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Peripheral neural serotonin receptors: Identification and characterization with specific antagonists and agonists

(autonomic nervous system/enteric nervous system/gut)

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ABSTRACT **Serotonin (5-hydroxytryptamine, 5-HT) has been shown to be a neurotransmitter in the enteric nervous system (ENS). Although 5-HT is a mediator of slow excitatory postsynaptic potentials evoked by stimulation of interganglionic connectives, the precise role it plays in the physiology of the gut is unclear. Research has been hampered by an inadequate knowledge of the types of 5-HT receptor in the ENS and thus the lack of well-characterized antagonists. We now report the identification of two classes of enteric neural 5-HT receptor, the effects of activating these receptors on myenteric type II/AH neurons, and their characterization with specific agonists and antagonists. One class, which we propose to call 5-HT_{1P}, is characterized by a high affinity for [^{3}H]5-HT in radioligand binding assays. This class of receptor mediates a slow depolarization of myenteric type II/AH neurons associated with an increase in input resistance. Agonists at this receptor include, in addition to 5-HT (in order of potency), 5- and 6-hydroxyindalpine and 2-methyl-5-HT. 5-HT_{1P}-mediated responses are specifically antagonized by 5-hydroxytryptophyl-5-hydroxytryptophan amide. The other class of 5-HT receptor, which we propose to call 5-HT_{2P}, appears not to have a high affinity for [^{3}H]5-HT. This receptor mediates a brief depolarization of myenteric II/AH neurons associated with a fall in input resistance. 2-Methyl-5-HT, at low concentrations, is a specific agonist at this receptor and ICS 205-930 is a specific antagonist. Binding of [^{3}H]5-HT to enteric membranes is inhibited by 5-HT_{1P} receptor agonists and antagonists but not by the 5-HT_{2P} receptor antagonist ICS 205-930 or by MDL 72222, another compound reported to be an antagonist of 5-HT at peripheral receptors.**

Serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter in the enteric nervous system (ENS) (1–3). Nevertheless, its precise role in the control of gastrointestinal motility or secretion is not clear. In part, this uncertainty arises because 5-HT has many actions on the neurons, muscle, and epithelium of the gut and it has been difficult to determine which of these are of physiological significance. The neuronal effects of 5-HT include excitation of mucosal afferent fibers to initiate the peristaltic reflex (4, 5), a presynaptic inhibition of the release of acetylcholine (AcCho) from activated myenteric axons (6), stimulation of excitatory enteric neurons to cause a net release of AcCho resulting in smooth muscle contraction, and a relaxation of the bowel secondary to the activation of nonadrenergic, noncholinergic inhibitory neurons (7). Direct application of 5-HT onto myenteric type II/AH neurons may result in a rapid depolarization of the cells associated with an increase in membrane conductance (8), a slow depolarization associated with a decreased membrane conductance (9, 10), a hyperpolarization associated with an increase in membrane conductance, or, with different time courses, all of these responses (11–13). The development of an understanding of the physiological significance of the many effects of exogenous 5-HT has been hampered by the absence, until recently, of specific antagonists of the enteric neural actions of the amine.

To utilize 5-HT antagonists effectively it is necessary to characterize the types of 5-HT receptor present in the gut. Gaddum and Picarelli (14) first classified the 5-HT receptors of the bowel as "D" or "M" receptors because they found responses of the guinea pig ileum to 5-HT that could be blocked by phenoxybenzamine (dibenzyline) or morphine, respectively. Although neither of these compounds is a specific antagonist of 5-HT, the terms D and M are now well established and each denotes a distinct receptor population (15–18). The D receptors are located on smooth muscle, whereas the M receptors are entirely neural. It seems likely that D receptors are similar to 5-HT_2 receptors of the central nervous system (19); however, the category of M receptor seems unique to the periphery and has only recently begun to be characterized (18, 20, 21). Because this class of receptor appears to be responsible for mediating the painful effects of 5-HT in humans, considerable interest has been focused on M receptors (18, 22).

