PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

# The Nervous Release and the Action of Substances which Affect Intestinal Muscle Through Neither Adrenoreceptors nor Cholinoreceptors

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# The nervous release and the action of substances which affect intestinal muscle through neither adrenoreceptors nor cholinoreceptors

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Mammalian gastrointestinal muscle is supplied by non-adrenergic, intrinsic inhibitory neurons. The substantial evidence which exists to suggest that adenosine triphosphate is the transmitter released from these nerves is discussed briefly. It is shown that the intrinsic inhibitory neurons compose the efferent link in a cascade of descending reflexes extending from the oesophagus to the anal sphincter.

Gastrointestinal muscle is also supplied by non-cholinergic excitatory nerves. The pharmacology of transmission from a group of such nerves to the smooth muscle of the guinea-pig proximal colon is described and evidence is presented to suggest that 5-hydroxytryptamine (5-HT) may be the transmitter substance. The contraction is blocked by phentolamine and methysergide which both antagonize the contractile action of 5-HT. When the muscle is desensitized by continued exposure to 5-HT, the non-cholinergic contraction can no longer be elicited.

This article deals with a group of substances which are released from nerves within the gut wall and which affect the motility of gastrointestinal smooth muscle. Although they have been identified only tentatively, there is enough knowledge of their modes of action to distinguish them from acetylcholine, noradrenaline and several other substances which are known to act on the muscle of the gut. The work is principally concerned with outlining the characteristics of intrinsic, non-adrenergic, inhibitory neurons and explaining their role in the control of gastrointestinal muscle. Transmitter release from non-cholinergic excitatory nerves is dealt with more briefly.

## Intrinsic inhibitory nerves

The most extensively examined of the non-adrenergic, non-cholinergic nerves are those often described as intrinsic inhibitory neurons (Bennett, Burnstock & Holman 1966; Burnstock, Campbell & Rand 1966; Bülbring & Tomita 1967; Kuriyama, Osa & Toida 1967; Campbell 1970). This terminology is slightly misleading because it is now known that intrinsic inhibitory *adrenergic* neurons are present in some parts of the gastrointestinal tract (Costa, Furness & Gabella 1971; Furness & Costa 1971). The electrical response to the stimulation of intrinsic inhibitory nerves is quite distinctive (figure 1); single pulses give inhibitory junction potentials (i.j.p.) which reach maximum amplitude in 150 to 250 ms and have a total duration of about 1 s (800 to 1500 ms in most cases). These potentials have been recorded from the stomach, jejunum, caecum, colon and internal anal sphincter of the guinea-pig (Bennett *et al.* 1966; Kuriyama *et al.* 1967; Furness 1969*b*; Beani, Bianchi & Crema 1971; M. Costa, unpublished), from the caecum and distal colon of the rabbit (Furness 1969*b*; Small 1972) and from the sheep small intestine and pig colon (J. B. Furness, unpublished). The corresponding mechanical changes have been recorded in different parts of the gut of several mammalian species (Holman & Hughes 1965; Martinson 1965*a*; Campbell 1966*a*; Bülbring & Gershon 1967; Day & Warren 1967, 1968; Goldenberg 1968; Rikimaru 1971*a*), including man (Bucknell 1965; Crema, del Tacca, Frigo & Lecchini 1968) and the monotreme *Tachyglossus aculeatus* (J. B. Furness, unpublished).

Intrinsic inhibitory nerves were first thought to be non-adrenergic because of the persistence of the effects of their stimulation in the presence of drugs which antagonize the release or action of noradrenaline (Martinson 1965*a*; Burnstock *et al.* 1966; Campbell 1966*a*). In addition, membrane potential changes caused by stimulation of adrenergic nerves supplying the intestine are difficult to detect at low frequencies of stimulation, whereas those in response to stimulation of the intrinsic inhibitory nerves are large and distinctive. However, drugs which antagonize transmission from adrenergic nerves to the gut do cause some reduction in the response to

