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5-HT antagonists in rat hippocampus

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The monoamine serotonin (5-hydroxytryptamine, 5-HT) is presumed to be a neurotransmitter in the central nervous system[1,12,15]. Despite the existence of a large number of antagonists of serotonin[6,8,10,17,20,22], most of them have not thus far gained acceptance as satisfactory antagonists of serotonin in the brain[9]. The present study attempted to evaluate serotonin antagonism on the pyramidal cells of the hippocampus (HPC) which receive serotonin containing afferents from the raphe nuclei[12,21]. Certain serotonin antagonists which appeared to be most effective were also tested for their effects on hippocampal cellular responses to stimulation of the raphe nuclei[19].

Ninety-four adult (150–250 g) male Sprague–Dawley rats (obtained from Zivic-Miller Laboratories, Allison Park, Pa.) were used. The surgical and electrophysiological methods employed are presented elsewhere[19].

The following drugs were used for iontophoresis: acetylcholine chloride (ACh, Merck, 2.5 *M*); bicuculline (Pierce Chem., 5 mg/ml, pH = 4.0); bromlysergic acid diethylamide bitartrate (BOL-148, 10 mg/ml, DHEW, NIMH); lysergic acid diethylamide bitartrate (LSD, 1 mg/ml in 0.165 *M* NaCl, NIMH, Bethesda); methysergide (MS, Sandoz, 10 mg/ml in 0.165 *M* NaCl); 4′,2-isopropylamine-1-hydroxylmethyl methane sulphonanalide (MJ1999, Mead Johnson, 0.5 *M*); L-norepinephrine HCl (NE, 1.0 *M*, Aldrich); picrotoxin (50 mg/ml in 0.165 *M* NaCl, K and K); methiothepin maleate (Hoffman-La Roche, 10 mg/ml); cyproheptadine HCl (0.01 *M*, Merck); mianserin (0.01 *M*, Squibb); serotonin creatinine sulphate (5-HT, Aldrich, 0.5 *M*, pH = 5); cinnanserin (0.01 *M*, Organon, Oss, Holland). All drugs were dissolved in distilled water or in saline and had a pH range of 4.0–8.0. In some cases, when recording was made from a very slow-firing cell (less than 1–2 spikes/sec) continuous leakage of ACh was used in order to maintain a stable and faster firing rate (5–10 spikes/sec). This procedure enhanced detection of inhibitory responses, and protected against pre-selection of spontaneously rapid-firing cells for study. Care was taken to exclude the possibility that some drug effects could be due to interactions with ACh by recording from spontaneously active cells and by comparing drug effects on some cells in the presence and absence of ACh leakage effects. When drug effects on the

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cellular responses to serotonin were tested, it was applied in pulses of 10–20 sec at constant interpulse duration and current magnitude for several repetitions before, during and after the application of the antagonist. There were three or more tests with each cell for a given antagonist. Further methodological details of iontophoresis test procedures and decisions concerning detection of antagonistic action is as presented elsewhere[7,19].

When applied iontophoretically with ejection currents of 50–100 nA, serotonin inhibited spontaneous and acetylcholine-sustained hippocampal pyramidal cellular activity (in 207 out of 224 cells recorded from 94 rats), as has been previously reported[3,19]. This inhibition had a short latency (5–10 sec) and short duration (5–10 sec after termination of ejection current) and was occasionally followed by a rebound excitation.

The effects of LSD[6] were tested in 29 cells in 9 rats. Twenty-seven of these were directly slowed by the drug when ejected with 50–100 nA. Despite this 'local anaesthetic' action, which was typically attributed to a drug when it caused a reduction in spike amplitude along with slowing of firing rate, the responses to serotonin of 23 of the 29 cells tested were at least partially antagonised by LSD. The antagonism was demonstrated with current range of 10–100 nA and it was in effect within 2 min from the onset of LSD ejection (Fig. 1). The effects of LSD on norepinephrine (NE)-induced inhibition were tested in 8 cells in 4 rats. No antagonistic action of LSD against NE-induced inhibition was seen in any of the cells tested when equivalent (50–100 nA) currents were used.

BOL[5,10] was tested on 9 cells in 3 rats. On 3 of these cells a reduction of spike amplitude was observed. The responses to serotonin of 4 of the 9 cells was antagonised by BOL (Fig. 1). The antagonistic action of BOL was only partial when ejection currents which produced no noticeable 'local anaesthesia' were applied.

