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COMMENTARY
ON THE POSSIBILITY THAT AN INDOLEAMINE IS A NEUROTRANSMITTER IN THE GASTROINTESTINAL TRACT

M. COSTA and J. B. FURNESS

Centre for Neuroscience and Departments of Human Physiology and Morphology, Flinders University of South Australia, Bedford Park, 5042, South Australia, Australia

The enteric nervous system, which is embedded within the wall of the gastrointestinal tract, contains sensory neurons, excitatory and inhibitory interneurons and both excitatory and inhibitory neurons which act on the smooth muscle of the intestine [1–4]. Acetylcholine is established as a transmitter from excitatory nerves to the smooth muscle, but the nature of other transmitters is still in doubt. In almost all parts of the intestine there are no noradrenergic cell bodies within the enteric nervous system, although the axons of noradrenergic neurons whose cell bodies are in prevertebral ganglia, extrinsic to the intestine, impinge on the enteric ganglia [5].

Because 5-HT * has readily demonstrable effects on intestinal movements and it is found in high concentration in the intestine [6, 7], it is not surprising that its possible physiological role in modifying intestinal movements has been under scrutiny for some time. Some twenty years ago it was shown that 5-HT applied to the serosal surface of the intestine inhibited peristalsis [8, 9], and following these observations the effects of 5-HT on the peristaltic reflex in the guinea-pig ileum and colon were examined in some detail [10–12]. It was found that 5-HT was released from the intestinal mucosa during peristalsis and that when 5-HT was added to the fluid bathing the mucosa, peristalsis was facilitated. Bülbring and Crema [10] attributed the blocking of peristalsis by serosal application of 5-HT to an antagonism of ganglionic transmission. Bülbring and her colleagues concluded that 5-HT has a physiological role in sensitizing mucosal stretch receptors and that it is released from the mucosa, presumably from enterochromaffin cells, into the vicinity of the sensory nerve endings. Later results, which show that enterochromaffin cells are depleted when intraluminal pressure is increased, support this interpretation of the origin of 5-HT [13].

The first suggestion that 5-HT might actually be a neurotransmitter in the intestine was made by Gershon, Drakontides and Ross [14]. They found that radiolabelled 5-HTP, when injected into mice, was taken up into the intestine and stored as 5-HT. Autoradiographic studies showed the label to be concentrated in the myenteric plexus. Soon after, Bülbring and Gershon [15] made a study of the pharmacology of transmission in the vagal inhibitory pathway to the stomach, a study which could be regarded as the real starting point for a serious consideration of 5-HT as an intestinal neurotransmitter. In brief, they found that transmission through the inhibitory pathway could be partly blocked by nicotinic antagonists and partly blocked by desensitization with 5-HT. Inhibition was completely blocked only when antagonism of nicotinic receptors and desensitization of 5-HT receptors were combined. Before the pharmacological evidence is considered in more detail, questions concerning the presence of 5-HT and associated enzymes in intestinal nerves should be answered.

Are there pre-existing stores of 5-HT in intestinal nerves?

A biochemical answer to this question is almost impossible to obtain because the nerves are embedded almost inextricably in the wall of the intestine. Measurements in extracts of the whole intestinal wall are certainly inadequate because of the concentration of 5-HT in enterochromaffin cells of the mucosa [6, 16]. Estimates from animals whose mast cells contain 5-HT (such as mice and rats) are unreliable. In preparations of longitudinal muscle, with myenteric plexus attached, from the guinea-pig ileum 5-HT concentrations of 81 ± 11 ng/g [17] and 110 ± 20 ng/g [18] have been reported. However, in the remaining layers of intestine, which includes the mucosa, concentrations of 6350 ± 860 to 8900 ± 600 ng/g were found [18, 19]. Therefore if only 10 to 20 mg of the mucosal layers were to contaminate each gram of longitudinal muscle plus myenteric plexus, the contamination could account for all the 5-HT measured. Even without contamination, the longitudinal muscle plus myenteric plexus contains (in addition to nerves) connective tissue, Schwann cells, muscle, blood vessels, the interstitial cells of Cajal and mast cells: some of these could conceivably contain traces of 5-HT, either as an endogenous substance or absorbed from mucosal stores liberated during dissection.

