Brain Research, 506 (1990) 62–69
Elsevier

BRES 15086

Serotonin (5-HT) induces IPSPs in pyramidal layer cells of rat piriform cortex: evidence for the involvement of a 5-HT_2-activated interneuron

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(Accepted 6 June 1989)

Key words: Inhibitory postsynaptic potential; Interneuron; Piriform cortex; Serotonin

In a slice preparation of rat piriform cortex, both intracellular and extracellular techniques were used to examine the pharmacological and electrophysiological actions of serotonin (5-HT). Bath application of 5-HT resulted in either depolarization (57%), hyperpolarization (34%) or no change (9%) in membrane potential of cells in the pyramidal cell layer (layer II) of piriform cortex. Additionally, when KCl-containing electrodes were used, 5-HT induced an increase in depolarizing synaptic potentials in 41% of these cells. It was concluded that these potentials were reverse inhibitory post-synaptic potentials (IPSPs) because they were blocked by bicuculline and tetrodotoxin. The induction of IPSPs by 5-HT was blocked by the 5-HT_2-selective antagonist ritanserin. By recording extracellularly in the presence of 5-HT, a group of 5-HT-activated, putative interneurons was found at the border of layers II and III of piriform cortex. 5-HT but not norepinephrine activation was blocked by ritanserin. The actions of 5-HT were mimicked by the 5-HT_2 agonist α-methyl-5-HT; the 5-HT_2 partial agonist, 2,5-dimethoxy-4-methyl-amphetamine had a small agonist action of its own and blunted the effect of 5-HT. Activation of a larger group of putative interneurons by the more universal excitant *N*-methyl-D-aspartate showed that the 5-HT-activated interneurons represented 23% of the interneurons located on the border between layers II and III. We conclude that 5-HT induces IPSPs in layer II pyramidal cells by activating a subpopulation of interneurons at the border of layers II and III of piriform cortex.

INTRODUCTION

Serotonin (5-HT)-containing neurons of the midbrain raphe nuclei (dorsal and medial) project extensively throughout the cerebral cortex of the mammalian brain[18,29–31]. Furthermore, 5-HT possesses multiple, distinct binding sites in the cerebral cortex as well as other brain regions. Evidence for the presence of multiple binding sites derives from the finding that the neuroleptic agent [^{3}H]spiperone labels 5-HT binding sites in the frontal cortex[8] that exhibit features distinct from those sites labeled by [^{3}H]5-HT itself. Briefly, 5-HT shows high affinity for the [^{3}H]5-HT site and low affinity for the [^{3}H]spiperone site. These sites have been termed the 5-HT_1 and the 5-HT_2 site, respectively[35]. The 5-HT_1 site has been further subclassified into the 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{1C} sites based upon pharmacological differences[36]. The 5-HT_2 receptor is pharmacologically similar to the 5-HT_{1C} receptor in that both show high affinity for many of the same 5-HT antagonists and for the phenethylamine and indolamine hallucinogens[12,17] and both stimulate phosphoinositide (PI) turnover[39]. However, the anatomical distribution of the latter two receptors is dissimilar, as exemplified by the fact that in the cerebral cortex the levels of 5-HT_2 receptors are extremely high and the levels of 5-HT_{1C} receptors are relatively low[34].

The electrophysiological actions of 5-HT in the cerebral cortex at various 5-HT receptor subtypes have not yet been well characterized. An in vivo study in the cerebral cortex found that the inhibitory effects of microiontophoretically applied 5-HT are enhanced by a selective 5-HT_2 antagonist[19]. In brain slices from guinea pig cortex, Davies et al.[10] have shown that 5-HT can have both hyperpolarizing and depolarizing actions on pyramidal cells. The hyperpolarizing effects appear to be mediated by 5-HT_{1A} receptors, whereas the depolarizing actions appear to be mediated by 5-HT_2 receptors. In cat cortical slices 5-HT and norepinephrine (NE) have been reported to have modulatory actions on principal neurons, in that they potentiate the actions of glutamate but have no apparent effect of their own[32]. However, the receptor subtypes mediating these actions were not determined.

