sparse. The administration of atropine (1·4 $\mu$mol kg$^{-1}$ I.V., usually followed by an I.V. infusion of 0·7 $\mu$mol kg$^{-1}$ h$^{-1}$) caused only a modest decrease in tone in eighteen experiments. At the time of

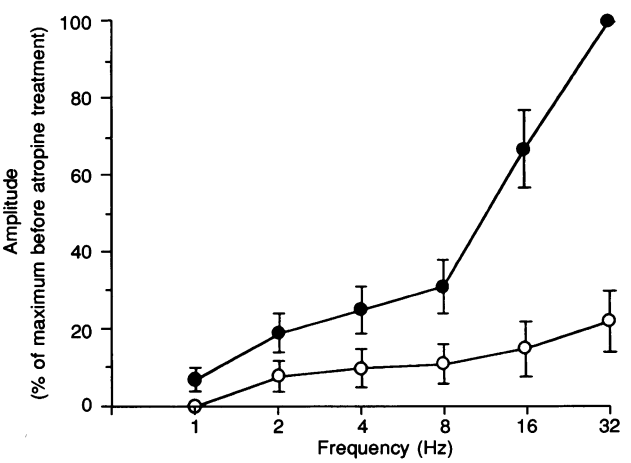


**Figure 1. Vagally induced jejunal contractions**
Frequency–effect relationship with respect to the peak amplitude of vagally induced jejunal contractions ($n = 10$). Stimulation (5 ms; 10–30 V) for 30 s elicited frequency-dependent hypermotility. In each animal, the maximal response before the administration of atropine was set to 100%. The values are presented as means ± S.E.M. ●, before atropine; ○, after atropine.

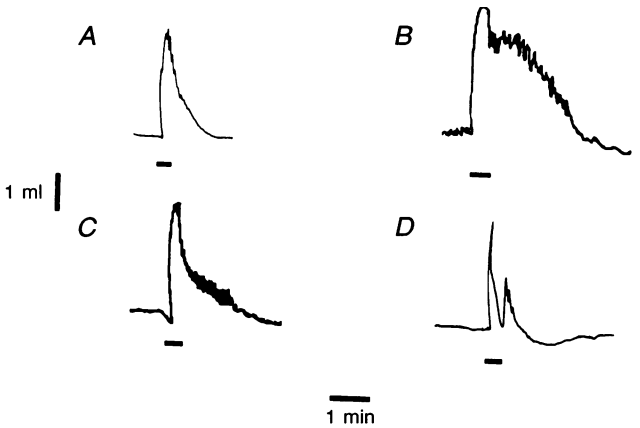


**Figure 2. Motor patterns before atropine treatment**
Different types of motor patterns observed in response to vagal nerve stimulation (8–32 Hz; 5 ms; 10–30 V) for 30 s before atropine treatment. The most common response consisted of a single contraction with little or no superimposed phasic activity (*A*). At frequencies > 8 Hz, phasic contractions were occasionally noted and the duration was then prolonged (*B* and *C*). In some animals, a relaxation preceded the contractile phase (*C*). Furthermore, a fading of the tone during stimulation, followed by an excitatory 'breakthrough' was sometimes observed, as well as a late-relaxatory event (*D*).

subsequent renewed vagal stimulation (10–20 min after atropine injection), this decrement amounted to 0·3 ± 0·1 ml. This indicates that the cholinergic contribution to gut muscle tone was minor in the experimental system used here. Immediately prior to the vagal stimulation series there was no spontaneous activity, although in four cats, such activity did appear in the course of the experiment.