It could be anticipated that 5-HT in intestinal nerves would be detected with fluorescence histochemical methods which have successfully demonstrated 5-HT in neurons of the CNS and in invertebrate nervous systems. In the normal intestine, noradrenergic axons which give a fluorescence reaction run in the intrinsic

* The following abbreviations are used in the text: AADC, aromatic l-amino acid decarboxylase; DBH, dopamine β-hydroxylase; 5,6-DHT, 5, 6-dihydroxytryptamine; 5,7-DHT, 5,7-dihydroxytryptamine; 5-HT, 5-hydroxytryptamine; 5-HTP, 5-hydroxytryptophan; LSD, lysergic acid diethylamide; MAO, monoamine oxidase; NA, noradrenaline; TH, tyrosine hydroxylase; TPH, tryptophan hydroxylase.

plexuses, but fortunately these are of extrinsic origin in most areas of the intestine [5] and are also susceptible to depletion by drugs having little or no effect on 5-HT neurons (at least on those 5-HT neurons in the CNS). Robinson and Gershon [17] used α-methyl paratyrosine to deplete NA from guinea-pig small intestine and could find "no evidence that 5-HT contributes at all to the control pattern of fluorescence". Other work on the guinea-pig small intestine supports this conclusion: extrinsic denervation or depletion with 6-hydroxydopamine, reserpine or guanethidine causes all nerves which can be revealed by histochemical methods for aromatic amines to disappear [20–23]. Similar results have been obtained in other areas of intestine and in other species [5, 21, 24, 25], although none of these have been examined as thoroughly as the guinea-pig small intestine.

When the guinea-pig ileum was depleted of NA with α-methylparatyrosine or reserpine and the animals were further treated with an inhibitor of MAO, a faint yellow fluorescence was seen in the myenteric plexus; this fluorescence failed to appear if the animals were pretreated with an inhibitor of TPH, parachlorophenylalanine [17]. Similar results were obtained in extrinsically denervated segments of the ileum of the cat after treatment with an inhibitor of MAO [26, 27]. Amine fluorescence cannot be detected in the small intestines of cats which have not been given inhibitors of MAO [24, 26–28]. Contrasting results after inhibition of MAO have been reported for the guinea-pig ileum; the animals were treated with reserpine and inhibitors of MAO but no induction of fluorescence was detected in the myenteric plexus [23].

Thus all recent histochemical studies are consistent in finding that there are no pre-existing stores of histochemically detectable 5-HT. The reported presence of 5-HT in intestinal neurons after inhibition of MAO could represent enhanced levels in pre-existing stores or the inhibition of MAO could allow 5-HT from other sources to be artificially retained. Retention of 5-HT derived from another source would be analagous to the retention of 5-HT released from pinealocytes in noradrenergic axons supplying the pineal [29].

Is there neurochemical evidence for intrinsic neurons which could utilize 5-HT as a transmitter?

If some of the neurons within the wall of the intestine were to utilize 5-HT as a neurotransmitter, then, by analogy with other systems, they would be expected to possess an uptake mechanism for 5-HT and to contain the following enzymes: AADC, TPH and MAO. In fact, an uptake mechanism and each of these enzymes have been found in intrinsic neurons.

Uptake mechanism for 5-HT. 5-HT and related indoleamines can be taken up into neurons which utilize catecholamines as transmitters as well as into those which are presumed to utilize indoleamines. However, experiments on the intestine have clearly demonstrated an uptake into neuronal elements which are not noradrenergic.

Robinson and Gershon [17] reported that when MAO was inhibited and 5-HT was administered to guinea-pigs after the depletion of NA, a diffuse yellow fluorescence, which was more widespread than that of noradrenergic axons in the normal, could be observed throughout the myenteric plexus when it was prepared using a histochemical method for aromatic amines. They attributed this to the presence of elements, presumed to be neuronal, which take up 5-HT. Further evidence for non-adrenergic elements which could take up indoleamines was obtained by Gershon and Altman [30] who reported a high affinity uptake mechanism for 5-HT ($K_m = 7.4 \times 10^{-7}$ M, $V_{max} = 0.22$ nmoles/g/min) which was not competitively inhibited by NA. This work was later confirmed and it was also found that NA uptake and 5-HT uptake were differentially affected by drugs, chlorimipramine being the most effective against 5-HT uptake and desmethylimipramine the most effective in inhibiting NA uptake [31]. It was also shown that axons with a propensity to take up 5-HT are found in the intestine in foetal life before axons which take up NA are apparent [32]. Further studies, using sections of intestine grown in tissue culture for three weeks, demonstrated that the elements which take up 5-HT are intrinsic to the intestine, and by electron microscope autoradiography these elements were shown to be neuronal [33]. The close analogue of 5-HT, 6-hydroxytryptamine, was taken up into intrinsic nerve cell bodies and axons after noradrenergic axons were caused to degenerate by 6-hydroxydopamine or extrinsic denervation [22, 31].