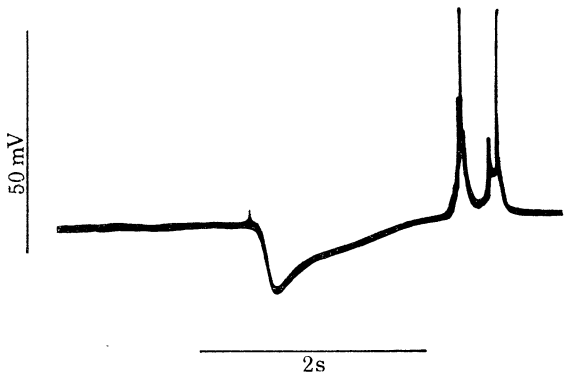


FIGURE 1. An intracellular record of the response to stimulation of intrinsic inhibitory neurons supplying the circular muscle of the guinea-pig distal colon. There was no fluctuation in the membrane potential of this cell before stimulation. A single pulse initiated a transient hyperpolarization, the inhibitory junction potential, which was followed by a rebound excitation. (From Furness 1970*a*).

stimulation of the intrinsic inhibitory nerves (Burnstock *et al.* 1966; Campbell 1966*a*), and it has been suggested that the latter might indeed be adrenergic (Paton & Vane 1963; Semba, Fujii, Kimura & Ohya 1968; Weisenthal, Hug, Weisbrodt & Bass 1971). Compelling evidence for the nerves being non-adrenergic is the persistence of responses to their stimulation after the degeneration of adrenergic nerves supplying the guinea-pig distal colon (Furness 1969*a*; Crema 1970). Histochemical examination shows that no adrenergic nerves are detectable after denervation and it would appear from our other experiments that there could be no intrinsic adrenergic neurons in this part of the gut with catecholamine levels too low to be detected (Costa & Furness 1971). Furthermore, transmission from intrinsic inhibitory neurons of the taenia coli is observed after 3 weeks in tissue culture, whereas adrenergic neurons, which are of extrinsic origin, degenerate within 4 days (Rikimaru 1971*b*). In addition, transmission from intrinsic inhibitory neurons can be demonstrated in parts of the gut which are contracted by catecholamines, such as the avian gizzard (Bennett 1969) and the mammalian anal sphincter (Costa & Furness 1972*b*; Garrett & Howard 1972).

The decay phase of the i.j.p. has a long time course compared with other post-junctional potential changes elicited in smooth muscle by nerve stimulation. This time course is approximately the same in different parts of the gut and in different species. In contrast, transmission from adrenergic nerves to smooth muscle gives excitatory junction potentials (e.j.p.) of different time courses in different organs, or in the same organ from different species (Furness & Burnstock 1969; Holman 1970). The constancy of the time course of the i.j.p. and the fact that it is considerably longer than that of the cholinergic e.j.p. (which can be recorded from the same cells in the intestine) suggest that the inhibitory transmitter continues to act throughout the i.j.p. The i.j.p. is capable of obliterating the e.j.p. even when the e.j.p. would be expected during the


PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

decay phase of the i.j.p. (Furness 1969*b*). The occlusion is probably at the muscle cell membrane because transmural stimulation, which excites both cholinergic and intrinsic inhibitory neurons postganglionically (Paton & Zar 1968; Furness 1970*a*) elicits i.j.p. in most cells of the taenia coli and distal colon even though they also receive a cholinergic innervation (Bennett 1966*a*; Furness 1969*b*). Moreover, acetylcholine is released from the proximal colon when the only response during stimulation is relaxation (M. Costa, unpublished). The dominance of intrinsic inhibitory neurons over excitatory neurons in the colon of the dog was recognized by Bayliss & Starling (1900), who found that 'descending inhibition was more than sufficient to counteract the excitatory effects of local stimulation'. In the stomach, the same type of occlusion may not apply, probably because fewer cells receive both a cholinergic and a non-adrenergic inhibitory supply. When non-adrenergic neurons were activated by direct vagal stimulation, i.j.p. were observed in only 18 % of gastric smooth muscle cells (Beani *et al.* 1971). This contrasts with the situation in the large bowel, where i.j.p. are observed in most cells (Bennett 1966*a*; Bennett *et al.* 1966; Furness 1969*b*). In the stomach, the spontaneous appearance of action potentials was blocked by stimulation of the non-adrenergic nerves, even in cells in which i.j.p. were not observed (Beani *et al.* 1971). The inhibitory neurons may selectively innervate pacemaker regions in the stomach.

