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The enteric neural receptor for 5-hydroxytryptamine

by M. D. Gershon, M. Takaki, H. Tamir and T. Branchek

Departments of Anatomy and Cell Biology and Psychiatry, Columbia University, College of Physicians and Surgeons, 630 West 168th Street, New York (New York 10032, USA)

Summary. An enteric neural receptor for serotonin (5-HT) has been characterized. This receptor was assayed, using ^{3}H-5-HT as a radioligand, by rapid filtration of isolated enteric membranes and by radioautography. In addition, intracellular recordings were made from ganglion cells of the myenteric plexus. High affinity, saturable, reversible, and specific binding of ^{3}H-5-HT was demonstrated both to membranes of the dissected longitudinal muscle with adherent myenteric plexus and the mucosa-submucosa. Radioautographs showed these ^{3}H-5-HT binding sites to be in myenteric ganglia and in a broad unresolved band at the mucosal-submucosal interface. Antagonists active at receptors for other neurotransmitters than 5-HT, at either of the two known types of CNS 5-HT receptor, and at 5-HT uptake sites on serotonergic neurons failed to inhibit binding of ^{3}H-5-HT. The structural requirements of analogues for binding to the enteric 5-HT receptor matched the known pharmacology of M or neural 5-HT receptors. A novel 5-HT antagonist was found. This compound, N-acetyl-5-hydroxytryptophyl-5-hydroxytryptophan amide (5-HTP-DP), antagonized the action of 5-HT on type II/AH cells of the myenteric plexus but did not affect the release or actions of acetylcholine (nicotinic or muscarinic) or substance P. 5-HTP-DP was also an equally potent displacer of ^{3}H-5-HT from its binding sites on enteric membranes. It is concluded that the sites responsible for specific binding of ^{3}H-5-HT are enteric M or neural 5-HT receptors. These receptors differ from those now known to be present in the CNS.
Key words. 5-hydroxytryptamine; serotonin; receptors; enteric nervous system; gut; radioautography.

Serotonin (5-hydroxytryptamine, 5-HT) has many actions in the gastrointestinal tract. These include a variety of neural effects[5,7,10,24,26,30,32,40], stimulation of smooth muscle[7,21,34], as well as actions on the crypt epithelium of the intestinal mucosa[6,9,20]. The neural effects of 5-HT are themselves multiple. These include an action on mucosal afferent nerve fibers to initiate the peristaltic reflex[3,4], a presynaptic inhibition of the release of acetylcholine (ACh) that reduces the amplitude of fast excitatory postsynaptic potentials (epsps) in myenteric ganglia[32], activation of cholinergic neurons of the myenteric plexus to release ACh[39], activation of non-adrenergic, non-cholinergic inhibitory neurons to relax the bowel[5], a fast depolarization associated with an increased membrane conductance of type II/AH myenteric ganglion cells[24], and a slow depolarization associated with a decreased membrane conductance of other (type II/AH) myenteric ganglion cells[24,40]. Needless to say, the abundance of its actions has proved to be an embarassment of riches and it has not been easy to determine which of the many actions of 5-HT are of physiological importance. Probably the major impediment for research in this area is a lack of adequate knowledge about the nature of the receptors acted upon by 5-HT to initiate its many enteric effects. It seems likely that some of the actions of 5-HT on the gut will be more than simple pharmacological curiosities. The gut is a major 5-HT storage organ. Within the alimentary tract the amine is found in enteroendocrine cells (EC cells) of the mucosa[12] and in neurons of the enteric nervous system (ENS)[8,11,13,14,18,25,33]. This dual localization would suggest that 5-HT might function as a local hormone and as a neurotransmitter (or neuromodulator). If so, that would make mucosal or mucosal-submucosal receptors and receptors in the myenteric plexus particularly interesting.

In order to develop tools for the exploration of 5-HT receptors, we have designed radioligand binding assays for them[2]. Our strategy was first to obtain membrane fractions that specifically, reversibly, and saturably bound ^{3}H-5-HT. This binding activity was assayed by rapid filtration. Second, we developed a method for radioautographic analysis that permitted the localization of the ^{3}H-5-HT binding sites. Third, we determined the structure-activity requirements for the ability of 5-HT analogues to compete with ^{3}H-5-HT for its recognition sites. Fourth, we sought to develop a potential antagonist from data derived from these studies. Fifth, and finally, we compared the results on 5-HT binding derived from radioligand filtration assays and radioautography with electrophysiological studies of myenteric ganglion cells to provide evidence that the binding sites studied were actually 5-HT receptors.