Methysergide (MS)[2,6] was tested on 32 cells in 9 rats. In 14 of these a reduction of spike size was observed when the drug was administered with high currents (100 nA). These 'local anaesthetic' effects were in most cases weaker than those of the former compounds (Fig. 2). The responses to serotonin of 19 of the 32 cells were at

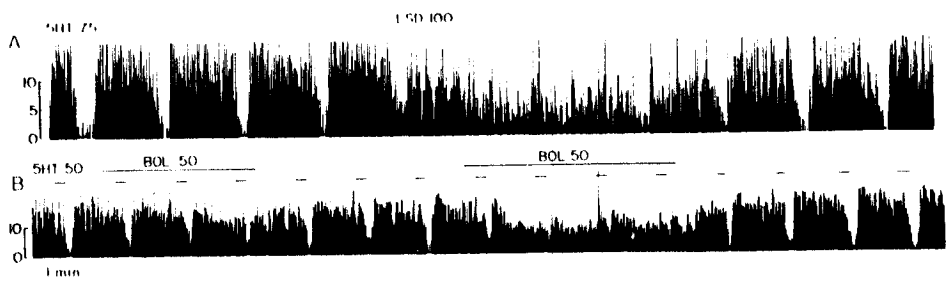

Fig. 1. Effects of LSD (A) and BOL (B) on 2 hippocampal cellular responses to 5-HT applied in 75 (A) or 50 (B) nanoamperes (nA) pulses at constant intervals. Note the presence of a potent direct action of LSD in addition to its antagonistic effect against 5-HT. BOL (B) has also an antagonistic action and its direct effect is much less pronounced, with the current used for ejection. In this and following figures abscissa — time and ordinate — spikes/sec.

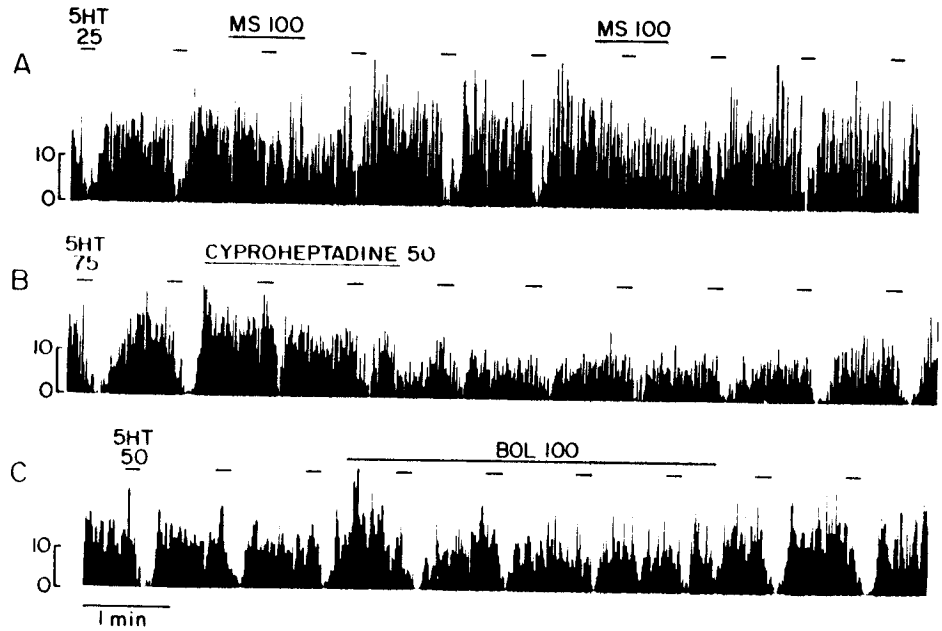

Fig. 2. The effects of methysergide (MS, A) cyproheptadine (B) and BOL (C) on cellular responses to 5-HT in 3 different cells. MS has a potent antagonistic action which outlasts the current ejection interval (*i.e.*, the response to 5-HT right after termination of the MS ejection currents was still antagonised) with only a minor direct effect in the current range used. Cyproheptadine has a potent direct effect in addition to its blocking of 5-HT responses. Note, though, the dissociation of the direct from the antagonistic action. The antagonistic action takes place earlier and disappears before the cell has recovered from the direct action of cyproheptadine. BOL (C) in this case has no direct action, but its antagonistic action is only partial.

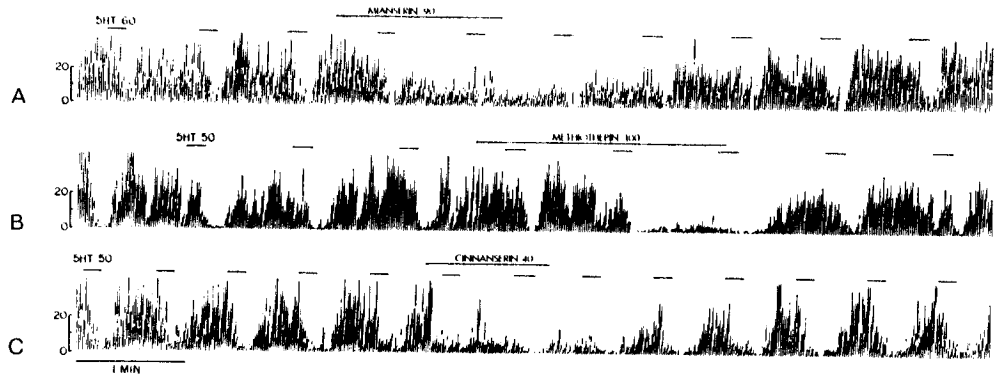

Fig. 3. The absence of 5-HT antagonistic action by some putative 5-HT antagonists: mianserin (A), methiothepin (B) and cinnanserin (C). Each of these compounds has a potent direct antagonistic action which has a shorter time course than previous compounds tested (Figs. 1 and 2), but they have no noticeable antagonistic action towards 5-HT.

least partially antagonised by MS, even when low (10–50 nA) currents of MS were used (Fig. 2). The effects of MS on GABA-induced inhibition were tested in 8 cells and none were affected. A similar lack of effect on NE inhibition were found in 2 cells.