5,6-Dihydroxytryptamine (5,6-DHT) and 5,7-dihydroxytryptamine, close analogues of 5-HT, are taken up by neurons which take up 5-HT in the central nervous system and cause these neurons to degenerate [34]. Evidence for the degeneration of axons in the myenteric plexus after the injection of 5,6-DHT and 5,7-DHT has been obtained by electron microscopy [35–37]. These axons were not noradrenergic because 5,6-DHT caused no impairment of NA uptake into the tissue whereas 5-HT uptake was reduced [35] and in some experiments extrinsic noradrenergic nerves were eliminated by autotransplantation of the intestine [36]. The nerves degenerated by 5,6-DHT contained vesicles of 120 to 160 nm in diameter which contained dense cores [36].

Presence of AADC in intrinsic neurons. Observations that radiolabelled 5-HT could be detected in the intestine after the injection of [^{3}H]5-HTP into mice and that radioactivity was concentrated in the myenteric plexus imply that AADC in the intestine converts 5-HTP to 5-HT and suggested the enzyme to be in nerves [14, 38]. However, autoradiographic studies showed that nerves in the vas deferens and in the pineal, which were probably noradrenergic, became labelled after the injection of [^{3}H]5-HTP into rats [39, 40]. Robinson and Gershon [17] therefore designed experiments to distinguish between noradrenergic nerves and other nerves in the intestine which might contain AADC and have other characteristics of nerves utilizing an indoleamine as a transmitter. They found that, after depletion of catecholamines with α-methylparatyrosine, a yellow fluorescence could be detected in the myenteric plexus after the administration of 5-HTP. The presence of AADC in intrinsic neurons was later more clearly demonstrated with improved histochemical methods [41]. Segments of intestine were incubated with 6-hydroxydopamine to destroy noradrenergic nerves and were then incubated with *l*-dopa and an inhibitor of monoamine oxidase. After this treatment, dopamine could be detected histochemically in intrinsic neurons. The inhibitor of AADC, Ro 4-4602, pre-

vented the appearance of the fluorescence. Experiments using segments of guinea-pig ileum in which the noradrenergic nerves had previously degenerated following interruption of their axons where they run through the mesentery or following the injection of 6-hydroxydopamine have confirmed these observations [22]. It has also been shown biochemically that segments of intestine grown in tissue culture and lacking both enterochromaffin cells and noradrenergic axons contain AADC [42].

Presence of TPH in intrinsic neurons

Immunoreactivity for TPH in intrinsic neurons of the intestine was demonstrated histochemically using antiserum raised in rabbits against rat TPH [43]. The enzyme was located in the cell bodies of intrinsic neurons of the myenteric and submucous plexuses of guinea-pigs, mice and rats. Biochemical experiments confirmed the presence of TPH in longitudinal muscle/myenteric plexus preparations [42]. Moreover, using a fluorescence histochemical method for aromatic amines, these authors found a diffuse yellowish fluorescence throughout the myenteric plexus after inhibition of MAO and injection of *l*-tryptophan. After treatment with *p*-chlorophenylalanine, an inhibitor of TPH, much of the diffuse fluorescence was no longer induced and fluorescence in nerve cell bodies was more clearly seen. The authors attribute this result to a greater sensitivity of TPH in axons, as compared with cell bodies, to inactivation by *p*-chlorophenylalanine.

Presence of MAO in intrinsic neurons

This enzyme has been demonstrated histochemically in intrinsic neurons of both the myenteric and submucous plexuses [44]. In order to demonstrate the retention of amines such as 5-HT in intrinsic neurons it is necessary to inhibit MAO, which indicates that neurons which take up indoleamines and neurons which contain the enzymes AADC and TPH also contain MAO [17, 22, 41, 42].