The piriform cortex is a simple cortical system which contains only 3 layers all of which are easily visualized at

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low magnification, thus rendering it an advantageous area to use for the in vitro slice preparation. In an early study, the predominant effect of 5-HT on neurons in the piriform cortex in vivo was shown to be inhibitory[20]. However, the latter study predated the availability of specific serotonergic compounds for receptor classification. The levels of 5-HT_2 receptors in the piriform cortex are among the highest in the brain[28,34] and this region has been extensively characterized with respect to both electrophysiology[15,16,42] and anatomy[14].

The present study uses both intracellular and extracellular recording techniques to examine the physiology and pharmacology of responses to 5-HT in the rat piriform cortex in vitro in a brain slice preparation.

MATERIALS AND METHODS

Slice preparation

Slices were prepared from a total of 250 male Sprague–Dawley rats as described previously[2]. Briefly, animals were anesthetized with chloral hydrate (400 mg/kg, i.p.) and then perfused intracardially with ice-cold (4 °C) modified artificial cerebrospinal fluid (ACSF) in which sucrose was substituted for NaCl (in mM: KCl 5, sucrose 252, NaH_2PO_4 1.25, glucose 10, $NaHCO_3$ 24–31, $CaCl_2$ 2 and $MgSO_4$ 2). Animals were then decapitated, and the brain was removed rapidly. In ice-cold sucrose–ACSF a 4 × 4 × 4 mm block of piriform cortex was dissected and 500 μm coronal sections were cut on a vibrating-knife microtome (Vibraslice; WPI). A slice containing piriform cortex was then transferred to the stage of a fluid-gas interface slice chamber which had a constant flow of humidified 95% O_2, 5% CO_2. The chamber was then heated slowly from room temperature to 33 °C. Sucrose–ACSF was used during the first hour post-decapitation, after which normal–ACSF (sucrose–ACSF in which sucrose is replaced by 126 mM NaCl) was used. The substitution of sucrose for NaCl in slice preparation and recovery improved the yield of healthy neurons in the slice[2]. Slices were allowed a second hour for recovery before use in recording.

Electrophysiological recordings

Intracellular recordings were obtained from cells in the pyramidal cell layer (layer II) of piriform cortex using glass microelectrodes filled with either 2 M KCl, KAcetate, or $KMeSO_4$. KCl-filled electrodes were used to visualize chloride-mediated inhibitory postsynaptic potentials (IPSPs). When Cl^--filled, as opposed to $MeSO_4$–filled, electrodes are used, the E_{rev} for chloride is shifted from approximately −70 mV to +15 mV and IPSPs (which would be barely detectable with $KMeSO_4$ or KAcetate-filled electrodes) appear as large depolarizing postsynaptic potentials (PSPs)[23]. Cells were considered to have an increase in IPSPs only when the average number of induced IPSPs exceeded baseline by at least 100% sampled for 30 s before and after administration of 5-HT (excepting those cells in which there were no baseline IPSPs, in which case any IPSPs which occurred reproducibly in the presence of 5-HT were considered to be induced). IPSPs ranged in amplitude from 0.5 to 5 mV; potentials smaller than 0.5 mV were not discriminable from baseline. Electrodes were pulled from starbore glass (1.4 mm; Radnotti Glass, Inc.) by a Brown-Flaming electrode puller (Sutter Instruments) to resistances of 20–50 MΩ. Only cells having a resting potential between −60 to −80 mV and an input resistance of 10–50 MΩ were used for recordings.

Single-unit extracellular recordings were obtained using glass microelectrodes filled with 2 M NaCl and 2% Pontamine sky blue (5–8 MΩ). In experiments where cells were located by excitation with *N*-methyl-D-aspartate (NMDA), NMDA was bath-applied and after a cell was located the effects of 5-HT were tested by applying a solution containing both NMDA and 5-HT. When 5-HT was excitatory, the NMDA was then discontinued and the effects of 5-HT were tested alone. Cells were tentatively identified as interneurons (see below) if they were activated by 10 μM NMDA and had low amplitude (0.5–3 mV), short duration (0.1–0.25 ms) extracellular action potentials. The electrode site was marked by passing a negative current of 25 μA through the electrode for 20 min. The slides were then fixed in formaldehyde, cut into 50 μm frozen sections on a sliding microtome and stained with Cresyl violet.