In the intestine, activation of intrinsic inhibitory neurons is followed by a rebound excitation of the muscle, which has been demonstrated by both electrical (Bennett 1966*b*; Furness 1970*a*; figure 1) and mechanical recording (Campbell 1966*b*; Day & Warren 1968; Furness 1971; figure 2). The rebound excitation is myogenic, although nerve-mediated contractions may sometimes outlast the period of stimulation and be superimposed on, or follow, the rebound contraction (see, for example, Furness 1971; Costa & Furness 1972*a*). Mechanical records from the stomach show only a slow recovery of muscle tone following stimulation of non-adrenergic inhibitory nerves and evidence of rebound is seldom apparent (Martinson 1965*a*, *b*; Campbell 1966*a*; Ohga, Nakazato & Saito 1970; Beani *et al.* 1971). On the other hand, electrical evidence for rebound excitation can be obtained from individual gastric smooth muscle cells (Beani *et al.* 1971).

Burnstock (1972) has reviewed the extensive evidence which now exists to suggest that adenosine triphosphate (ATP) is the inhibitory transmitter. The following arguments were brought forward: ATP is stored and synthesized in the terminals of intrinsic inhibitory nerves and is released when the nerves are stimulated; low concentrations of ATP mimic the effects of stimulation of the nerves; enzymes capable of degrading ATP to less active compounds are found in tissues supplied by these nerves; various drugs have parallel actions on transmission from intrinsic inhibitory nerves and on the action of ATP. Burnstock, Campbell, Satchell & Smythe (1970) found that quinidine (1 to $5 \times 10^{-5}$ g/ml) antagonized the relaxations caused by catecholamines ($10^{-8}$ to $10^{-7}$ g/ml) or sympathetic nerve stimulation but that a concentration of $2 \times 10^{-4}$ g/ml was required to reduce the responses to ATP ($10^{-6}$ to $2 \times 10^{-5}$ g/ml) or intrinsic inhibitory nerve stimulation. In the rabbit ileum, high doses of ATP ($10^{-6}$ to $2 \times 10^{-5}$ g/ml) reduced the response to its subsequent application and to the stimulation of non-adrenergic inhibitory nerves but not to the stimulation of adrenergic nerves. Dipyridamole ($10^{-6}$ to $5 \times 10^{-8}$ g/ml), a drug which is known to inhibit adenosine uptake in several tissues, increased the amplitudes and durations of relaxations of the guinea-pig taenia coli caused by stimulation of intrinsic inhibitory nerves or by exogenous ATP, but did not enhance the relaxations in response to adrenergic nerve stimulation or exogenous noradrenaline (Satchell, Lynch, Bourke & Burnstock 1972).


PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

ATP mimics the effect of stimulation of intrinsic inhibitory nerves in most parts of the gut of several species (Burnstock, Satchell & Smythe 1972). Nevertheless, in some areas it does not do this. Transmural stimulation elicits a nerve-mediated non-adrenergic, inhibition of the guinea-pig ileum (Holman & Hughes 1965; figure 2) but, ATP has been reported to cause contraction, relaxation or no response (Burnstock *et al.* 1970; Iso, Yamauchi, Uda & Toshioka 1971; Burnstock *et al.* 1972). ATP (1 to $3 \times 10^{-4}$ g/ml) contracted the longitudinal muscle of the rabbit distal colon (McKay & McKirdy 1972), although this is innervated by intrinsic inhibitory neurons (Furness 1969*b*), and there is a similar disparity in the case of the rat stomach (Heazell 1969, personal communication; Burnstock *et al.* 1970). The circular muscle of the rabbit caecum is supplied by non-adrenergic inhibitory neurons but ATP gave a biphasic


FIGURE 2. Length changes in a segment of guinea-pig ileum in response to stimulation of intramural nerves at frequencies of 5 and 50 Hz in the presence of hyoscine ($2 \times 10^{-7}$ g/ml). Stimulation at 5 Hz for 10 s caused a relaxation which was followed, after the stimulus was stopped, by a contraction of the muscle. At 50 Hz, the stimulus elicited a non-cholinergic contraction of the muscle without any prior relaxation. Note that the non-cholinergic contraction of the guinea-pig ileum is markedly briefer than that observed in the proximal colon (figure 4*a*).

response, contraction followed by relaxation (Small 1972, personal communication). Failure to mimic in these few cases is not necessarily strong evidence against the hypothesis of ATP as a transmitter because, as Burnstock (1972) has pointed out, there may well be different receptor types for ATP. Furthermore, the transmitter released from terminal varicosities of nerves may reach more limited areas of muscle in higher concentrations than the exogenously applied ATP. Nevertheless, further comparison of the effects of stimulation of intrinsic inhibitory nerves and of ATP in these tissues is warranted.