Absence of TH and DBH from intrinsic neurons

There is biochemical, histochemical and functional evidence which shows that the origins of noradrenergic nerves are extrinsic to the intestine [5, 22]. However, there is evidence for a neurotransmitter in the intestine which is pharmacologically similar to dopamine and is released from intrinsic neurons [3, 45]. If the transmitter is also chemically related to dopamine the nerve plexuses from the extrinsically denervated intestine would be expected to contain tyrosine hydroxylase. However, assayable TH disappeared from the wall of the small intestine when extrinsic nerves were interrupted and their terminals allowed to degenerate [46]. This study also showed that there is no DBH associated with intrinsic neurons.

Distribution of intrinsic nerve cell bodies and axons which could synthesize indoleamines

Furness and Costa [22] have reported that the distributions of cell bodies and axons of intrinsic neurons which contain both AADC and MAO and of those which take up indoleamines (and also dopamine) are apparently the same. Because biosynthetic pathways for catecholamines are apparently absent, it seems likely that the same neurons contain TPH, AADC and MAO. It was found that most of the cell bodies are in the submucous plexus (about 10 per cent of the total in this plexus) and that a smaller proportion (about 0.5 per cent) are found in the myenteric plexus. These figures are approximately equivalent to a total of 30,000 of the (presumed) indoleamine neurons in the submucosa of the small intestine and 3,000 in the myenteric plexus. Considering that not all cells might have loaded successfully, these figures must be regarded as estimates of the minimum numbers of amine-handling neurons in the guinea-pig small intestine. The axons of these neurons were very numerous, considerably more numerous than those of noradrenergic nerves. They ramified extensively amongst the nerve cell bodies of both plexuses and also contributed to the deep muscular plexus in the circular muscle. A few of the axons ran with arterioles. In fact, on the basis of their widespread distribution, a number of functional roles would be feasible.

Receptors for 5-HT and their antagonists

The classification of receptors for 5-HT in the intestine, which has been substantiated in numerous investigations, has been reviewed [7, 47–49]. 5-HT acts on receptors on the smooth muscle (muscle receptors) and on receptors on enteric neurons (nerve receptors); it causes the muscle to contract and excites both cholinergic and enteric inhibitory neurons. LSD and its analogues bromolysergic acid and methysergide are effective and selective antagonists of muscle receptors for 5-HT. Neural receptors are blocked by biguanides and by quaternary derivatives of 5-HT, although there appear to be species differences in the effectiveness of biguanides [50]. For both types of receptor continued exposure to 5-HT causes desensitization (tachyphylaxis) and this ability of 5-HT to inactivate its own receptors has been used as a pharmacological tool in some of the experiments described below. Nerve receptors for 5-HT were formerly called M receptors, because the contraction of the ileum caused by the stimulation of cholinergic neurons by 5-HT is substantially reduced by morphine. It is now quite clear that morphine was not acting on the receptors for 5-HT, but that it acted on other receptors on the cholinergic neurons to reduce the output of acetylcholine.

5-HT and vagal inhibitory pathways

The hypothesis that there are two groups of preganglionic neurons, one which releases 5-HT and another which releases acetylcholine, in the vagal pathway which causes gastric inhibition, has been advanced [15]. It was found that acetylcholine, 5-HT and vagus nerve stimulation all activated enteric inhibitory nerves to the guinea-pig stomach. The effects of acetylcholine and its analogues were selectively blocked by pentolinium or hexamethonium while the effects of 5-HT were selectively blocked by desensitizing the receptors with 5-HT itself. Pentolinium or hexamethonium by themselves, or desensitization of 5-HT receptors by itself, only partly blocked the inhibitory effect of vagal stimulation. Complete block was only achieved when antagonists of acetylcholine and desensitization of 5-HT receptors were combined. It was also found that transmural stimulation of the isolated stomach of the mouse, after the mucosa had been largely destroyed by asphyxiation, caused the release of a 5-HT-like sub-

stance and that this release was blocked by tetrodotoxin [15]. Beani, Bianchi and Crema [51] repeated the desensitization experiments of Bülbring and Gershon [15] using a slightly different arrangement. They prepared broad (15 mm) strips of stomach supplied by the vagus and removed the mucosa from these strips. They found that relaxations in response to vagal stimulation were only partly blocked by hexamethonium but, unlike Bülbring and Gershon, they found no reduction of the hexamethonium insensitive component when receptors for 5-HT in the stomach were desensitized.