TABLE I

5-HT effects on membrane potential and synaptic activity (IPSPs) in pyramidal layer cells

Potential change	*Number of cells*	*5-HT-induced IPSPs*		*IPSPs (% of n)*
		Yes	*No*	
Depolarization	50	32	18	64%
Hyperpolarization	29	0	29	0%
No change	8	4	4	50%
% Total *n*	87	41%	59%	

Chemicals

NMDA and tetrodotoxin (TTX) were obtained from Sigma Chemical Co., serotonin and norepinephrine from Regis Chemical Co., ritanserin from Janssen, bicuculline methiodide from Pierce Labs., α-Me-5-HT from R.B.I. and 2,5-dimethoxy-4-methylamphetamine (DOM) from NIDA. All compounds were bath-applied.

RESULTS

As previously reported for somatosensory cortical neurons[9], in pyramidal layer (layer II) cells of piriform cortex we found that bath application of 5-HT resulted in either depolarization (57%), hyperpolarization (34%) or no change (9%) in membrane potential (Table I). In all cases these responses were small (1–4 mV) and none ever brought the cell to threshold for firing as resting potentials were generally in a deeply hyperpolarized range (V_{rest} = −72.6 ± 5.2 mV). In addition, the number of spikes induced by constant current depolarizing pulses was increased in all of the neurons with a depolarizing response (Fig. 1). This increase in number of spikes was probably due to the small depolarization, though other factors could have contributed to the change in excitability. When KCl-filled electrodes were used, many pyramidal layer cells displayed small potentials which resembled the spontaneous IPSP activity described by Galvan et al.[11] in guinea pig piriform cortex. In 41% of the pyramidal layer cells, application of 5-HT produced an increase in this apparent synaptic activity (Fig. 1, Table I). In agreement with previous results[11], we found that both the fast Na^+-channel blocker TTX (n = 5) and the $GABA_A$ antagonist bicuculline (n = 5) blocked the increase in synaptic activity, indicating that the effect was both transsynaptic and GABA-mediated (Fig. 2). Also,

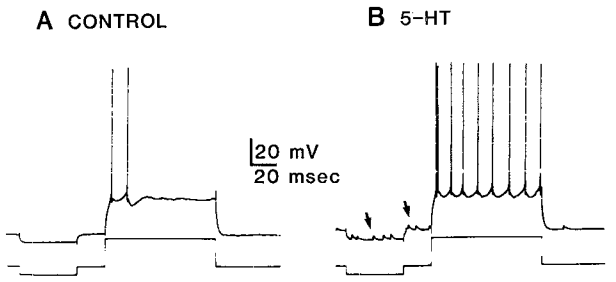

Fig. 1. Oscilloscope tracings of an intracellular recording with a KCl-containing electrode showing an increase in synaptic activity in response to bath application of 5-HT. In the control, A, there are no ostensible PSPs, whereas in B application of 5-HT (100 μM) causes a marked increase in apparent PSPs which is marked by the arrows. These are presumed reverse IPSPs because they are seen only with KCl-containing electrodes and not with KAcetate or $KMeSO_4$-containing electrodes (see text for details). The cell was given a hyperpolarizing pulse (0.17 nA) followed by a depolarizing pulse (0.5 nA). In all cells which were depolarized by 5-HT, as was this one, 5-HT caused an increase in spikes during the depolarizing current pulse.

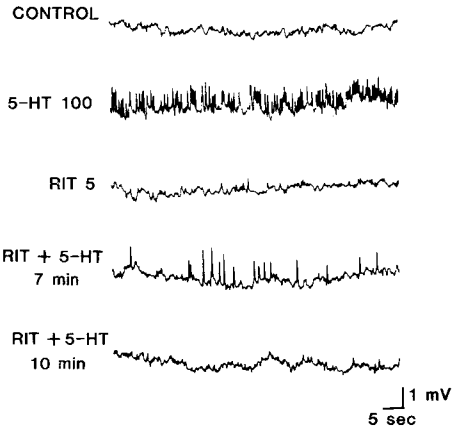

Fig. 3. Ritanserin (5 μM) blocks the induction of IPSPs by 5-HT. Serotonin (100 μM; lane 2) causes a large increase in IPSPs as compared to control (lane 1). Ritanserin has no effect of its own (lane 3) but blocks the induction of IPSPs by 5-HT in a time-dependent manner; at 7 min (lane 4) there is only a partial block of 5-HT-induced IPSPs, while at 10 min (lane 5) there is a complete block. 5-HT applications were 3 min each.

when KAcetate or $KMeSO_4$-filled electrodes were used, activity in the presence of 5-HT was virtually indistinguishable from baseline (n = 10); see Methods. For these reasons, it was concluded that the PSPs induced by 5-HT and seen with Cl^--filled electrodes were reverse IPSPs. Table I shows that the induction of IPSPs occurred only in those cells that were either depolarized or unchanged by 5-HT. However, there appeared to be no causal relationship between the two phenomena, since blockade of IPSPs by TTX or bicuculline had no effect on depolarization (n = 7) and not all cells that were depolarized had 5-HT-inducible IPSPs.