Branchek *et al.* (20) described high-affinity, saturable, reversible binding sites for [^{3}H]5-HT in enteric membranes. These sites are different from either the 5-HT_1 or the 5-HT_2 class of central nervous system 5-HT receptor and the structure–activity requirements of indoles for affinity to the enteric [^{3}H]5-HT binding sites parallel their requirements for pharmacological activity at M receptors. Moreover, radioautographic studies of the localization of enteric [^{3}H]5-HT binding sites showed that they are present in neural structures of the bowel wall but not on the smooth muscle. These observations led to the suggestion that the high-affinity binding of [^{3}H]5-HT represented binding to enteric neural 5-HT receptors. Subsequently, Takaki *et al.* (12) confirmed this suggestion when they demonstrated that dipeptides of 5-hydroxytryptophan are able both to block the high-affinity binding of [^{3}H]5-HT to enteric neural membranes and to antagonize, specifically, the 5-HT-mediated slow depolarization of myenteric type II/AH neurons. Since the dipeptides were also found to abolish the slow excitatory postsynaptic potentials (EPSPs) elicited in myenteric type II/AH neurons by stimulation of interganglionic neural connectives, their use established that 5-HT is a mediator of slow EPSPs in the myenteric plexus.

Additional compounds, besides dipeptides of 5-hydroxytryptophan, have also been reported to be antagonists of 5-HT at M receptors. These include benzoyltropine analogues (23) and indoletropanyl esters (18). One indole-

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Abbreviations: 5-HT, serotonin (5-hydroxytryptamine); ENS, enteric nervous system; AcCho, acetylcholine; EPSP, excitatory postsynaptic potential; 5-HTP-DP, *N*-acetyl-5-hydroxytryptophyl-5-hydroxytryptophan amide; 5- and 6-OHIP, 5- and 6-hydroxy[(indolyl-3)-2-ethyl]-4-piperidine (5- and 6-hydroxyindalpine).

tropanyl ester in particular, ICS 205-930 (Sandoz), has been described as especially potent and specific in inhibiting atropine- and tetrodotoxin-sensitive contractions of the guinea pig ileum in response to the addition of 5-HT (18). Since these effects of 5-HT are neurally mediated they were presumed to be due to activation of M receptors. Nevertheless, since the myenteric plexus is complex, with many different types of interneuron, it is difficult to interpret the results of pharmacological experiments in which a neurally active drug is added to an organ bath and its action is assessed by measuring muscle contraction. The output of the final common excitatory myenteric neurons, reflected in smooth muscle contraction, may be the net result of a complicated series of actions of the added substance on a variety of the interneurons that comprise enteric circuits. This is especially true of a compound such as 5-HT, which may exert more than one kind of action on individual myenteric neurons (8, 10) and for which there may be more than one kind of neural receptor. To best characterize enteric 5-HT receptors, therefore, it is necessary to study each of the actions of the amine on individual neurons.

To characterize enteric neural 5-HT receptors we have used electrophysiological techniques to investigate the responses of individual myenteric neurons to the application of the amine. A single type of neuron, the type II/AH cell, was selected for study because this type of neuron appears to receive a serotonergic input (11–13). Types of response were classified physiologically and by their susceptibility to antagonism by a dipeptide of 5-hydroxytryptophan [*N*-acetyl-5-hydroxytryptophyl-5-hydroxytryptophan amide (5-HTP-DP)] or ICS 205-930. In addition, indolic compounds were analyzed for their ability to act as agonists at the 5-HT receptors for which 5-HTP-DP or ICS 205-930 were found to be antagonists. Finally, the physiological characterization of the effects of 5-HTP-DP, ICS 205-930, and agonists on enteric neural receptors for 5-HT was related to the ability of the same compounds to antagonize the high-affinity binding of [^{3}H]5-HT to enteric membranes. The binding of [^{3}H]5-HT was studied for this purpose by radioautography and by rapid filtration of isolated membranes. Preliminary reports of some of these observations have been presented (24, 25).