In all animals vagal stimulation produced NANC contractions while no bradycardia was elicited. No difference was noted between the two vagal nerves. In ten of the intact gut experiments, an identical quantitative analysis was performed as before atropine treatment. Maximal amplitude was significantly lower than before atropine (0·7 (0·2–2·4) *vs.* 3·5 (1·8–8·8) ml; $P < 0·001$), whereas the responses had a longer latency (30 (18–36) *vs.* 9 (6–18) s; $P < 0·001$). On the other hand, the duration of the responses did not differ (150 (66–408) *vs.* 141 (60–240) s; $P > 0·05$). Moreover, in a further analysis, maximal effect in each animal obtained before and after atropine administration was set to 100%, respectively. After plotting these frequency–effect relationships, the frequency producing half-maximal response ($F_{50}$) was estimated by linear interpolation. The $F_{50}$ after atropine treatment was 13·3 (1·5–22·6) Hz, as compared to 11·7 (2·6–21·5) Hz before addition of this drug ($P > 0·05$). In one cat (intact gut), the voltage–duration relationship for a minimal excitatory response was investigated. In concert with our previous report (Gustafsson & Delbro, 1986), the stimulation thresholds before and after atropine treatment were essentially the same, suggesting equal fibre diameter for the two pathways.

Due to a long latency to onset, the NANC contractions often appeared as rebounds at a stimulation of 30 s duration (Fig. 3). However, when the stimulation was prolonged ($n = 17$), the contractile response usually started before the stimulation had ceased. Due to the long latency, a possible fading of the contraction could not be observed

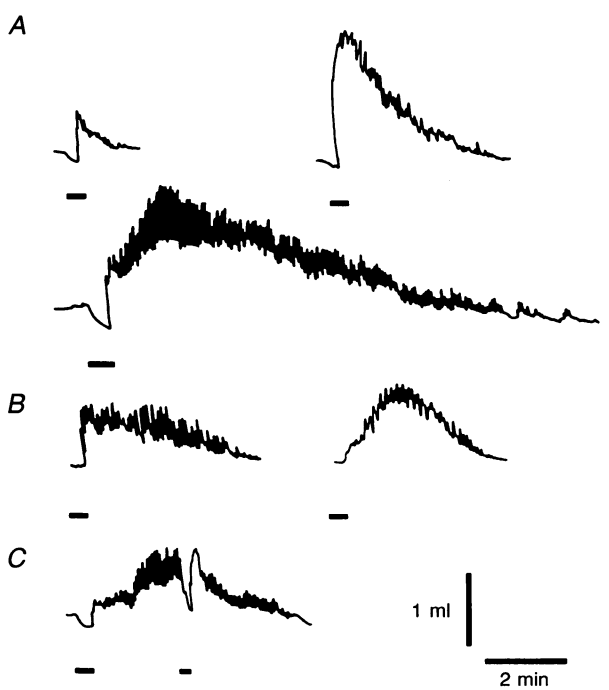


**Figure 3. Motor patterns after atropine treatment**
Typical NANC motor responses elicited by vagal nerve stimulation (parameters as in Fig. 2). Generally, these exhibited a more pronounced phasic contractile activity than the cholinergic ones. The shape of the responses and the duration were more variable (*A* and *B*). Relaxation could be observed, either initially (*A*) or by a renewed stimulation on a vagally induced hypermotility (*C*). No post-stimulatory relaxation was noted.

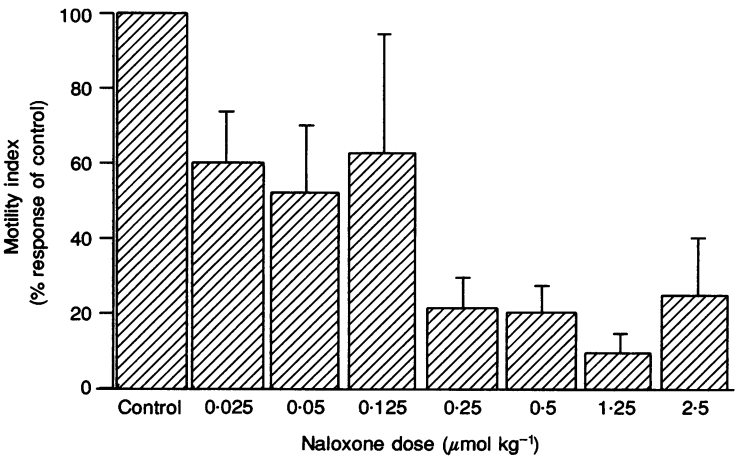


**Figure 4. Effect of naloxone**
Dose–effect relationship (means ± S.E.M.) for the inhibitory action of naloxone (0·025–2·5 μmol kg$^{-1}$ I.V. at 15 min intervals) on NANC jejunal contractions as elicited by a standardized vagal stimulation.

at 30 s stimulation. If stimulation was continued for ⩾ 2 min ($n = 5$), a plateau was reached which, in most cases, waned slowly during the rest of the stimulation.