To analyze the pharmacological profile of the 5-HT-induced IPSPs we tested various 5-HT antagonists. The non-selective 5-HT antagonist spiperone (1–5 μM) blocked 5-HT-induced IPSPs relatively rapidly (5–10 min; not shown)[3]. The selective 5-HT_2 antagonist ritanserin (5 μM; Fig. 3; n = 5) also blocked the induction of IPSPs by 5-HT. As illustrated in Fig. 4, the continuous perfusion of ritanserin blocked the 5-HT-induced IPSPs at an earlier time-point than it blocked the 5-HT-induced depolarization (n = 4): the frequency of induced IPSPs was reduced by 75% in 6.6 ± 1.14 min, whereas 19.6 ± 5 min was required for a 75% reduction in the depolarization.

That 5-HT produces IPSPs in cells of the pyramidal

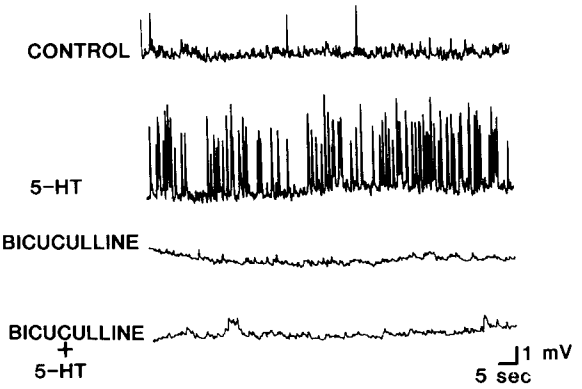

Fig. 2. Application of bicuculline (100 μM) blocks the induction of IPSPs by 5-HT (100 μM). In lane 1 there is a moderate level of spontaneous synaptic activity, this activity is greatly increased by application of 5-HT, lane 2. Application of bicuculline abolishes both the spontaneous IPSPs, lane 3, and the 5-HT induced IPSPs, lane 4. 5-HT applications were 3 min each; bicuculline was applied for 3 min prior to application of 5-HT.

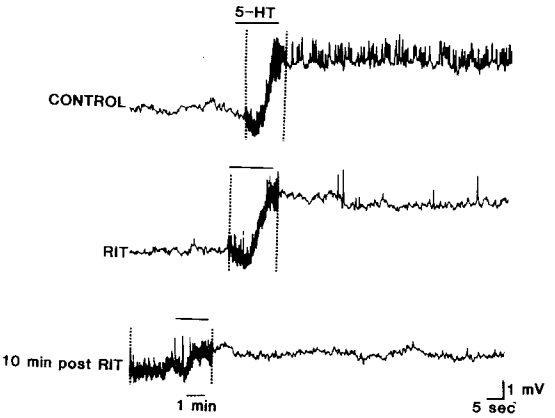

Fig. 4. Ritanserin (5 μM) blocks 5-HT (100 μM)-induced IPSPs more potently than it blocks 5-HT-induced depolarization. In lane 1 5-HT causes both a depolarization as well as large induction of IPSPs. Following 7 min bath application of ritanserin, virtually all of the 5-HT-induced IPSPs have been blocked while the 5-HT-induced depolarization remains unchanged. It is only at 10 min after ritanserin application was discontinued, lane 3, that the 5-HT-induced depolarization is blocked. Ritanserin often shows this type of progressive blockade even after application is discontinued. Note that the area between the dotted lines represents a different time scale.