Rikimaru, Fukushi & Suzuki (1971) reported that imidazole ($3.5 \times 10^{-3}$ g/ml) and phentolamine ($5 \times 10^{-5}$ g/ml) both block the inhibitory action of ATP (1 to $5 \times 10^{-6}$ mol/l) on the guinea-pig taenia coli but that neither blocks the relaxation caused by stimulation of intrinsic inhibitory nerves at 5 to 10 Hz. It should be noted that the concentrations used are unusually high and


PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

that phentolamine does not always have such a specific effect as is suggested by Rikimaru *et al.* (1971). In fact, the same concentration of phentolamine markedly reduced the response to stimulation of intrinsic inhibitory nerves at 0.2 to 0.8 Hz and ATP ($5 \times 10^{-6}$ mol/l) overcame the blocking action of phentolamine (Burnstock 1972). Imidazole and phentolamine are both antagonists of the relaxing action of catecholamines on intestinal muscle, so the results lend support to the hypothesis that adrenaline and ATP relax the intestine through a common mechanism (Bueding *et al.* 1967; Bülbring 1967; Bowman & Hall 1970). Bueding *et al.* (1967) have reported that the actions of adrenaline and ATP are similarly affected by an altered Ca environment. Reduction of $[Ca]_o$ to one tenth substantially reduces the action of adrenaline (Bülbring & Tomita 1969) and if $[Ca]_o$ is reduced to a very low value by placing the taenia coli in a Ca-free solution, ATP even at a concentration of $1.5 \times 10^{-3}$ mol/l no longer inhibits spontaneous electrical activity although $10^{-7}$ mol/l is effective in a normal environment (Axelsson & Holmberg 1969). In contrast, reduction of $[Ca]_o$ to one tenth does not influence the amplitude of the i.j.p. elicited by stimulation of intrinsic inhibitory nerves (Hidaka & Kuriyama 1969). It would be valuable to compare reversal potentials for ATP, adrenaline and the i.j.p. to further clarify the points raised above.

*The role of intrinsic inhibitory nerves: descending inhibitory reflexes*

The role of intrinsic inhibitory nerves in the control of gastrointestinal muscle is now partly determined. Vagal fibres, with their cell bodies in the brain stem, form excitatory synapses with intrinsic inhibitory neurons of the stomach in cats, dogs, mice and guinea-pigs (Campbell 1966*a*, 1970; Martinson 1965*b*; Bülbring & Gershon 1967; Fukuda 1968; Ohga, Nakazato & Saito 1969). The cell bodies of some of the inhibitory neurons supplying the stomach may be extrinsic (Ohga *et al.* 1969; Beani *et al.* 1971). Intrinsic inhibitory neurons are found in the colon, but it is unlikely in the cat, guinea-pig and rabbit, and possibly in other mammals, that these receive any excitatory connexions through the sacral parasympathetic nerves (Bianchi, Beani, Frigo & Crema 1968; Furness 1969*b*; Hultén & Jodal 1969). Inhibitory responses to the stimulation of mesenteric nerves have been shown to be adrenergic in innumerable experiments (see Kosterlitz & Lees 1964). Thus the only extrinsic efferent pathway demonstrated to activate the non-adrenergic neurons is the vagus, although our recent experiments suggest an extrinsic connexion with inhibitory neurons in the rectum and internal anal sphincter.

When the pharynx and upper oesophagus are stimulated by the passage of a bolus of food a reflex, 'receptive', relaxation of the stomach occurs, mediated through the vagus nerves (Cannon & Lieb 1911; Abrahamsson & Jansson 1969). The nature of the efferent pathways in this reflex has recently been examined and it has been shown to involve non-adrenergic inhibitory neurons of the type described above (Abrahamsson & Jansson 1969; Jansson 1969; Ohga *et al.* 1970).