In eight animals, the NANC responses to stimulation at ⩾ 4 Hz for 30–60 s were biphasic, the contractile component being preceded by an inhibition with a duration of 30–60 s (Fig. 3*A* and *C*). The maximal amplitude of these inhibitions ranged between 0·1 and 0·8 ml, amounting to 9–62% of the ensuing contraction. A NANC inhibitory effect after vagal stimulation could be further demonstrated in four animals. Thus, on the near peak of a vagally induced contraction, a renewed stimulation resulted in a transient relaxation (Fig. 3*C*). In no experiment was any post-stimulatory relaxation noted.

## The effect of naloxone on vagally induced NANC jejunal contractions

The NANC contractile response, as observed during a prolonged vagal stimulation, was abruptly abolished by the I.V. injection of naloxone (0·025 μmol kg$^{-1}$), as

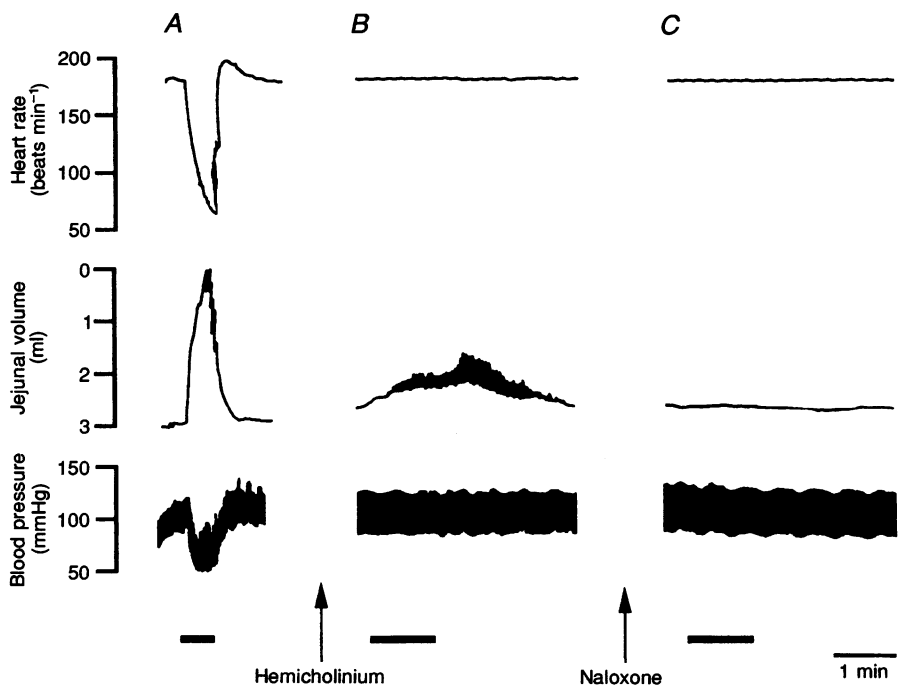


**Figure 5. Effect of hemicholinium**
*A*, control before hemicholinium administration. Vagal nerve stimulation (10 Hz; 5 ms; 10 V; denoted by horizontal bar) elicits bradycardia, hypotension and a jejunal contraction (i.e. a decrease in jejunal volume). *B*, renewed stimulation (32 Hz; 5 ms; 10 V) 140 min after the injection of hemicholinium (8·7 μmol kg$^{-1}$ I.V.) results in a 'NANC-like' excitatory motor response while the cardiovascular effects are abolished. *C* (5 min subsequent to *B*), after naloxone (2·5 μmol kg$^{-1}$ I.V.) the same vagal stimulation as in *B* has no effect on the variables investigated.