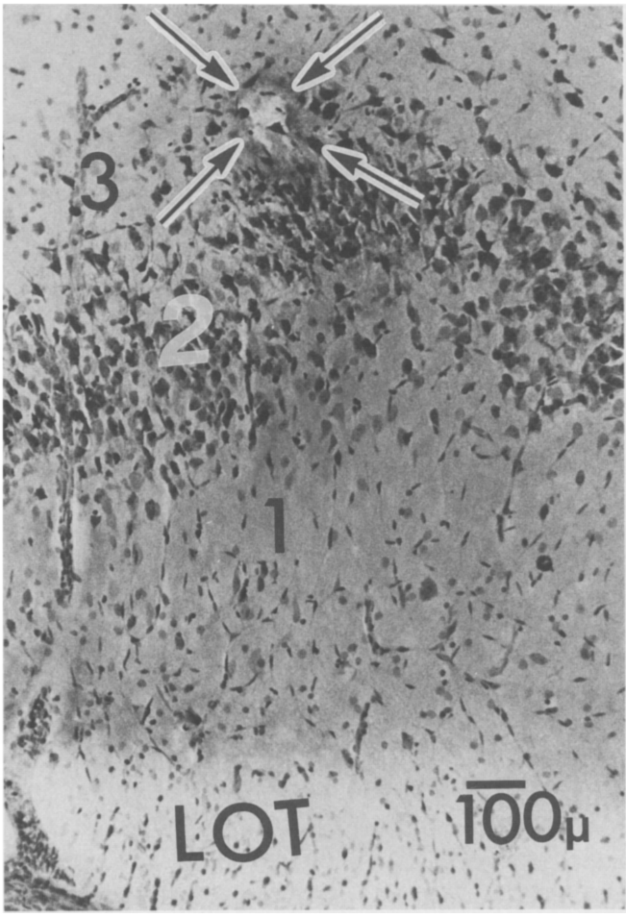

Fig. 5. Photomicrograph of the recording site where the 5-HT-activated putative interneurons are located. Arrows show an electrolytic lesion at the center of a dye spot (dark halo) caused by passing a 25-μA negative current through the electrode for 20 min in order to stain the recording site with Pontamine sky blue. Cells were located by bath-applying 20 μM 5-HT to the slice and making successive drops (50–100 μm apart) with an extracellular electrode beginning at the LOT and descending through Layer III. LOT, lateral olfactory tract.

layer implies that some cells elsewhere in the slice, presumably GABAergic interneurons owing to the characteristics of the IPSPs, are directly excited by 5-HT. By extracellularly searching the slice for cells which were firing in response to bath-applied 5-HT, a group of 5-HT-activated cells was found on the border of layers II and III of piriform cortex (Fig. 5). To assess the relationship between the 5-HT-activated cells and the 5-HT-induced IPSPs some pharmacological characteristics of these responses were examined. Fig. 6 shows that the excitatory actions of 5-HT on these cells were blocked by ritanserin (5 μM; n = 5). This blockade was a selective effect because the effect of NE, which was also excitatory on these cells, was not blocked by ritanserin. Fig. 7 shows that the actions of 5-HT were mimicked by the 5-HT_2 agonist[13] α-Me-5-HT (12 μM) (n = 3) and that the hallucinogen and 5-HT_2 partial agonist[36] DOM (1 μM) had only a small agonist effect of its own and blunted the effect of 5-HT (n = 7). In addition to blunting the effect of 5-HT, the partial agonist nature of DOM is indicated by the fact that concentrations up to 10 μM had no greater agonist activity than that of 1 μM. Because 5-HT induced IPSPs in only 41% of pyramidal layer cells, we sought to determine whether this was because in some pyramidal cells IPSPs were too small to detect or whether 5-HT indeed induced IPSPs in only a fraction of the population. We found that bath application of the more universal excitant NMDA (10 μM) produced bicuculline-sensitive IPSPs in all pyramidal layer cells tested (n = 17). Furthermore, 10 μM NMDA never brought any of these neurons to firing threshold (mean depolarization = 3.1 ± 2 mV), indicating that at low concentrations only non-pyramidal cells, which we presumed to be GABA-ergic interneurons, were induced to fire. The finding that pyramidal cells were not activated by 10 μM NMDA allowed a survey of a greater population of putative interneurons than was afforded by use of 5-HT alone. Firing rates of neurons activated with NMDA ranged from 1 to 10 spikes per second. Extracellular recording in the presence of bath-applied NMDA showed that the cells activated by 5-HT represented 23% of the NMDA-