Filtration assays[2]

The entire small intestine was removed from male New Zealand white rabbits (0.5–1 lbs). The longitudinal muscle with adherent myenteric plexus (LM-MP) was dissected free and homogenized with 20 volumes of iced buffer (50 mM Tris-HCl, pH 7.4). The remaining mucosa-submucosa (M-S) was separately homogenized in a similar fashion. The resulting homogenates were then subjected to differential centrifugation to obtain a membrane fraction. An intermediate pellet was obtained by centrifugation at 27,000 × g for 30 min. Since preliminary experiments revealed little specific binding of ^{3}H-5-HT in this fraction, this pellet was discarded and the supernatant was centrifuged at 100,000 × g for 60 min to obtain the final fraction of membranes for binding assay. In the LM-MP preparation, the intermediate (27,000 × g) fraction is one that contains isolated varicosities (autonomic synaptosomes) from the myenteric plexus[25]. These isolated varicosities, unlike CNS synaptosomes, do not pellet with postsynaptic membranes attached to them. Ganglionic postsynaptic membranes, as well as other membranes, therefore, are found in the material sedimenting at 100,000 × g.

Aliquots of sedimented membranes (containing 1 mg protein/ml) were re-suspended and the binding of ^{3}H-5-

HT to them was evaluated by a modification[2] of the rapid filtration method of Bennett and Snyder[1]. The membranes were incubated with ^{3}H-5-HT for 10 min at 37 °C. Following incubation the membranes were collected by vacuum filtration on glass fiber filters (Whatman GF/B). ^{3}H-5-HT was extracted from the filters with 70 % ethanol and radioactivity was counted by liquid scintillation. Non-radioactive 5-HT was used as a displacing agent (no specific antagonist being available at the outset of our studies) and specific binding was defined as that displaced by a 1000-fold excess of non-radioactive 5-HT.

In initial experiments, the specific binding of ^{3}H-5-HT was examined as a function of the concentration of 5-HT in the incubating medium. Membranes of the LM-MP and M-S preparations were separately analyzed. Saturation isotherms were constructed. At low concentrations of 5-HT, saturation of binding was rapidly approached. At higher concentrations of 5-HT (above 50 nM), a second lower affinity binding site was also noted. Scatchard-type analysis of the high affinity binding site for the LM-MP preparation revealed an equilibrium dissociation constant (K_D) of 2.7 ± 0.2 nM and a B_{max} of 92.3 ± 19.4 fmol/mg. This K_D was not significantly different from that of the M-S preparation (1.4 nM) but the B_{max} of the M-S membranes was only about ⅓ that of the LM-MP (31.6 fmol/mg protein). The similar affinities of the LM-MP and M-S ^{3}H-5-HT binding sites for ^{3}H-5-HT are compatible with the hypothesis that the same 5-HT receptor type is present in both locations. The M-S preparation, however, contains much more protein from a variety of tissues than does the LM-MP. The lower concentration of specific ^{3}H-5-HT binding sites in membranes of this preparation than in those of the LM-MP, in which neural elements from the myenteric plexus are a more significant component, would be anticipated if the 5-HT receptor in both preparations were a neural one. The Hill coefficient of ^{3}H-5-HT binding to the high affinity site was found to be 0.99 ± 0.7. It seems likely, therefore, that there is only a single category of high affinity ^{3}H-5-HT binding site in the gut with probably no positive or negative cooperativity.

The high affinity binding of ^{3}H-5-HT was not only saturable, but dissociable as well. The rate of dissociation of ^{3}H-5-HT from its enteric binding site was measured as a function of time following the addition of a 1000-fold excess of non-radioactive 5-HT. The rate constant for the dissociation of ^{3}H-5-HT from enteric membranes was estimated to be about 0.1 min^{-1}. The corresponding association constant of ^{3}H-5-HT binding to membranes (K_{+1}) was also estimated. The experimentally observed rate constant (K_{ob}) for the specific binding of ^{3}H-5-HT as a function of time following its addition to a suspension of membranes to the achievement of equilibrium was measured. The relationship of K_{ob} and the previously determined K_{-1} yielded a K_{+1} of approximately 7.5×10^7 mol^{-1} min^{-1}. The ratio of the dissociation constant to the association constant (K_{-1}/K_{+1}) provided a kinetic estimate of the equilibrium dissociation constant, K_D, and was 1.5 nM. This kinetic estimate of K_D is in good agreement with the estimates derived from Scatchard-type analysis of saturation isotherms (1.4 nM for the M-S and 2.7 nM for the LM-MP); therefore, it seems likely that the assumptions that underlie the analysis of binding data by the methods used are valid and the behavior of the ^{3}H-5-HT binding site is that expected for a receptor.