observed in one cat. This confirmed our earlier finding that this response is, at least in part, opioidergic (Gustafsson & Delbro 1986). The dose–effect relationship (expressed as MI; see Methods) of such naloxone antagonism was investigated in six animals after atropine injection (intact gut experiments). Two vagal stimulations (16 Hz; 5 ms; optimal voltage) for 60 s (or, in one cat, 90 s) were performed with an interval of 15 min. The MI of the response elicited by the second stimulation was 123 (76–160)% of that of the first one ($P > 0.05$), which demonstrates the reproducibility of the response. The mean MI was then calculated and used as control. Naloxone (0·025 $\mu$mol kg$^{-1}$ I.V.) was injected 10 min after the second stimulation and, subsequent to a 5 min equilibration period, a renewed stimulation was performed. This sequence was repeated with increasing doses of naloxone (0·05, 0·125, 0·25, 0·5, 1·25 and 2·5 $\mu$mol kg$^{-1}$ I.V., Fig. 4.). There was a dose-dependent inhibition of the contractile response (Spearman rank-order correlation coefficient test, $P < 0.02$, $n = 6$). The maximal inhibitory effect in the individual experiment was 98 (80–100) %, obtained at 0·88 (0·025–2·5) $\mu$mol kg$^{-1}$. Notably, the effect of any specific dose varied considerably between the experiments. In three cats, a complete inhibition was never achieved. Naloxone seemingly had no effect on basal motility.

### The effect of hemicholinium on vagally induced NANC jejunal contractions

A partial non-cholinergic ganglionic transmission mechanism for the vagally induced NANC jejunal contractions, as inferred from previous experiments using nicotinic receptor blocking drugs (Delbro & Gustafsson, 1989; Gustafsson & Delbro, 1990), was investigated in five cats by the use of hemicholinium. Hemicholinium reversibly depletes acetylcholine stores in cholinergic axon terminals by competitively blocking choline uptake and therefore attenuates acetylcholine release. Hence, this compound is useful for the investigation of the role of acetylcholine as a mediator in various neuronal pathways (see Carpenter & Woodruff, 1987, for references). When vagal stimulation (in the absence of atropine) produced jejunal contractions in a reproducible fashion, hemicholinium was administered (injected as repeated I.V. boluses during 20 min, to a total dose of 8·7 $\mu$mol kg$^{-1}$). At the onset of hemicholinium administration, continuous low-frequency vagal stimulation (5 Hz; 5 ms; optimal voltage) was begun in order to deplete (preferentially) the preganglionic acetylcholine content. This resulted in bradycardia but did not affect jejunal motility. The bradycardia waned within 40–45 min, by which time the heart rate had returned to pre-hemicholinium level. Thereafter, repeated stimulation at 32 Hz (60–120 s duration) produced progressively less bradycardia (which ultimately vanished) and likewise greater jejunal contractions which were qualitatively indistinguishable from the NANC responses as characterized above. These 'NANC-like' contractile effects obtained after hemicholinium were abolished by naloxone (2·5 $\mu$mol kg$^{-1}$ I.V., $n = 4$; Fig. 5).

## DISCUSSION

The present study was aimed at investigating the vagal influence on jejunal motility in the cat. Particular interest was focused on the atropine-resistant (NANC) effects, which have been sparingly characterized to date.

The anatomical pathway for the vagally induced cholinergic as well as the atropine-resistant contractions of the small intestine is confined to the mesenteric nerves (Bayliss & Starling, 1899; Kewenter, 1965; Gustafsson & Delbro, 1986). With respect to relaxation, on the other hand, Mir, Mason & Ormsbee (1978) suggested that vagal inhibitory fibres may reach the upper small intestine via an intramural route by crossing the gastroduodenal junction from the gastric antrum. In the present study, however, the vagally induced inhibitory motor effects obtained before or after atropine application seem to be conveyed via the paravascular route, since they were qualitatively similar in both the pedicle and intact gut experiments.