MATERIALS AND METHODS

Electrophysiology. Intracellular recordings were made from myenteric neurons exposed by dissection of the longitudinal layer of smooth muscle with attached myenteric plexus from adult guinea pig jejunum. Standard techniques, previously described in detail, were used for dissection of the tissue, recording from neurons, and application of drugs (11, 12). Preparations were maintained in a low-volume chamber that was perfused with Krebs solution at 37°C and gassed with 95% oxygen/5% carbon dioxide. Glass microelectrodes, used for intracellular recording, were filled with 3 M KCl and had resistances of 40–80 MΩ. To measure membrane resistance, constant anodal current pulses were passed into the cells through the recording electrode. Afferent inputs to ganglia were activated by applying 1- to 2-sec trains of 200-μsec rectangular pulses at a frequency of 10 Hz to interganglionic connectives. This was done with monopolar stimulating electrodes made from platinum wire (25 μm in diameter) insulated with Teflon.

Drugs were applied by superfusion and/or pressure microejection. All drugs were dissolved in Krebs solution except 5-HTP-DP, which was dissolved in 100% ethanol and then diluted to 10% ethanol in Krebs solution. When pressure microejection was used, drugs were applied from pipettes (1.0 mM; 15- to 20-μm tip diameter) by pulses of nitrogen gas (300 kg/cm^2; 10–999 msec in duration). The distance between the tip of the pipette and the recording electrode was 50–100 μm. Solutions containing drugs were never recirculated.

Binding of [^{3}H]5-HT. For radioautographic studies of the binding of [^{3}H]5-HT, frozen sections of guinea pig small intestine were thaw-mounted, dried, and incubated with [^{3}H]5-HT [26.3–29.1 Ci/mmol (1 Ci = 37 GBq); New England Nuclear] for 30 min at room temperature in the presence or absence of nonradioactive 5-HT or an experimental compound. After being thoroughly rinsed and air dried, the tissue was exposed to ^{3}H-sensitive film (Ultrofilm, LKB) for 1–2 weeks at 25°C. This method has been described in detail (20).

The binding of [^{3}H]5-HT to isolated membranes was studied by rapid filtration. Longitudinal muscle with attached myenteric plexus was dissected from adult male rabbit intestines and homogenized in 50 mM Tris·HCl buffer at pH 7.4. Membranes were prepared by differential centrifugation and incubated with [^{3}H]5-HT in the presence or absence of a 1000-fold excess of nonradioactive 5-HT or an experimental compound for 10 min at 37°C in 50 mM sodium phosphate buffer (pH 7.4) as described (20). Following incubation, the membranes were collected by rapid filtration on Whatman GF/B filters and radioactivity was counted in a Tracor (Austin, TX) 6895 liquid scintillation counter. Specific binding was defined as that displaced by a 1000-fold excess of nonradioactive 5-HT and was ≈80% of total binding.

Drugs Used. 5-Hydroxytryptamine creatinine sulfate (5-HT) and acetylcholine chloride (AcCho) were purchased from Sigma. 5-HTP-DP was graciously supplied by Hadassah Tamir. ICS 205-930 and 2-methyl-5-HT were obtained from Sandoz. 5- and 6-Hydroxyindalpine {5- and 6-hydroxy-[(indolyl-3)-2-ethyl]-4-piperidine} (5-OHIP, 6-OHIP) as well as the nonhydroxylated parent compound, indalpine, were provided by Adam Doble, Pharmuka (Gennevillers, France). MDL 72222 (3,5-dichlorobenzoyltropine ester) was obtained from Merrell Dow (Strasbourg Center, France). Tetrodotoxin was purchased from Calbiochem.