Radioautography[2]

Small intestines were removed from rabbits, guinea pigs, and mice. Segments were rapidly frozen, sections were cut on a cryostat-microtome, and thaw-mounted onto glass slides. The sections were preincubated for 15 min at 37° in 300 mM Tris maleate buffer (pH 7.4) containing pargyline, and then incubated with ^{3}H-5-HT (2–100 nM) for 30 min at 25 °C. Following incubation, the slide-mounted sections were rapidly washed, dried, and exposed to tritium-sensitive film (LKB Ultrofilm), typically for 1–2 weeks. Again, specific binding was defined as that displaced by an excess (1000-fold) of non-radioactive 5-HT. This is a modification of the method Meibach[28]. Binding sites for ^{3}H-5-HT were found in the same locations in all three species. Silver grains denoting the specific binding of ^{3}H-5-HT were found as localized clusters over the ganglia of the myenteric plexus and as a broad band covering the mucosa-submucosa junction (fig. 1, 2). Not labeled by ^{3}H-5-HT were the villus epithelium, the deep connective tissue of the submucosa, the smooth muscle of the villi or the muscularis externa, or the serosa. The M-S zone of labeling did not have a resolution adequate to distinguish between possible labeling limited to the epithelial cells of the crypts of Lieberkuhn, the nerve terminals in the lamina propria just underneath this epithelium, the submucosal plexus, or some combination of these elements. All of these, in consequence, on the basis of radioautographic data alone, have to be considered potential ^{3}H-5-HT binding sites. In any case, the location of ^{3}H-5-HT binding in the bowel is appropriate for a receptor acted upon by EC cell 5-HT to initiate the peristaltic reflex[5] (the M-S sites) or a receptor to receive 5-HT released by a serotonergic interneuron within the ENS (the myenteric ganglion sites). The ^{3}H-5-HT binding sites are not appropriately located for them to be receptors mediating the direct actions of 5-HT on smooth muscle.

Moreover, the absence of binding sites from villus epithelium and, more importantly, the observation of Gaginella and co-workers that isolated intestinal epithelial cells do not specifically bind ^{3}H-5-HT[17] suggest that the ^{3}H-5-HT binding site is not an epithelial 5-HT receptor.

Structure-activity requirements for antagonism of the binding of ^{3}H-5-HT[2]

Analogues were tested for their ability to displace ^{3}H-5-HT from its binding sites on enteric membranes. Although LM-MP and M-S membranes were separately analyzed, identical results were obtained with each, supporting the view that binding sites in each location represent the same type of receptor. For screening, all analogues were tried against a fixed concentration (10 mM) of ^{3}H-5-HT. In essence, all effective displacing agents were ring-hydroxylated indoles. The order of potency of these compounds relative to 5-HT, assigned an arbitrary value of 1, was 5-HT > 6-hydroxytryptamine (6-HT; 0.77) > bufotenin [5-OH-N,N,-dimethyltryptamine] (0.42) > 5-hydroxyindole acetic acid (0.29) > 5,6-dihy-

droxytryptamine (0.15) > 5-hydroxyindole (0.07). Other compounds able to antagonize the binding of 10 nM ^{3}H-5-HT when present at a 1000-fold higher concentration (10 μM) included (in order of potency) 7-hydroxytryptamine, 5-hydroxy-6-methyltryptamine, L-alpha-methyl-5-hydroxytryptamine, 4-hydroxytryptamine, D-alpha-methyl-5-hydroxytryptamine, 5-hydroxy-7-methoxy-tryptamine, 5,6,7-trihydroxytryptamine, and 4-hydroxyindole. In contrast to these compounds, non-ring-hydroxylated indoles including tryptamine, 5-methoxytryptamine and 5-aminotryptamine had no effect at all on the binding of 10 nM ^{3}H-5-HT even when present at 10 μM. It can be concluded from these observations that ring hydroxylation of indoles preferably at the five or six positions is essential for affinity at the ^{3}H-5-HT-binding site. The aliphatic side chain of the tryptamines seems to be less essential for affinity than the indole ring of the molecules, although modifications of the side chain did moderately reduce potency for binding relative to 5-HT. The relatively lower affinity of 5,6-dihydroxytryptamine or 5,6,7-trihydroxytryptamine relative to the singly hydroxylated compounds, 5-HT or 6-HT, suggests some steric hindrance can occur. Alternatively, the apparently lower potency of the di- and tri-hydroxylated compounds could reflect their chemical instability.