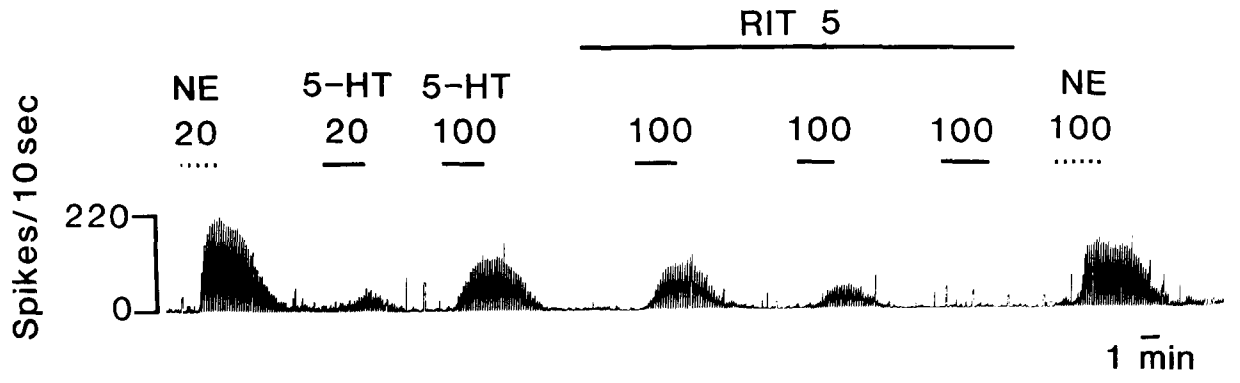

Fig. 6. Antagonist action on putative interneurons. The excitatory effects of 5-HT but not NE are blocked by ritanserin; note that 5-HT has a dose-dependent excitatory effect. Concentrations in μM.

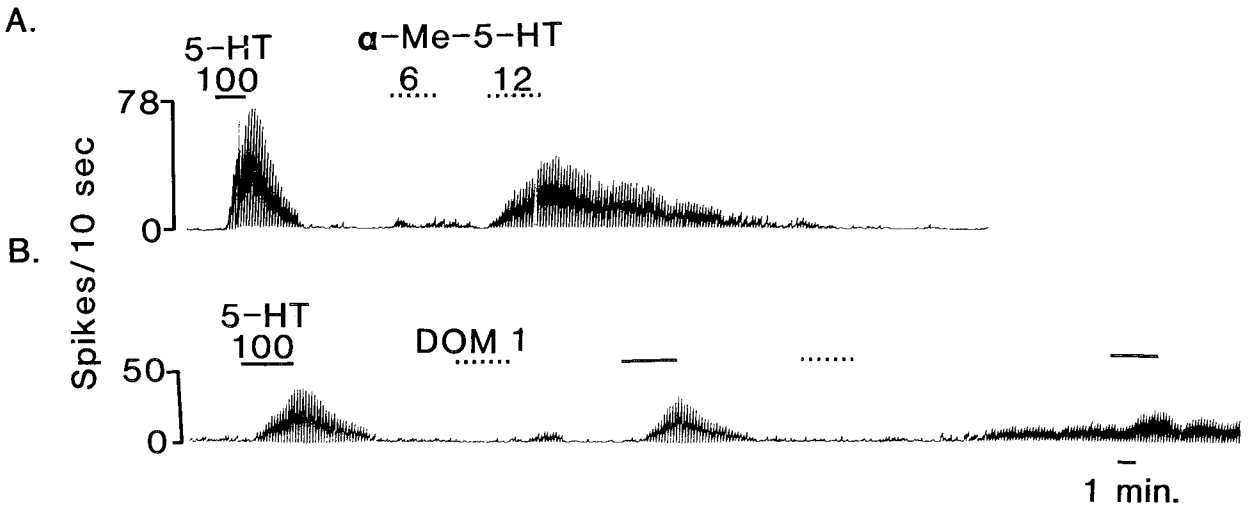

Fig. 7. Agonist actions on putative interneurons. A: the 5-HT_2 agonist α-Me-5-HT mimics the actions of 5-HT in a dose-dependent manner. B: the 5-HT_2 agonist DOM acts as a partial agonist, having a small activating effect of its own and blunting the effect of 5-HT. Note the prolonged action of DOM following the second application. Concentrations in μM.

activated cells at the layer II–III border (Table II).