RESULTS

All records were made from type II/AH cells (26) of the guinea pig myenteric plexus. Criteria for the identification of neurons as type II/AH included a resting membrane potential of >60 mV, absence of anodal break excitation, the presence of a prolonged hyperpolarizing afterpotential (the AH), and the failure of cells to spike repetitively when injected with depolarizing current pulses (12). Control responses to 5-HT were elicited in 20 cells; 4 showed only a brief depolarization associated with a decrease in input resistance (fast response), 1 showed only a prolonged depolarization associated with an increase in input resistance (slow response), and 15 showed fast and slow responses (Fig. 1*A*). The long duration of the slow response to 5-HT permitted it to be detected when fast and slow responses were obtained simultaneously because the slow outlasted the fast response. When this occurred, cells usually were excited to fire action potentials during the fast response and these were followed by a hyperpolarizing afterpotential, which preceded the appearance of the slow response to 5-HT. Superfusion of ICS 205-930 (0.1 μM) reversibly blocked the fast but not the slow response to 5-HT (Fig. 1 *B* and *C*) in 10/10 cells tested. ICS 205-930 did not block nicotinic or muscarinic responses to microejected AcCho in 2/2 cells tested (data not illustrated).

Slow EPSPs were evoked by fiber tract stimulation to evaluate the effect of ICS 205-930 on endogenously released 5-HT. 5-HT is a mediator of this response and slow EPSPs are reproducibly blocked by 5-HTP-DP (12). Slow EPSPs have the characteristics of the slow response to 5-HT (Fig. 1*D*). ICS 205-930 did not antagonize slow EPSPs elicited by stimulation of interganglionic connectives (Fig. 1*E*).

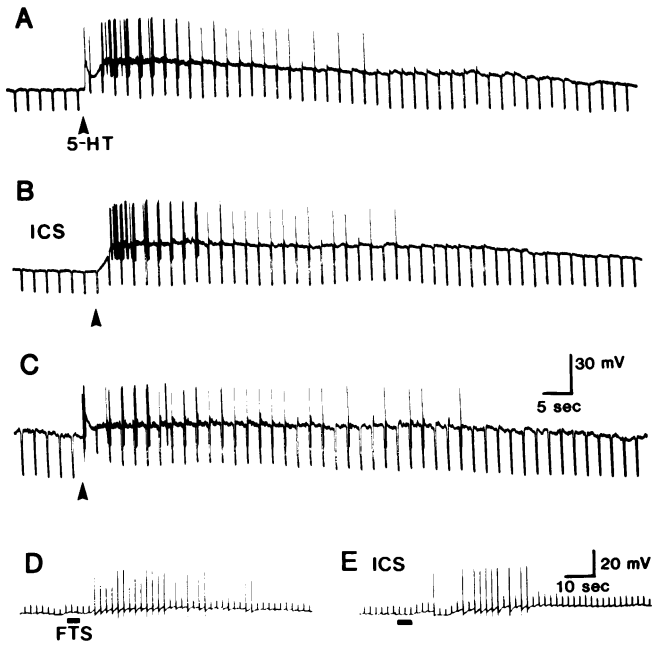

FIG. 1. Effect of ICS 205-930 on responses to 5-HT and on slow EPSPs evoked by stimulating an interganglionic connective. Membrane potential of two myenteric type II/AH neurons is illustrated. Constant hyperpolarizing (*A–C*) or depolarizing (*D* and *E*) current pulses were injected into the cells through the recording microelectrode. (*A*) Control response to microejection of 5-HT (arrowhead): there is a transient depolarization (the fast response) and a partial recovery of the membrane potential, followed by a prolonged depolarization of the membrane (the slow response). During the slow response the neuron is hyperexcitable and spikes frequently. Note the increase in input resistance during the slow response to 5-HT. (*B*) Superfusion with ICS 205-930 (1.0 μM) blocks the initial fast response to 5-HT but does not affect the slow response. (*C*) One hour following the wash out of ICS 205-930 the fast response to microejection of 5-HT (arrowhead) has recovered. Note that input resistance is diminished during the fast response and the partial recovery of the membrane potential that follows it. The resting membrane potential was −71 mV. (*D*) A slow EPSP is evoked by fiber tract stimulation (FTS). Note that although there is only a modest depolarization of the membrane (5 mV), the cell is hyperexcitable throughout the response, which long outlasts the application of stimuli. (*E*) Superfusion of ICS 205-930 (1.0 μM) does not affect the slow EPSP (6 mV). The resting membrane potential was −68 mV.