More recently, Rattan and Goyal [52] have produced evidence for 5-HT as a transmitter in preganglionic vagal axons impinging on enteric inhibitory nerves supplying the lower esophageal sphincter in the possum. They found that stimulation of the vagus relaxed the sphincter in the combined presence of hexamethonium and atropine. 5-Methoxydimethyltryptamine, which antagonized the action of 5-HT, abolished this relaxation of the sphincter caused by vagal stimulation. However, attempts to detect 5-HT axons in the vagus have not been successful. Dreyfus, Sherman and Gershon [33] were able to detect green (noradrenaline) fluorescence of axons proximal to a ligature of the vagus in the guinea-pig. However, after the fluorescence of the noradrenergic axons had been abolished by 6-hydroxydopamine or α-methylparatyrosine, no 5-HT-containing axons were revealed, even when monoamine oxidase was inhibited and the animals were injected with tryptophan. Uptake experiments showed that the vagus nerves did not concentrate 5-HT. Furthermore, no tryptophan hydroxylase could be found in the nerves with immunohistochemical methods.

An explanation for the apparently contradictory results of Bülbring and Gershon [15] and Beani *et al.* [51] and for the apparent inconsistency of the pharmacological results (which imply that 5-HT fibres are in the vagus) and the neurochemical results (which show that there are probably no vagal 5-HT fibres) is suggested by the observation that stimulation of noradrenergic axons running with the vagus releases 5-HT from enterochromaffin cells (see below). Perhaps, in Bülbring and Gershon's [15] experiments, 5-HT was released from the enterochromaffin cells and diffused to the inhibitory neurons. In the experiments of Beani *et al.* [51] this would not have been possible as the mucosa containing the enterochromaffin cells was removed. The mucosa was present in the experiments of Rattan and Goyal [52].

Pharmacological evidence for release of endogenous 5-HT from intramural nerves

Contractions elicited by transmural stimulation of circular muscle strips taken from the most distal 1.5 cm of the canine esophagus have been reported to be blocked by bromolysergic acid and by exposure to 5-HT [53], suggesting that the transmitter released from these nerves acts on the same receptors as 5-HT. The contractions were not modified either by atropine or eserine. These experiments do not appear to have been repeated by other investigators, although Christensen [54] has reviewed the literature on the esophagus which confirms that transmission to the circular muscle is atropine-resistant in other species.

Some experiments suggest that nicotine might release 5-HT or a similar substance from intestinal nerves [55]. It was found that nicotine caused relaxations of the guinea-pig distal colon which were blocked by tetrodotoxin. These relaxations were also blocked by desensitizing receptors for 5-HT and by metoclopramide. 5-HT had no direct effect on the muscle. It did, however, stimulate inhibitory nerves to the muscle and this effect was blocked by 5-HT desensitization of its own receptors as well as by metoclopramide. A simple explanation of these results would be that nicotine causes the release of 5-HT which then stimulates enteric inhibitory nerves which relax the muscle. The question which is unsolved is the source of the 5-HT-like substance: it could be cells other than neurons.

Transmural stimulation of the proximal colon of the guinea-pig at frequencies of 5 to 50 Hz causes release of an excitatory substance which might be 5-HT. Such stimulation in the presence of hyoscine resulted in a relaxation of the longitudinal muscle which was followed, after the end of stimulation, by a rapid and then by a slow contraction [56]. The slow contraction is nerve-mediated in that it was blocked by tetrodotoxin and was considered to be non-cholinergic because it was unaffected by hyoscine in concentrations up to 10^{-5} g/ml. In the presence of tetrodotoxin, 5-HT caused similar contractions indicating that it acts directly on the muscle. Methysergide and phentolamine were both effective antagonists of the slow contraction and of the action of 5-HT on the muscle [1]. When the muscle was desensitized to the contractile action of 5-HT by sustained exposure, the slow contraction in response to transmural stimulation was abolished, although the excitability of the muscle was not impaired. These experiments indicate that the substance which causes the slow contraction acts through the same receptors as 5-HT.