Kewenter (1965) observed fading of the contractile response at frequencies above 10–12 Hz and stated that this resulted from an inability of the motor nerves to fire repetitively at high frequencies. At variance with this proposal, we suggest that the relaxatory responses, as observed before atropine administration, could be due either to inhibitory (non-adrenergic) neurons under vagal control, or to blockade of the excitatory (i.e. cholinergic) fibres via prejunctionally located inhibitory muscarinic receptors (cf. Kilbinger & Nafziger, 1985). However, a simultaneous activation of excitatory and presumably inhibitory neurons may not represent a physiological event, since in the present experimental set-up the entire vagal trunk was stimulated. Moreover, a muscarinic suppression of the NANC excitatory effect as exerted by the concomitant stimulation of cholinergic motor fibres (cf. North, Slack & Surprenant, 1985) could explain the apparent absence of naloxone-sensitive contractions before atropine application. This absence in our view is thus not physiological, but a consequence of the present method of nerve stimulation. Furthermore, continuous electrical stimulation does not correspond to the natural firing of the nerve, which instead occurs in 'bursts' (cf. Miolan & Roman, 1974). Stimulation that mimics such a pattern may elicit a more optimal response (cf. Edwards, Andersson, Järhult & Bloom, 1984).

In this study, the NANC contractions had lower amplitudes than the cholinergic ones, in concert with the results of Collman *et al.* (1983) in the ferret, but at variance with our previous report (Gustafsson & Delbro, 1986). However, when these early data were re-examined by a

non-parametric statistical method, the cholinergic responses (at 8 and 16 Hz) were indeed significantly greater than those obtained after atropine treatment.

The longer latency of the NANC excitatory response (cf. Collman *et al.* 1983) may be due to different kinetics for the transmitter or because the action of an inhibitory substance must first be overcome. Although the NANC contractions seem to be off-responses at stimulations for 30 s, they are not 'true' rebounds but are instead independent of the preceding inhibitions.

We have shown earlier that the vagally induced NANC jejunal contractions are sensitive to naloxone (Gustafsson & Delbro, 1986), and this is confirmed in this study. The interpretation of the dose–effect relationship for such inhibition by naloxone was ambiguous, since the dose required to achieve an antagonism of $\geqslant 80\%$ varied considerably. When taken together with our previous observations that vagally induced NANC contractions could be mimicked by morphine (Gustafsson & Delbro, 1993*a*), an involvement of $\mu$-opioid receptors seems likely, although $\delta$-receptors cannot be ruled out (cf. Ouyang, Clain, Snape & Cohen, 1982; for opioid receptor nomenclature, see Paterson, Robson & Kosterlitz, 1983). In some animals, a residual response was observed also after the highest dose, which could indicate the release of another NANC transmitter leading to excitation. Interestingly, substance P of myenteric origin mediates NANC excitation of the longitudinal as well as the circular muscle layer of the small intestine *in vitro* (see Furness & Costa, 1987, for references).

Naloxone-sensitive contractions in response to vagal stimulation were also demonstrated in the feline pylorus (Edin *et al.* 1980) and ileocolonic sphincter (Ouyang *et al.* 1982). In further support of the existence of enteric opioidergic pathways, there is abundant histochemical evidence for the presence of opioid peptide-containing

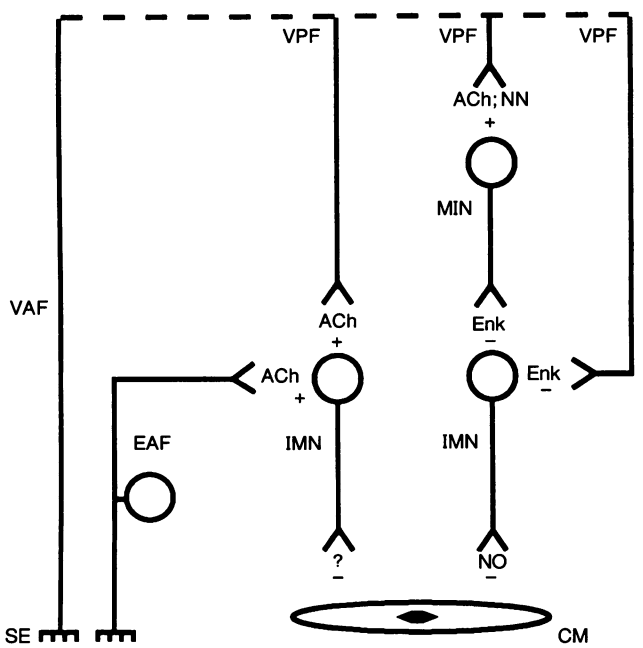


**Figure 6. Hypothetical neuronal arrangement with respect to non-adrenergic, non-cholinergic (NANC) mechanisms involved in the control of the circular muscle layer of the small intestine (CM)**