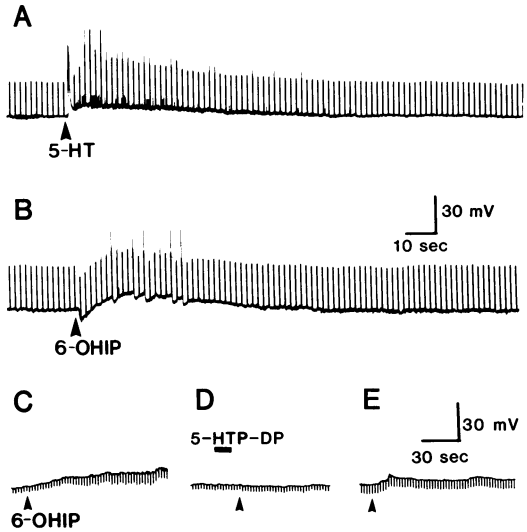

FIG. 2. Responses to the microejection of 6-OHIP. Constant depolarizing (*A* and *B*) or hyperpolarizing (*C–E*) current pulses were injected into the cell through the recording microelectrode. (*A*) Control: the cell responds to the microejection of 5-HT (200 μsec; arrowhead) with fast and slow responses. (*B*) Microejection of 6-OHIP (200 μsec; arrowhead) leads to an initial hyperpolarization followed by a prolonged membrane depolarization associated with an increase in cell excitability similar to the slow response to 5-HT. A fast response like that that accompanies the application of 5-HT is not evoked by 6-OHIP. The resting membrane potential was −65 mV. (*C*) Control response of another cell to 6-OHIP. Note that the prolonged depolarization is associated with an increase in input resistance. (*D*) The response to 6-OHIP is abolished by microejection of 5-HTP-DP (10 × 100 μsec). (*E*) Twenty minutes following washout of 5-HTP-DP the response to 6-OHIP has returned. The resting membrane potential was −73 mV.

An effect similar to the slow response to 5-HT was produced by microejection of 5-OHIP and 6-OHIP (Fig. 2 *A–C*). These compounds evoked a prolonged depolarization of II/AH cells associated with an increase in input resistance. This response was occasionally preceded by a transient hyperpolarization of the membrane (Fig. 2*B*) and was not blocked by the addition of tetrodotoxin (0.5 μM) to the bath. As with 5-HT, neurons were hyperexcitable during the slow depolarization evoked by 5-OHIP or 6-OHIP. The response to 6-OHIP was blocked reversibly by 5-HTP-DP (Fig. 2 *D* and *E*) but not by ICS 205-930. 6-OHIP mimicked only the slow response to 5-HT (11/11 cells tested); however, 5-OHIP also mimicked the fast response in 2/4 cells tested.

An effect similar to the fast response to 5-HT was produced by the microejection of 2-methyl-5-HT. This compound evoked a brief depolarization of II/AH cells associated with a decrease in input resistance (Fig. 3*A*) in 5/5 cells tested. Neurons were hyperexcitable while they were depolarized; however, a pronounced hyperpolarization, also associated with a fall in membrane resistance, followed the depolarizing response. None of these 5 cells showed the prolonged depolarization associated with a decreased conductance characteristic of the slow response to 5-HT, even though a slow response was part of the effect evoked in each cell by control microejections of 5-HT. ICS 205-930 reversibly blocked the effects of 2-methyl-5-HT (Fig. 3 *A–C*). Superfusion of 2-methyl-5-HT in high concentration (10.0 μM) antagonized fast and slow responses to 5-HT. When the amount of 2-methyl-5-HT to which neurons were exposed was increased by lengthening the duration of the ejection pulse 3- to 5-fold, 2-methyl-5-HT elicited a two-phased response (data not illustrated). The first phase of this effect resembled the fast response and the second phase resembled the slow response to 5-HT.