The 77% of cells at the border of layers II and III which were activated by 10 μM NMDA but not by 5-HT either were not reactive to 5-HT (14%) or were inhibited by it (63%). The cells that were inhibited by 5-HT were also inhibited by 8-hydroxy-(di-*N*-propyl-amino)tetralin (8-OH-DPAT; 1 μM), a 5-HT_{1A} agonist which inhibits serotonergic neurons of the dorsal raphe nucleus[1]. The inhibition by 8-OH-DPAT was blocked by the non-selective 5-HT antagonist spiperone (5 μM; $n = 4$). This type of cell was found throughout layers I–III (Table II).

DISCUSSION

Bath application of 5-HT caused an increase in PSPs in 41% of neurons in the pyramidal cell layer (layer II) of piriform cortex. These PSPs fit the previously described characteristics for GABAergic IPSPs[11,27,38] since they were both TTX- and bicuculline-sensitive. Galvan et al.[11] have reported the presence of spontaneous IPSPs in piriform cortex. The present study shows that in piriform cortex IPSPs of this type can be induced by 5-HT, as has been shown for other transmitters in various brain regions[22,27]. For example, in somatosensory cortex of the guinea pig, acetylcholine causes an increase in IPSPs[27] and, in rat hippocampus, NE causes an increase in IPSPs[22]. As previously shown in guinea pig cortex[9], in the pyramidal cell layer of piriform cortex we found that the application of 5-HT also caused either a small depolarization, hyperpolarization or no change in membrane potential. The induction of IPSPs by 5-HT occurred in those cells which were either depolarized or unaffected by 5-HT. The induction of IPSPs by 5-HT was blocked by the 5-HT_2 antagonist ritanserin as well as the non-selective 5-HT antagonist spiperone.

Extracellular recording in the presence of a low concentration of 5-HT revealed a group of cells at the border of layers II and III of piriform cortex which could be induced to fire by 5-HT. This finding was not unexpected since the induction of IPSPs by 5-HT in cells of the pyramidal cell layer implies that some cells within the confines of the slice are directly activated by 5-HT. Although we have no direct evidence that these activations were direct, a transsynaptic mechanism seems unlikely since even when 5-HT was excitatory to pyramidal cells, it never depolarized these cells to firing threshold. The 5-HT, but not NE, activation of cells at the layer II–III border was blocked by ritanserin. Because the IPSPs were bicuculline-sensitive and had pharmacological characteristics similar to those of the directly excited cells, it was concluded that the directly excited cells were GABAergic interneurons. Though bicuculline has been reported to interfere with some serotonergic responses[24], the response, in this case, is specific because it blocks the 5-HT-induced IPSPs but does not block the 5-HT-induced depolarization. It is possible that the directly excited cells are excitatory

TABLE II

In a representative sample of NMDA-activated cells, 5-HT only excites cells located on the border of layers II and III of piriform cortex

	Excited	*Inhibited*	*No change*	*% Excited*
Layer I	0	6	3	0%
Layer II	0	7	2	0%
Layer II/III border	7	19	4	23%
Layer III	0	6	6	0%

interneurons which impinge upon inhibitory interneurons; however, this is unlikely in view of immunocytochemical evidence that most of the identified interneurons in this region of piriform cortex are GABAergic[15]. The excitatory actions of 5-HT on these putative interneurons were mimicked by the 5-HT_2 agonist α-Me-5-HT. The above pharmacological results suggest that the 5-HT activation of cells at the layer II–III border is mediated by a 5-HT_2 receptor. However, it is possible that these actions could also be mediated, at least in part, by the 5-HT_{1C} receptor whose pharmacological profile is similar to that of the 5-HT_2 receptor[40]. However, the relative density of 5-HT_{1C} receptors compared to 5-HT_2 receptors in cortical regions is very low[34].

Based on receptor binding and electrophysiological studies, it has been hypothesized that the phenethylamine hallucinogens, such as DOM, are agonists at the 5-HT_2 receptor[12,37]. However, in the present study DOM acts like a partial agonist, having a small but prolonged activating effect of its own while blunting the effect of 5-HT. Interestingly, DOM, in contrast to 5-HT itself, has been shown to be only a partial agonist in its ability to stimulate PI turnover[39]. We also found that α-Me-5-HT, which is a full agonist with respect to PI turnover[5], was fully effective in its ability to activate piriform interneurons.