Mall (1896) deduced that propulsion of intestinal contents involves inhibition below and excitation above a bolus, but it was the work of Bayliss & Starling (1899, 1900, 1901) which clearly defined this phenomenon. Relaxation of intestinal muscle ahead of a bolus moving aborally was described by Bayliss & Starling (1899) for the small intestine of the dog. It is quite clear that the inhibitory neurons involved in this descending inhibition are intrinsic; Bayliss & Starling made these observations in animals from which the coeliac and superior mesenteric plexuses were removed, the vessels to the intestines cleared of nerve filaments, the mesentery divided, the abdominal sympathetic chains bilaterally removed and the vagi and


PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

splanchnic nerves severed. They found that the descending inhibition involved both muscle coats and that it was blocked by nicotine. Relaxation of the muscle below the bolus was essential; if it did not occur, the wave of contraction simply passed over the bolus without advancing it along the intestine. Descending inhibition was subsequently demonstrated in the peristaltic reflex of the colon in the cat, dog and rabbit (Bayliss & Starling 1900; Langley & Magnus 1905) and in the small intestine of the cat and rabbit (Bayliss & Starling 1901) and is now recognized as generally occurring during the propulsion of intestinal contents (Crema 1970). Langley & Magnus (1905) confirmed that the neurons involved are intrinsic by their demonstration of the descending inhibition in the isolated colon of the rabbit during the propulsion of a bolus. They also observed normal peristalsis between 5 and 14 days following the division of extrinsic nerves; this time is sufficient for the degeneration of adrenergic nerves supplying the gut (Furness 1969*a*, 1970*b*; Furness & Costa 1971).

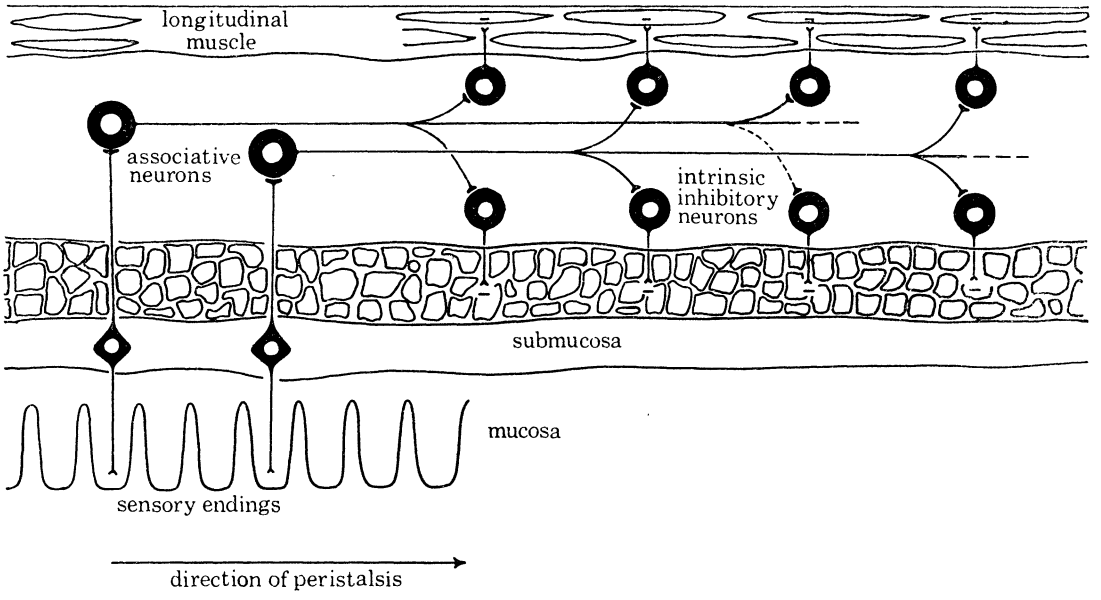


FIGURE 3. Diagrammatic representation of the arrangement of the neurons involved in the descending inhibitory reflex in the intestine. Neurons with sensory endings in the mucosa form excitatory connexions with associative neurons whose processes run aborally in Auerbach's plexus. Impulses passing down the axons of associative neurons are transmitted to inhibitory cells whose processes innervate both the longitudinal and circular muscle coats. The transmitter released by the inhibitory neurons has yet to be identified.