The ability of compounds to act physiologically at 5-HT receptors was correlated with their ability to antagonize the high-affinity binding of [^{3}H]5-HT to enteric membranes (K_d = 2.7 ± 0.2 nM) (20). ICS 205-930 did not inhibit the binding of [^{3}H]5-HT even when present at 10,000 times the concentration of 5-HT. On the other hand, 5-OHIP (K_i = 2.1 ± 0.8 nM), 6-OHIP (K_i = 10.6 ± 1.7 nM), and 2-methyl-5-HT (K_i = 34.0 ± 10.2 nM) antagonized the binding of [^{3}H]5-HT. 5-OHIP was significantly more potent than 6-OHIP ($P < 0.002$) and 2-methyl-5-HT ($P < 0.02$). This ability to interfere with the binding of [^{3}H]5-HT was also seen in radioautographs of frozen sections of gut incubated with [^{3}H]5-HT (0.01 μM) in the presence or absence of the test compounds (Fig. 4). Again, ICS 205-930 (10 μM) failed to inhibit the binding of [^{3}H]5-HT, whereas specific binding was blocked by 5-OHIP, 6-OHIP, and 2-methyl-5-HT (10 μM). Thus, all of the [^{3}H]5-HT binding sites in the wall of the gut appear to have the same properties with respect to these compounds. In additional experiments indalpine was found to differ from its hydroxylated analogues in that it was unable to inhibit the binding of [^{3}H]5-HT. Similarly, MDL 72222, another drug reputed to be an antagonist of peripheral neural 5-HT

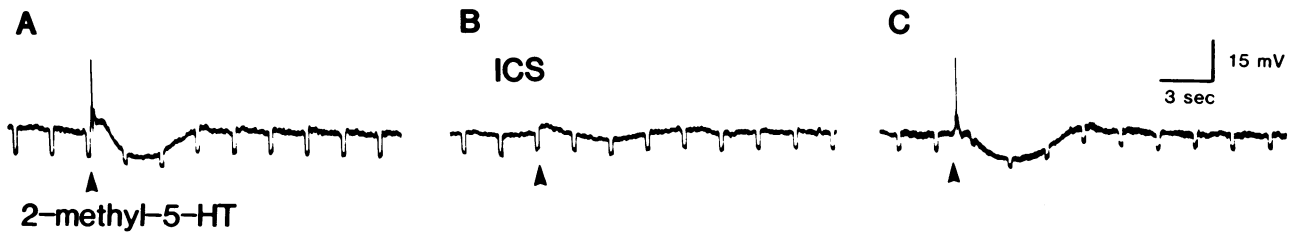

FIG. 3. Responses to the microejection of 2-methyl-5-HT. Constant hyperpolarizing current pulses were injected into the cell through the recording microelectrode. (*A*) Microejection of 2-methyl-5-HT (200 msec; arrowhead): there is a transient depolarization (similar to the fast response to 5-HT) followed by a hyperpolarization of the membrane associated with a fall in input resistance. (*B*) Responses to 2-methyl-5-HT are blocked by superfusion with ICS 205-930 (1.0 μM). (*C*) Forty minutes following washout of ICS 205-930 responses to 2-methyl-5-HT return. The resting membrane potential was −65 mV.

receptors (23) with properties that resemble those of ICS 205-930, did not antagonize the binding of [^{3}H]5-HT.