The structure-activity requirements for affinity at the ^{3}H-5-HT binding site are those that would be predicted for an enteric neural 5-HT receptor. These receptors were first classified on the basis of pharmacological experiments with the guinea pig ileum by Gaddum and Picarelli[16]. They identified an 'M' and a 'D' receptor. M receptor-mediated effects were antagonized by morphine and D receptor-mediated actions were blocked by phenoxybenzamine. It was subsequently pointed out that neither morphine nor phenoxybenzamine are specific 5-HT antagonists[10]. Rather, they are physiological antagonists; however, morphine does prevent neurally mediated contractions of the bowel in response to 5-HT because it interferes with the release of the excitatory nerve-muscle transmitter, acetylcholine (ACh)[35]. The M and D receptor terminology, therefore, is probably inappropriate; nevertheless, it is established in the literature. The neural receptors for 5-HT are usually known, therefore, as M receptors while direct muscular actions are considered to be mediated via D receptors[15,22,23]. M receptors require hydroxylation of the indole ring in order to be activated[10,15,19] while D receptors are at least as potently stimulated by tryptamine as by 5-HT[15,19]. The requirements for affinity at the ^{3}H-5-HT binding site, therefore, are those of an M and not a D receptor. These requirements at the M site, interestingly, are very similar to those at other peripheral ganglia acted upon by 5-HT[19].

Structure-activity studies done with filtration assays were repeated, with identical results, using radioautography. This supports the view that the same type of ^{3}H-5-HT binding site is detected by radioautographic and filtration assays.

Specificity of ^{3}H-5-HT binding sites[2]

The specificity of the enteric sites able to bind ^{3}H-5-HT was investigated using ligands known to be antagonists or agonists at a variety of receptors. Potential displacers of ^{3}H-5-HT binding were incubated with 10 nM ^{3}H-5-HT and preparations of enteric membranes. Quantitative results obtained with filtration assays were qualitatively duplicated using radioautography. Compounds evaluated included drugs known to be antagonists at alpha and beta adrenoceptors (phentolamine and propranalol), nicotinic and muscarinic ACh receptors (hexamethonium and atropine), dopamine receptors (butaclamol, domperidone, metoclopramide, spiroperidol), histamine H_1 and H_2 receptors (cyproheptadine, diphenhydramine, mianserin, and cimetadine), and opiate receptors (naloxone). In addition, no inhibition of ^{3}H-5-HT binding was manifested by compounds that specifically antagonize the high affinity neuronal uptake of ^{3}H-5-HT (fenfluramine, fluoxetine, trazodone, and zimelidine).