When recordings of electrical activity in enteric neurons are made with suction electrodes, direct activation of the neurons can be distinguished from synaptic activation by differences in latency and in ability to follow a train of stimuli and by the susceptibility of synaptic transmission to removal of calcium [57]. Brief trains of stimuli (40 Hz for 0.5 sec) caused a repetitive synaptic activation of neurons lasting 10 to 30 sec. This barrage of activity which followed stimulation was abolished if receptors for 5-HT were desensitized by exposing the tissue to 10^{-5} M 5-HT. The repetitive activity was not significantly affected by hexamethonium (10^{-4} M), although hexamethonium and curare, another antagonist acting at nicotinic receptors, are effective antagonists of excitatory synaptic transmission in the intestine when the stimuli are single pulses [58, 59]. The repetitive synaptic activation was examined in preparations devoid of mucosa which therefore did not contain enterochromaffin cells which could be sources of 5-HT. These results, like those for the proximal colon (see above) suggest that activity in neurons releasing 5-HT or a similar substance continues after a brief high frequency train of stimuli.

Possible involvement of nerves releasing 5-HT in peristalsis

The pharmacology of the events underlying the peristaltic reflex have been examined by Costa and Furness [4] using a system in which the contraction which occurs on the oral side of a point of distension and the relaxation occurring on the anal side are recorded

separately. The reflex which results in a contraction on the oral side has been called the ascending excitatory reflex. In the guinea-pig colon, it was found that the ascending excitatory reflex was partly blocked by hyoscine and was also partly blocked by methysergide or by desensitizing the preparation to 5-HT. Complete block was obtained when hyoscine and either methysergide or 5-HT desensitization were combined. The reflex was entirely blocked by tetrodotoxin. These results indicate that when the ascending excitatory reflex is activated, a stimulant of the smooth muscle, other than acetylcholine, is released, and suggest that it could be 5-HT. However, when exogenous 5-HT was applied to the circular muscle in concentrations up to 10^{-4} g/ml it failed to have any direct effect on the muscle in more than 90 per cent of experiments [50]. When a direct response was observed it was a weak contraction. In the ileum the ascending excitatory reflex was completely blocked by hyoscine ([60] and unpublished observations). However, methysergide and desensitizing the preparation to 5-HT also caused a partial antagonism. This would suggest that the final transmitter in the pathway, which acts on the smooth muscle, is acetylcholine, and that another transmitter whose action is sensitive to 5-HT desensitization acts at a neuro-neuronal synapse. The action of methysergide is difficult to explain, in that it is not an antagonist of the action of 5-HT on intestinal neurons [49, 50]. The effect of drugs on the excitatory reflex could be explained by assuming that a transmitter pharmacologically similar to 5-HT is released at a neuro-neuronal synapse in the pathway and that both 5-HT and methysergide also bind to receptors for this substance and can act as antagonists. In the isolated colon the unknown transmitter is released in sufficient quantity to diffuse to the circular muscle which also contains receptors for the transmitter. In the ileum, either the substance is not released in sufficient quantity, or there are not receptors for it in the circular muscle.

Antagonism of reflex contractions of the circular muscle by 5-HT has also been reported in investigations using other methods of recording peristalsis [8–10, 12].

If nerves releasing 5-HT were essential for peristalsis, it would be expected that the peristaltic reflex would be inhibited when 5-HT stores were depleted. When reserpine was used to reduce 5-HT to 2 per cent or less of control, the reflex was unimpaired [61]. Similarly, when tryptophan-free diets were used to reduce 5-HT to undetectable levels the peristaltic reflex remained intact [62]. In both these studies, the assays were of mucosal 5-HT: attempts to separately estimate 5-HT in the muscle layers were not made.