DISCUSSION

Experiments were done to characterize enteric neural receptors for 5-HT. Our observations indicate that there are at least two classes of such receptor on type II/AH neurons of the myenteric plexus. One, for which ICS 205-930 is a specific antagonist, mediates a short-lived depolarization of these cells associated with a decrease in input resistance, the fast response to 5-HT. The other, for which 5-HTP-DP is a specific antagonist, mediates a prolonged depolarization associated with an increase in input resistance, the slow response to 5-HT. Since these two receptor classes can occur in the same neuron, the response of II/AH cells to 5-HT may have fast and slow components blocked by ICS 205-930 or 5-HTP-DP, respectively. 2-Methyl-5-HT appears to be a relatively specific agonist at the ICS 205-930-sensitive receptor. Thus, at a moderate concentration it mimics the fast but not the slow response to 5-HT and its effects are antagonized by ICS 205-930. On the other hand, 6-OHIP appears to be a specific agonist at the 5-HTP-DP-sensitive 5-HT receptor. Thus, it mimics the slow but not the fast response to 5-HT and its effects are antagonized by 5-HTP-DP. At high concentrations of 2-methyl-5-HT, selectivity for the ICS 205-930-sensitive 5-HT receptor is diminished. The slow response to 5-HT, as well as the fast response, may be mimicked by long pulses of microejected 2-methyl-5-HT. Moreover, superfusion of the compound at 10 μM desensitizes both types of receptor, blocking all responses to 5-HT. 5-OHIP appears to be a less selective agonist at the 5-HTP-DP-sensitive 5-HT receptor than is 6-OHIP. Unlike 6-OHIP, 5-OHIP sometimes evokes an effect similar to the fast as well as the slow response to 5-HT.

It seems likely that only the 5-HTP-DP-sensitive receptor is responsible for slow EPSPs mediated by 5-HT. The slow response to 5-HT, which is antagonized by 5-HTP-DP but is insensitive to ICS 205-930, resembles slow EPSPs. As would be predicted from these effects, ICS 205-930 does not inhibit the generation of slow EPSPs when they are evoked by stimulation of interganglionic connectives, but these responses are antagonized by 5-HTP-DP (12). Neither ICS 205-930 nor 5-HTP-DP (12) inhibits muscarinic or nicotinic responses of myenteric neurons to AcCho. Fast EPSPs have been shown to be mediated by AcCho and to be nicotinic not serotonergic (26); therefore, the physiological significance of the ICS 205-930-sensitive fast responses to 5-HT remains unclear. ICS 205-930 does block hyperpolarizing responses to 5-HT or 2-methyl-5-HT (see Fig. 3*B*). Hyperpolarizing responses to fiber tract stimulation were not encountered often enough to test the effect on them of ICS 205-930; thus, it remains possible that serotonergic neurites may play a role in the mediation of hyperpolarizing inhibitory postsynaptic potentials. Nevertheless, the only response for which a definite role for 5-HT has been established is the slow EPSP.

Electrophysiological observations made in this and other studies (12, 13) are consistent with the idea that the high-