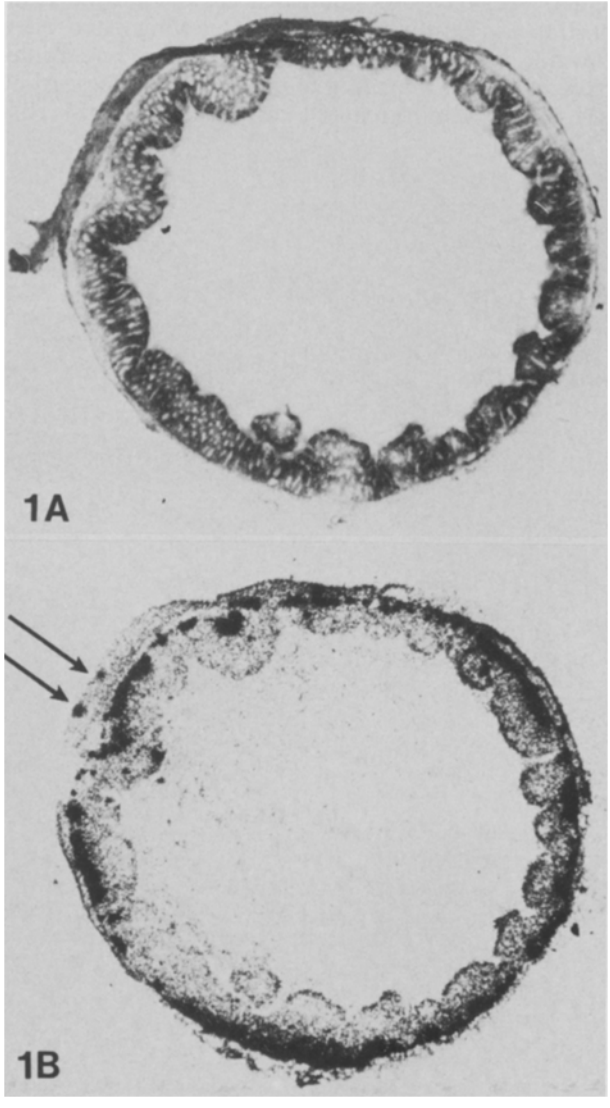

Figure 1. Low power dry mount radioautographs showing the binding of ^{3}H-5-HT to frozen sections of the guinea pig small intestine. *A* The tissue stained with toluidine blue. *B* The overlying ^{3}H-sensitive film showing the location of ^{3}H-5-HT binding sites. Only a small strip of muscularis externa appears at the upper left in (A). Note the labeling of ganglia within it (arrows). Note also the broad band of labeling at the mucosal-submucosal interface. Muscle, connective tissue and villus epithelium are not labeled. Magnification × 17.

Finally, many compounds able to bind to one or the other of the two types of CNS 5-HT receptor were evaluated and none were able to displace enteric binding of ^{3}H-5-HT. S_1 compounds tested included cinanserin, d-lysergic acid diethylamide (d-LSD), metergoline, 5-methoxytryptamine, methysergide, 6-nitroquipazine, quipazine and trazodone. S_2 compounds included cyproheptadine, ketanserin, d-LSD, mianserin, and spiroperidol. These data indicate that the enteric ^{3}H-5-HT binding sites are not a known category of receptor for ACh, a catecholamine, histamine, or an opioid. Moreover, the enteric ^{3}H-5-HT binding site is probably not the membrane 5-HT transporter of serotonergic neurons or an S_1 or S_2 CNS 5-HT receptor subtype. When ^{3}H-d-LSD or ^{3}H-spiroperidol were used as radioligands in place of ^{3}H-5-HT, no specific binding was noted; therefore, not only is the enteric ^{3}H-5-HT binding site different from the CNS S_1 and S_2 5-HT receptors, but these receptors were not demonstrated to exist in the gut. It is interesting that d-tubocurarine, in contrast to hexamethonium, was a weak inhibitor of ^{3}H-5-HT binding to enteric membranes. At 10 μM, d-tubocurarine reduced the binding of 10 nM ^{3}H-5-HT by about 30%. D-Tubocurarine has been shown to be a weak antagonist of neural actions of 5-HT at this concentration[7].