Although 5-HT produced IPSPs in only 41% of pyramidal cells, the more universal excitant NMDA (10 μM) produced IPSPs in all pyramidal cells. Furthermore, at this concentration of NMDA, no pyramidal cells were brought to firing threshold, indicating that only putative interneurons were induced to fire. Because of this differential effect, NMDA was used to study a greater population of putative interneurons. However, because the concentration of NMDA was relatively low this greater population of interneurons probably does not represent all interneurons in the slice. We showed that the cells excited by 5-HT represent only 23% of the interneurons in this NMDA-excitable population which occur at the border of layers II and III (Table II). The subpopulation of NMDA-excitable cells which was not excited by 5-HT was either inhibited (63%) or unaffected (14%) by this transmitter. In layers I, II and III all NMDA-excitable cells (except for the cells at the layer II–III border) were either inhibited or unaffected by 5-HT. The cells inhibited by 5-HT were also inhibited by the 5-HT_{1A} agonist 8-OH-DPAT, an effect which was blocked by the non-selective 5-HT antagonist spiperone. Recently, Ropert[38] has shown that 5-HT decreases spontaneous IPSPs in the hippocampus via a 5-HT_{1A} receptor.

Although it has been shown previously that ritanserin blocks the depolarizing effects of 5-HT on pyramidal cells[9], in the present study we found that the depolarizing effect of 5-HT on pyramidal cells was more resistant to blockade by ritanserin than were the induced IPSPs. This finding raises the issue as to the relative physiological significance of 5-HT actions at the 5-HT_2 receptor on pyramidal cells as compared to interneurons. The depolarizing effect of 5-HT on pyramidal cells was small (i.e. it produced only a 1–4 mV depolarization), whereas its action on the putative interneurons was comparatively large (i.e. it robustly activated the cells). Although the magnitude of the depolarizing effect on the interneurons may be the same as on the pyramidal cells, the relative consequences of that depolarization are much different, possibly because the resting potential of the interneurons is closer to firing threshold. Alternatively, the interneurons may be more sensitive to 5-HT than are the pyramidal cells. In vivo, 5-HT has been reported to have primarily inhibitory effects on cortical neurons. For instance, in one study[33] stimulation of the dorsal raphe nucleus produced a transsynaptic inhibition of neurons in the cingulate cortex. This effect was blocked by methysergide and cyproheptadine, both of which are known to be much more selective for 5-HT_2 receptors than for 5-HT_1 receptors. This result would seem incongruous because actions at the 5-HT_2 receptor tend to be excitatory[9,10], whereas actions at the 5-HT_1 receptor tend to be inhibitory[1,3,38]. However, a transsynaptic inhibitory effect resulting from a direct excitatory effect of 5-HT on an inhibitory interneuron could explain how a 5-HT_2 antagonist might antagonize inhibitory effects of 5-HT. Anatomical evidence in support of this possibility comes from the finding that 5-HT terminals make synapses on non-pyramidal cells in the cortex[31]. Taken together, these results are consistent with the in vivo findings that the net effect of 5-HT on principal neurons in cerebral cortex tends to be inhibition.

Anatomically, there are two major types of principal neurons in the piriform cortex, typical pyramidal cells and semilunar cells[14,15]. Both of these cell types are found predominantly in layer II. They differ in that pyramidal cells have both apical and basal dendrites, whereas semilunar cells have only apical dendrites. It is not yet known whether there are any electrophysiological differences between the two types of cells. However, based on the present results, it would be of interest to investigate possible differences between these two types of principal cells of the piriform cortex with respect to differential sensitivity to 5-HT depolarization or induction of IPSPs. Possibly, each morphological type is uniquely wired to the interneuronal system or to afferent inputs. As an example of the latter, it has been shown[15] that the cholinergic input to piriform cortex terminates only on the basal dendrites of the pyramidal cells.

Alternatively, differential responsivity may be conferred by the intrinsic pharmacological and/or electrophysiological characteristics of the cell.

In conclusion, we propose that there are GABAergic interneurons located at the border of layers II and III in rat piriform cortex which are activated by 5-HT via 5-HT_2 receptors. These cells represent 23% of the NMDA-activated putative interneurons located in this area. Furthermore, we hypothesize that activation of putative GABAergic interneurons at the Layer II/III border induces IPSPs in principal neurons upon application of 5-HT. However, a definitive demonstration that the subpopulation of cells excited by 5-HT represent GABAergic interneurons will require intracellular double-labeling techniques.

Acknowledgements. We would like to thank Nancy Margiotta for her technical assistance. Supported by PHS Grants MH17871 and 5T32GMO7324-12.

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