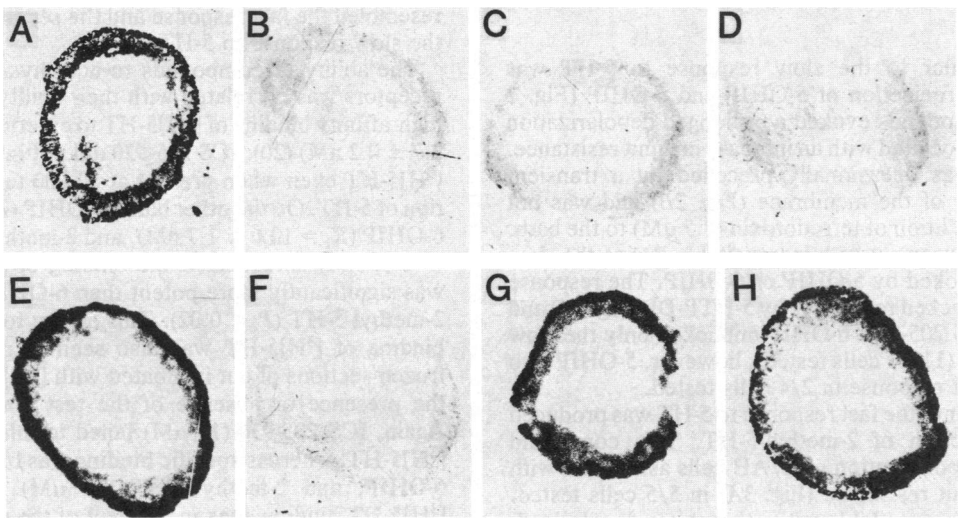

FIG. 4. Radioautographs showing the binding of [^{3}H]5-HT (10 nM) in 15-μm serial sections of guinea pig jejunum incubated in the presence or absence of experimental compounds (10 μM). (*A*) [^{3}H]5-HT alone. (*B*) With nonradioactive 5-HT. (*C*) With 2-methyl-5-HT. (*D*) With 5-OHIP. (*E*) With indalpine. (*F*) With 6-OHIP. (*G*) With ICS 205-930. (*H*) With MDL 72222. All compounds that were agonists or antagonists at the 5-HTP-DP-sensitive 5-HT receptor were able to compete with [^{3}H]5-HT for binding in the gut. Note that the nonhydroxylated indalpine, as well as the antagonists ICS 205-930 and MDL 72222, did not interfere with the binding of [^{3}H]5-HT.

affinity binding site for [^{3}H]5-HT in enteric membranes corresponds to the receptor responsible for the mediation of slow responses to 5-HT and slow EPSPs. Thus, the binding of [^{3}H]5-HT is inhibited by 5-HTP-DP but not by ICS 205-930. Moreover, the agonists, 5-OHIP, 6-OHIP, and 2-methyl-5-HT, are also effective competitors of [^{3}H]5-HT. Furthermore, the potency of these agonists in inhibiting [^{3}H]5-HT binding correlates with their efficacy in activating the 5-HTP-DP-sensitive receptors responsible for eliciting slow depolarizations associated with an increase in input resistance. The hydroxylated indalpines are more effective agonists at 5-HTP-DP-sensitive receptors than is 2-methyl-5-HT and they are also significantly more potent inhibitors of the binding of [^{3}H]5-HT. In contrast, 2-methyl-5-HT, which is more potent than 5- or 6-OHIP in eliciting an effect that resembles the fast response to 5-HT, mimics (or desensitizes) the slow response only at relatively high concentrations of the compound. The affinity of indolic compounds for the 5-HTP-DP-sensitive receptor requires hydroxylation of the indole ring; thus, indalpine, like tryptamine and 5-methoxytryptamine (12, 13, 20, 21), does not act at this site. Actions of these compounds, reported to be 5-HT-like from pharmacological studies of muscle contraction, may be due to effects on ICS 205-930-sensitive receptors, to other receptors, or to other types of action, such as the release of 5-HT by tryptamine (13). MDL 72222, like ICS 205-930, does not act at the 5-HTP-DP-sensitive receptors. Thus, the only antagonist with an action at a receptor that has been shown to be involved in the mediation of a physiological response to the release of endogenous 5-HT in the myenteric plexus is 5-HTP-DP.

A number of schemes have been proposed for the classification of peripheral 5-HT receptors (15, 18, 27, 28). Although the terms M and D are well established, they stand for the names of compounds that are not specific antagonists of 5-HT (15). Moreover, they do not correspond to classifications of central 5-HT receptors and there is not just a single enteric neural M receptor. The D and M terms, therefore, should probably be dropped, as has been suggested in the literature (15, 27). 5-HT_1 and 5-HT_2 classes of central 5-HT receptor have been characterized (29–31). The 5-HT_1 class has three subtypes but all have in common a high affinity for 5-HT (32–34). The 5-HT_2 class has a low affinity for 5-HT (35) but a high affinity for certain antagonists, such as ketanserin (36), that do not affect the enteric binding of [^{3}H]5-HT (20). It is proposed that the 5-HTP-DP-sensitive receptor be referred to as 5-HT_{1P} because it has a high affinity for 5-HT and is a peripheral receptor. Similarly, it is proposed that the ICS 205-930-sensitive receptor be referred to as 5-HT_{2P} because it does not have a high affinity for 5-HT while it too is a peripheral receptor. The possibilities that the 5-HT_{1P} and 5-HT_{2P} classes of receptor are found in the peripheral nervous system outside of the gut or in the central nervous system have not been tested. The ability of ICS 205-930 to block the activation of nociceptive nerve fibers by 5-HT in humans (18) and the observation that [^{3}H]5-HT binds to nerve fibers in perianal skin (37) suggest that both classes of peripheral neural receptor for 5-HT may have a widespread distribution.

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