Evidence for 5-HT release from nerves impinging on intestinal vasodilator nerves

Blood flow through the decentralized cat jejunum, *in vivo*, is increased when the mucosa is irritated [63]. This increase was blocked by injecting tetrodotoxin into the arterial supply to the jejunum, indicating that it was nerve-mediated. It was also blocked by the 5-HT receptor antagonist, bromolysergic acid, suggesting that the transmitter might be 5-HT. The responses were not affected by atropine, by blockade of α or β receptors for noradrenaline or by blocking nicotinic receptors. Similar vasodilator responses in the cat small intestine in response to electrical stimulation of intramural nerves have been reported [64]. The vasodilator responses to both mucosal irritation and transmural stimulation were blocked by a continuous intra-arterial infusion of 5-HT which was used to desensitize receptors for 5-HT [65]. It was found that the close intra-arterial injection of a bolus of 5-HT caused an increase in intestinal blood flow, but this increase was blocked and a decrease was observed in the presence of tetrodotoxin, indicating that 5-HT stimulates intramural vasodilator nerves as well as having a direct constrictor action [66]. Transmural stimulation was associated with a release of 5-HT which could be detected in the venous outflow from the intestine. However, it was not determined whether or not the release was affected by tetrodotoxin. The results so far have failed to identify the cell type from which a 5-HT-like substance which stimulates intrinsic vasodilator nerves is released. The release might be from enterochromaffin cells which would be expected to release 5-HT when irritated or depolarized by electrical stimulation. On the other hand release might be from nerves, in which case the transmitter seems to act through the same receptors as 5-HT.

The vessels of the proximal colon of the cat dilated when the mucosa was irritated or when 5-HT was injected into the superior mesenteric artery [67]. Both effects were blocked by dihydroergotamine. These results suggest that a similar reflex to that in the small intestine also exists for the cat colon.

Nerve-mediated release of 5-HT from enterochromaffin cells

In both cats and guinea-pigs it has been shown that stimulation of the vagus decreases the 5-HT content of enterochromaffin cells in the small intestine [68, 69]. The release appears to be due to the stimulation of sympathetic, noradrenergic nerves which have been shown in fluorescence histochemical studies to run with the vagus [70–72]. The response was prevented by destroying noradrenergic nerves by the injection of 6-hydroxydopamine or by injecting antibodies to nerve growth factor and was also prevented by prior removal of the superior cervical ganglia. These experiments illustrate that 5-HT can be released through nerve activity, even though the source of 5-HT is not the nerves themselves.

Collection of assayable 5-HT in response to nerve stimulation

A substance which contracts the rat fundus and whose action on the fundus is blocked by methysergide is released from the mouse stomach in response to stimulation of intramural nerves [15]. The collections were made from stomachs in which the mucosa had sloughed off and had been removed following three hours asphyxiation. Histological examination showed only small remnants of mucosa. These experiments leave several doubts. Firstly, could the 5-HT-like substance have come from enterochromaffin or mast cells? This is not possible to exclude, but it seems unlikely because the release was blocked by tetrodotoxin and even in the remnants of mucosa, no argentaffin (enterochromaffin) cells could be detected. Could 5-HT, liberated from the asphyxiated mucosa, have been taken up into nerves and subsequently released by nerve stimulation? This is possible because intestinal nerves have

been shown to take up [^{3}H]5-HT and release it in response to electrical stimulation, the release being blocked by tetrodotoxin [73]. Could the active substance be something other than 5-HT? This is also possible; a number of substances appear to be agonists acting on the same receptors as 5-HT and some of these contract the rat fundic strip [74, 75]. Other experiments to measure 5-HT output in response to stimulation are subject to similar doubts about the true source and/or the true identity of the 5-HT-like substance.

Conclusions

There is ample evidence that a class of intrinsic nerves exists in the gastrointestinal tract which can take up and retain indoleamines, which can synthesize indoleamines utilizing the enzymes TPH and AADC and which can inactivate indoleamines through MAO. Despite the fact that the intrinsic indoleamine handling neurons are numerous and their terminals ramify extensively within the gut wall, efforts to procure adequate evidence for the presence of endogenous 5-HT in intestinal nerves have been unsuccessful. This indicates that, if 5-HT is an intestinal neurotransmitter, it is stored in amounts which are too low to be reliably detected.

There is substantial pharmacological evidence for the release of a substance from intestinal nerves which acts through the same receptors as 5-HT. The experimental data indicates that this 5-HT-like transmitter is released at neuro-neuronal synapses. It stimulates cholinergic excitatory pathways involved in the peristaltic reflex and may also stimulate enteric inhibitory neurons in vagal pathways to the lower esophagus and stomach. When the neurons are stimulated at high frequencies sufficient transmitter can be released to overflow to the muscle and cause it to contract. As yet, there is not sufficient evidence to confidently identify this substance as 5-HT, although it seems likely that it is an indoleamine.

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