It is necessary for the relaxation at any point to change fairly rapidly to contraction as the bolus passes. The myogenic rebound which follows the action of the intrinsic inhibitory nerves may facilitate this change. Evidence for such a view comes from the work of Hukuhara & Miyake (1959), who noted that the inhibition below a point of irritation of the mucosa disappeared rapidly following stimulation and was replaced by a transitory excitation. Bayliss & Starling (1899) also observed that inhibition below a point of stimulation ended abruptly and was followed by strong contractions.

The postulated arrangement of the fibres involved in the descending inhibition is shown in figure 3. The mucosal localization of the sensory endings was demonstrated by Bülbring, Lin & Schofield (1958) who abolishes the reflex by ablation of the mucosa. The conduction of the response along the gut is prevented by division of Auerbach's plexus, but not by division of the submucous plexus, so the descending neurons must be in Auerbach's plexus (Cannon 1912;

Hukuhara, Yamagami & Nakayama 1958). The descending inhibition is observed immediately aboral to the point of irritation as well as considerably further away. In the dog small intestine, Bayliss & Starling (1899) observed the inhibition to extend for up to 0.9 m below the point of stimulation and Hukukara *et al.* (1958) observed it over a distance of 10 cm. In the rabbit, the reflex was restricted to 3 to 4 cm from the stimulus (Bayliss & Starling 1901). A similar species difference was noted in the colon; in the dog the whole of the colon below the stimulus was inhibited, whereas in the rabbit the inhibition extended only 2 to 3 cm (Bayliss & Starling 1900). In the guinea-pig, we have observed the inhibition to involve up to 11 cm of the colon. Ganglion blockade prevents the inhibition both close to and distant from a point of electrical stimulation, suggesting that the intrinsic inhibitory neurons which form the final pathway do not extend very far along the intestine (figure 3). The electrophysiological studies of Bülbring & Tomita (1967) indicate that the axons of intrinsic inhibitory neurons supplying the taenia coli are only a few millimetres long, and other experiments have confirmed that the cell bodies are situated in the myenteric plexus (Burnstock *et al.* 1966; Rikimaru 1971*b*). There is considerable overlap of the descending inhibition initiated from mucosal receptors; for any one point of irritation an extensive area of gut may be inhibited and, conversely, each area of muscle is influenced by irritation at different positions in the more proximal mucosa. The musculature at any point is influenced by up to six inhibitory neurons and its response is graded by the number of neurons which are stimulated (Bennett & Rogers 1967). Our observations suggest that the overlap results in a wave of inhibition extending ahead of the advancing bolus, with greatest inhibition immediately ahead of the bolus and lesser effects further along.

The descending intrinsic inhibitory fibres also extend to the muscle of the internal anal sphincter. We have found that stimulation of the anal canal in the absence of any extrinsic innervation results in a relaxation of the sphincter (Costa & Furness 1972*b*). The relaxation is not antagonized by drugs which block adrenergic transmission and, furthermore, noradrenaline contracts the sphincter muscle. Garrett & Howard (1972) have described the inhibition of sphincter muscle in the cat in response to rectal distension and have shown the response to be insensitive to the blockade of adrenoreceptors or of cholinoreceptors, but to be abolished by hexamethonium. In the human, reflex relaxation of the anal sphincter in response to rectal distension is normally observed, but in patients with Hirschprung's disease, in which intramural ganglia are absent, the descending inhibitory reflex cannot be elicited (Howard & Nixon 1968; Ehrenpreis 1971). Reflex relaxation also occurs at the oesophago-gastric junction in advance of a bolus in the oesophagus (Code & Schlegel 1968).

The observations outlined above indicate that the intrinsic inhibitory neurons compose the efferent link in a cascade of descending reflexes extending from the oesophagus to the anal sphincter. These reflexes prepare the way for material advancing through the alimentary tract by increasing gastric capacity, by opening sphincters and by dilating the intestine ahead of a bolus.

## Excitatory non-cholinergic nerves

Nerve mediated excitation of gastrointestinal muscle has been observed in several species and in different areas of the alimentary tract (see, for example, Ambache & Freeman 1968; Goldenberg & Burns 1968, 1971; Hultén & Jodal 1969; Furness 1971). In the ileum of the guinea-pig, the pharmacology of these responses has been examined by Ambache & Zar (1970) and Ambache, Verney & Zar (1970), but in no case has the transmitter involved been characterized.




PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The present description is concerned with the possible identification of an excitatory substance released from nerves within the wall of the guinea-pig proximal colon (Costa & Furness 1972*a*).

Parallel ring electrodes held around the tissue were used to stimulate the intramural nerves of isolated segments of proximal colon which were suspended in the oxygenated Krebs solution at 36 °C. In some experiments, the blood vessels supplying the proximal colon were drawn through ring electrodes so that paravascular nerves could be stimulated. Stimuli consisted of 10 s bursts of monophasic, rectangular pulses delivered at various frequencies. Pulse durations were 0.2 or 0.5 ms and strengths which were near those required to elicit non-cholinergic contractions of maximum amplitude were chosen. Except where stated, hyoscine ($10^{-7}$ g/ml) was included in the Krebs solution. Responses of the longitudinal muscle were recorded as length changes using a balanced lever and smoked drum or as tension changes using a mechano-electrical transducer.

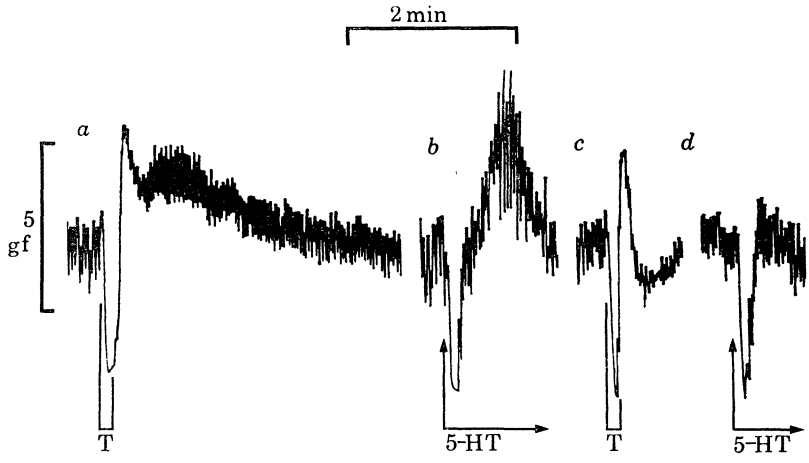


FIGURE 4. The effect of desensitization with 5-HT on the occurrence of the slow contraction in response to stimulation of intramural nerves in a segment of the proximal colon of the guinea-pig. In (*a*) intramural nerves were stimulated for 10 s at 10 Hz, marked by the horizontal bar. There was a relaxation of the muscle during stimulation and following its withdrawal there was a rebound contraction which preceded the slow contraction peculiar to this part of the gut. In (*b*) relaxation followed by contraction in response to 5-HT ($10^{-6}$ g/ml) applied at the arrow and left in the bath. In (*c*) and (*d*) the effect of stimulation of intramural nerves and of 5-HT ($10^{-6}$ g/ml) after the muscle had become desensitized. Note that both the slow contraction (*c*) and the contractile effect of 5-HT (*d*) are abolished, although the occurrence of the rebound contraction in (*c*) indicates that the muscle has retained its excitability.

Transmural stimulation elicited a mixed response, which was due to the combined actions of inhibitory and excitatory substances. This included the effect of stimulation of intrinsic inhibitory neurons which produced an initial relaxation which began within a second of the stimulus, persisted for most of its duration and was followed by a rebound contraction of the muscle. The rebound contraction subsided quickly, but instead of the original tone being restored, a second, very much more prolonged, contraction developed (figure 4*a*). The slow contraction often persisted for 4 to 5 min after a 10 s period of stimulation. It was most prominent at stimulation frequencies of 20 to 50 Hz but was observed at frequencies as low as 5 Hz.

The slow contraction is considered to be nerve mediated because it was blocked by tetrodotoxin ($10^{-8}$ or $10^{-7}$ g/ml) and non-cholinergic because it was not antagonized by hyoscine (up to $10^{-5}$ g/ml).

Guanethidine ($10^{-6}$ g/ml) and bretylium ($10^{-6}$ g/ml) both blocked the slow contraction, but this antagonism was readily reversed when the drug was washed from the organ bath.