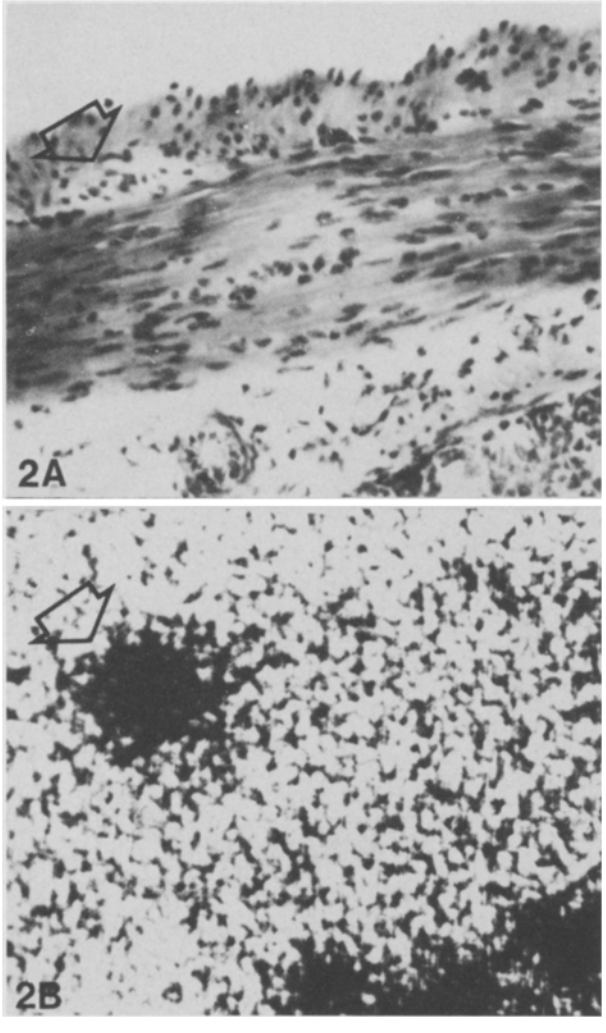

Figure 2. At higher magnification (of the section shown in fig. 1) the localized aggregations of grains (B) over the muscularis externa can be seen to correspond to the myenteric plexus (A). Magnification × 170.

In summary, studies of the enteric binding of ^{3}H-5-HT have revealed specific, saturable, reversible, high affinity binding of the amine to enteric membranes derived either from the LM-MP or M-S preparations. Properties of the binding sites in these two preparations are identical. Radioautographs prepared from fresh-frozen sections indicated that the ^{3}H-5-HT binding sites of the LM-MP are located in ganglia of the myenteric plexus, while those of the M-S preparation lie along a broad band, not specifically resolved, at the mucosal-submucosal junction. Structure activity requirements of molecules for affinity at the enteric ^{3}H-5-HT binding sites are compatible with the site being an M but not a D 5-HT receptor. The radioautographic location of the binding sites also supports this conclusion. 5-HT M receptor sites, like ^{3}H-5-HT binding sites, are located on nerve and not muscle. The D receptors, in contrast, are located on muscle, which does not specifically bind ^{3}H-5-HT. The observations are also consistent with the hypothesis that the ^{3}H-5-HT binding sites correspond to a unique, third type of 5-HT receptor, different in properties from those of other neurotransmitters, or either the S_1 or S_2 5-HT receptors of the CNS. The ability of hydroxylated analogues to displace ^{3}H-5-HT, and the apparent relative lack of importance of the aliphatic side chain for antagonizing the binding of ^{3}H-5-HT suggests that the indole nucleus is responsible for affinity to the binding site. In turn, it is possible that the aliphatic side chain could be involved in determining agonist efficacy. If so, a good antagonist would be a 5-hydroxylated indole compound, appropriately modified in the aliphatic side chain. We have recently investigated a dipeptide that meets these criteria and that seems to be a potent and specific antagonist at enteric neural receptors for 5-HT.

An antagonist of enteric neural actions of 5-HT

N-acetyl-5-hydroxytryptophyl-5-hydroxytryptophan amide (5-HTP-DP) is a synthetic dipeptide that inhibits the binding of 5-HT to its intravesicular storage protein[37]. This compound was previously studied and found to be analgesic when injected into the CNS[38]. We tested it in the gut against the action of 5-HT on ganglion cells of the myenteric plexus.

The LM-MP preparation was dissected from the guinea pig small intestine, pinned flat in a dish, and superfused with oxygenated Krebs solution, essentially as described by Wood and Mayer[41]. Ganglia were visualized using Nomarski differential interference contrast microscopy. Movement of individual ganglia was prevented by applying pressure with two metal bars (pressure feet) positioned on either side of the ganglion to be recorded from, perpendicular to the long axis of the longitudinal muscle fibers. Intracellular recordings were made from myenteric ganglion cells using electrodes filled with 3M KCl. At least three types of ganglion cell have been identified in the myenteric plexus[31,42]. In our studies, we considered type I/S cells to be cells that spiked repeatedly at a frequency proportional to injected depolarizing current. Type II/AH cells were those that displayed a long-lasting