Muscle excitation is primarily a result of a diminished discharge of presumably tonically active, inhibitory myenteric neurons (IMN). These nerves, possibly using nitric oxide (NO) as transmitter, can be controlled, either at the soma or (not shown) prejunctionally, by enkephalinergic (Enk) neurons. These constitute a subpopulation of the vagal preganglionic fibres (VPF), and/or the myenteric interneurons (MIN). The MIN, in turn, may be excited by a vago-vagal reflex. The reflex connection between the vagal afferent fibres (VAF) and the efferents is located in the brainstem region (dashed line). These VPF release acetylcholine (ACh), which acts at nicotinic ganglionic receptors, but there could also exist a non-nicotinic mediator (NN). The muscle activity can also be inhibited by another set of IMN using an unknown transmitter (denoted by ?). These nerves can be excited reflexly, via enteric afferent fibres (EAF), or vago-vagally. In this reflex arc, the VPF and EAF use ACh as the sole transmitter since their action is blocked by hexamethonium. The sensory endings (SE) may respond to chemical (i.e. luminal) stimuli but also to changes in intestinal wall tension. (+) and (−) denote excitatory and inhibitory effects, respectively.

neurons in several parts of the gastrointestinal tract, including the small intestine (see Furness, Llewellyn-Smith, Bornstein & Costa, 1988, for references).

In previous reports (Delbro & Gustafsson, 1989; Gustafsson & Delbro, 1990) we demonstrated a non-nicotinic component of the ganglionic transmission mechanism for the vagally induced opioidergic contractions. Non-nicotinic responses to vagal stimulation have been reported previously in the canine small intestine (Thomas & Kuntz, 1926; Gray *et al.* 1955). This phenomenon could hypothetically be due to an antidromic activation of afferents with excitatory collaterals to the myenteric plexus or to the gut muscle, as suggested for the feline stomach (Delbro, Fändriks, Lisander & Andersson, 1982). However, such an effect could not be observed in the canine gut (Neya, Mizutani, Yanagihara & Nakayama, 1990). Furthermore, the results obtained in the present study with stimulation after hemicholinium treatment strongly suggest that a population of the vagal preganglionic fibres are non-cholinergic. The transmitter released is unidentified, but could be an opioid, since the resulting motor effects were abolished by naloxone. Notably, vagal preganglionic fibres showing immunoreactivity for enkephalins have been demonstrated in the cat (Lundberg *et al.* 1978). Our results, however, do not preclude the possibility of non-opioidergic preganglionic fibres activating a postganglionic opioidergic pathway.

A possible mechanism by which endogenous or exogenous opioids cause gut hypermotility was discussed in a recent paper (Gustafsson & Delbro, 1993*a*). Thus, in keeping with the hypothesis proposed by Wood (1987), tonically active inhibitory neurons, seemingly under vagal opioidergic control, are of prime importance for the regulation of circular muscle activity. Nitric oxide may be a transmitter in such neurons (Gustafsson & Delbro, 1993*b*). In addition, the present study supports our earlier suggestion of another NANC inhibitory pathway (Gustafsson & Delbro, 1993*a*, *b*), which, in order to be evident, demands a certain level of intestinal tone. On the other hand, an inhibitory transmitter released on a low resting tone might cause some latency to onset of the ensuing contractions.

To summarize, the vagal influence on the motility of the feline jejunum is complex. Excitatory as well as inhibitory motor responses can be elicited by vagal stimulation before and after atropine treatment. The NANC contractions seem to involve opioid receptors. We hypothesize that the activation of such receptors cause excitation by disinhibition, since they inhibit tonically active nitroxergic neurons to the circular muscle. Moreover, there is evidence for non-cholinergic preganglionic vagal fibres involved in the opioid-mediated pathway. NANC relaxations involving a yet unknown transmitter can also be elicited by vagal stimulation. A tentative neuronal arrangement for the observed effects, when taken together with our earlier reported results (see Introduction), is presented in Fig. 6.

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