Phentolamine ($10^{-6}$ g/ml) blocked the slow contraction, although its action could be reversed only slowly by repeated washing. In spite of these actions of guanethidine, bretylium and phentolamine, it is unlikely that the transmission involves adrenergic neurons. First, the directly acting sympathomimetic amines, phenylephrine, adrenaline, noradrenaline and isoprenaline all relaxed the muscle and their actions were not reversed by $\alpha$- and $\beta$-antagonists. Secondly, stimulation of paravascular nerves caused a relaxation of the proximal colon mediated through adrenergic nerves which was blocked by guanethidine and bretylium but this blockade was not reversed by washing. These two drugs are known to block transmission across ganglionic synapses (Kosterlitz & Lees 1961; Rand & Wilson 1967) and this might be anticipated to be the way in which they blocked the slow contraction, because conventional ganglion blocking agents, pentolinium ($10^{-6}$ to $10^{-5}$ g/ml) and dihydro-$\beta$-erythroidin ($10^{-5}$ g/ml) also blocked it.

Present experiments suggest 5-hydroxytryptamine (5-HT) to be the substance causing the slow contraction. When injected into the organ bath, 5-HT ($10^{-7}$ to $10^{-6}$ g/ml) caused an initial relaxation of the proximal colon, followed by contraction. Tetrodotoxin ($10^{-8}$ g/ml), but not guanethidine ($10^{-6}$ g/ml), blocked the relaxation, which is therefore most likely to be due to the stimulation of intrinsic non-adrenergic inhibitory neurons by 5-HT, as in the guinea-pig stomach (Bülbring & Gershon 1967). 5-HT still contracted the proximal colon when nerve-mediated responses had been blocked by tetrodotoxin. The 5-HT antagonist, methysergide ($10^{-6}$ g/ml), substantially reduced and in some cases completely blocked both the slow contraction and the contractile effect of 5-HT. Phentolamine ($10^{-6}$ g/ml) also antagonized both. When the muscle was desensitized to the contractile action of 5-HT by an extended exposure to a high concentration, the slow contraction was abolished, although the excitability of the muscle was not impaired (figure 4). The action of 5-HT in stimulating the non-adrenergic inhibitory neurons was not readily abolished by desensitization. It is unlikely that 5-HT released from enterochromaffin cells could contribute to the slow contraction, because it was also obtained in preparations from which the mucosa and submucosa had been removed.

Bülbring & Gershon (1967) showed that 5-HT is released from vagal fibres which activate non-adrenergic inhibitory neurons in the stomachs of guinea-pigs and mice. It is conceivable that similar neuro-neuronal connexions exist in the proximal colon and that 5-HT excites the intrinsic inhibitory neurons in both parts but, in the proximal colon, the diffusion of 5-HT to the longitudinal muscle which occurs at moderate or high frequencies of stimulation *in vitro* may not occur *in vivo*. If 5-HT releasing neurons are involved in the activation of non-adrenergic inhibitory neurons in the gut, then they might be expected to operate at frequencies of less than 10/s. Maximum relaxations of intestinal muscle are obtained with stimulus frequencies less than 10 Hz and sometimes as low as 1 to 5 Hz (Burnstock *et al.* 1966; Furness 1971) and, as mentioned above, electrophysiological studies indicate that the i.j.p. is capable of preventing the appearance of spontaneous action potentials and of cholinergic e.j.p. during stimulation at 2 Hz (Bennett 1966*a*; Furness 1969*b*). The hyperpolarization caused by stimulation of intrinsic inhibitory nerves at frequencies greater than 10 Hz is not maintained (Bennett *et al.* 1966; Furness 1970*a*). Thus it is reasonable to propose that normal impulse frequencies in intrinsic inhibitory nerves do not exceed about 10/s.

Bülbring & Gershon (1967) described an extended release of 5-HT from vagal nerves supplying the stomach of the mouse which was not blocked by tetrodotoxin and which the authors ascribed to a slow diffusion of 5-HT from the tissue. However, the slow contraction in the proximal colon in response to transmural stimulation does appear to result from the repetitive firing of




PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

intramural neurons which continues after the stimulus has been stopped. When tetrodotoxin ($2 \times 10^{-7}$ g/ml) was injected into the organ bath immediately the stimulus ended, the slow contraction was reduced in both amplitude and duration.

A substance which has similar properties to that causing the slow contraction appears in the superfusate from the stimulated proximal colon. Experiments to identify this substance are in progress.

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