hyperpolarization following an initial action potential, that did not spike repeatedly in response to injection of depolarizing current, and which, in contrast to type I/S cells, did not display anodal break excitation. Type III cells were those that did not show action potentials. Interganglionic fiber tracts leading to the ganglion from which recordings were made were stimulated with a second extracellular electrode in order to evoke post synaptic potentials. Fast epsps were studied in type I/S cells and slow epsps were examined in type II/AH cells. The fast epsp has been shown to be mediated by ACh on nicotinic receptors. The slow epsp is mimicked by 5-HT[40] and 5-HT is a candidate, along with substance P[27], to be a neurotransmitter responsible for slow epsps. In response to 5-HT or during a slow epsp, type II/AH cells display a slow depolarization associated with a decreased membrane conductance. Both 5-HT and the slow epsp antagonize the after-hyperpolarization of type II/AH cells. These cells thus spike repeatedly when injected with depolarizing current while under the influence of applied 5-HT or during a slow epsp. This action of 5-HT can be elicited by applying 5-HT to cells from micropipettes by iontophoresis or by pressure (using a Picospritzer). The receptor responsible for mediating this action of 5-HT desensitizes rapidly; consequently, the action of the amine is not apparent when 5-HT is added to the superfusing medium. A response similar to that of 5-HT can also be elicited in type II/AH cells by ACh[29].

5-HTP-DP (1–20 μM) added to the medium, blocks the 5-HT induced slow depolarization, associated with a decreased membrane conductance, of type II/AH cells[36]. It does not itself appear to have an agonist action. Significantly, 5-HTP-DP does not block the action of substance P or ACh on type II/AH cells. Its action on these cells, therefore, seems to be limited to that of an antagonist active at 5-HT receptors. It does not directly prevent the decreased potassium conductance that is common to the activation of 5-HT, substance P, and muscarinic receptors; nor does it act as an antagonist at the receptors for substance P or ACh on type II/AH cells. Moreover, 5-HTP-DP fails to inhibit fast epsps in type I/S cells. This indicates that the dipeptide lacks presynaptic actions on cholinergic nerve terminals and postsynaptic actions on nicotinic receptors. In contrast, compatible with mediation of slow epsps by 5-HT, slow epsps can be antagonized by 5-HTP-DP. Thus, while it is true that further work needs to be done on 5-HTP-DP, the compound appears to be a good antagonist of neural responses to 5-HT.

Correlation between electrophysiology of myenteric neurons and ^{3}H-5-HT binding

If the ^{3}H-5-HT binding site is the receptor responsible for the 5-HT-induced slow depolarization of type II/AH cells associated with a decreased membrane conductance, then an antagonist of this response should be effective in inhibiting specific binding of ^{3}H-5-HT to enteric membranes. 5-HTP-DP, as noted above, does appear to be a good antagonist of the action of 5-HT on type II/AH cells. We have also found it to be a good inhibitor of the binding of ^{3}H-5-HT to isolated enteric membranes provided it is assayed in Na^+-containing media (50 mM sodium phosphate buffer at pH 7.4). Its ID_{50} is about 2 μM[36] and it fully eliminates binding of ^{3}H-5-HT at 10 μM. When examined by radioautography, 5-HTP-DP is equally effective, suppressing the binding of ^{3}H-5-HT both within myenteric ganglia and at the mucosal-submucosal interface[36]. The efficacy of 5-HTP-DP as a displacing agent for the binding of ^{3}H-5-HT agrees well with its efficacy as a 5-HT antagonist on type II/AH cells[36]. This correlation supports the view that the enteric ^{3}H-5-HT binding site is actually a physiologically significant neural 5-HT receptor.

Conclusions

In conclusion, we have characterized a ^{3}H-5-HT binding site in the gut. The site has properties that are compatible with its identification as a 5-HT receptor. Binding of ^{3}H-5-HT is of high affinity, saturable, reversible and specific. Analysis of the ability of analogues to compete with ^{3}H-5-HT binding indicates that the binding site has the characteristics expected for an M or neural, not a D or muscle, 5-HT receptor. Radioautographic studies confirm a neural location of ^{3}H-5-HT binding sites in the ganglia of the myenteric plexus. There are additional sites at the boundary between the mucosa and submucosa that have not yet been resolved; however, 5-HT receptors involved in the initiation of the peristaltic reflex are known to be located in this region of the intestine. 5-HTP-DP appears to be a specific antagonist of neural responses to 5-HT and is an effective inhibitor of the enteric binding of ^{3}H-5-HT. The good correlation between neural actions and ^{3}H-5-HT binding indicates that the ^{3}H-5-HT binding assays, by filtration or radioautography, are of enteric neural 5-HT receptors. Studies with antagonists of known specificity show that this enteric neural class of 5-HT receptor is different from either of the two types of 5-HT receptor that have previously been characterized in the CNS. No evidence for the presence of these receptors, furthermore, could be found in the gut. Further studies with 5-HTP-DP should help to reveal which of the abundant actions of 5-HT in the gut are of physiological significance.

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