Cyproheptadine[20] was tested on 9 cells in 3 rats. In three of these, 'local anaesthetic' effects were seen when the drug was administered with medium to high currents (50–100 nA). The responses to serotonin of those 3 cells were antagonised by cyproheptadine (Fig. 2). The time courses of the antagonistic action and the local anaesthetic action were different in that the former preceded the latter in both onset and recovery.

Mianserin[22] was tested on 8 cells in 2 rats. Four of these cells were potently inhibited by the drug (Fig. 3). These depressant actions of mianserin appeared with short latency (5–10 sec), for a short duration after termination of ejection current and with little reduction in the action potential height, indicating that mianserin inhibitions were not due to local anaesthetic actions. None of the serotonin responses of cells tested were significantly antagonised by mianserin, even when currents that produced potent direct action (50–100 nA) were used.

Methiothepine was tested on 10 cells in 3 rats. Seven of these cells were inhibited by the drug in a way similar to that produced by mianserin (Fig. 3). The inhibitory action was potent and short lasting; complete recovery from methiothepine effects took place less than 1 min after the termination of the ejection current. The responses to serotonin of only one of the cells were antagonised by methiothepine.

Cinnanserin[17] was tested on 6 cells in 2 rats. All of these cells were inhibited by the drug. The inhibitory action was similar to that of the two previous drugs (Fig. 3) in that its action had a rapid time course of onset and recovery. Lower currents were needed to get effects similar to those of mianserin and methiothepine. The responses to serotonin were not altered by cinnanserin in any of the cells tested, with currents (20–60 nA) that produced potent inhibitory action.

Picrotoxin, a presumptive specific GABA antagonist, was tested on 15 cells in 2 rats. The spike height of only two of these cells was affected by the drug. Surprisingly, the responses of serotonin were at least partially antagonised by picrotoxin in 12 of the cells tested (Fig. 4) when medium (25–50 nA) currents were used.

The compounds tested in the present study can be categorised according to their effects, into three main groups. The first contains those compounds that exhibited at

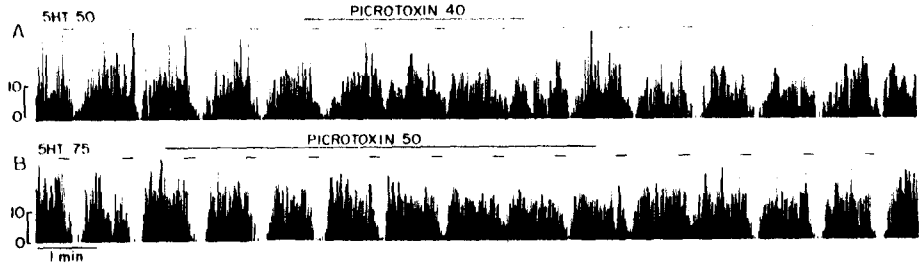

Fig. 4. Picrotoxin effects on the cellular responses to 5-HT in 2 different cells (A and B). Long ejection periods reduced firing rates only slightly, yet antagonism of 5-HT responses is evident.

least some serotonin antagonistic effects. Among these are LSD, BOL, cyproheptadine and methysergide (MS). All of these compounds had some additional local anaesthetic effect, which interfered to a certain extent with the estimation of the antagonistic function of the drug. It was possible, though, to dissociate the 'local anaesthetic' action from the antagonistic action, because the two actions usually had different time courses; the antagonistic effect took place faster and dissipated earlier than the anaesthetic effect (see Fig. 2). Moreover, there was no apparent correlation between the presence of an anaesthetic effect and an antagonistic effect.

The second group of compounds tested consists of those drugs which exhibited a potent depressant effect and therefore could not be tested against serotonin. Among these drugs are cinnanserin, methiothepine and mianserin. These drugs had inhibitory actions at dose levels that produced no noticeable antagonistic effects. There is a possibility that these drugs activated 5-HT receptors, and further tests of this possibility should be performed.

Finally, picrotoxin, a GABA antagonist, blocked (at least partially) the responses to 5-HT of most cells tested. This effect was rather specific because picrotoxin, in previous studies[18], did not antagonise the inhibition produced by NE. This is not to say that the response to 5-HT is mediated by a GABA receptor since another GABA antagonist, bicuculline, did not affect 5-HT responses in several cells tested (unpublished).

One of the difficulties in comparing results obtained in several laboratories is the different testing methods used, as well as the different responses to 5-HT reported. When applied iontophoretically 5-HT either inhibits or excites or maintains both actions, depending on the brain area tested and on the pH of the 5-HT solution[11,16]. Comparisons between iontophoresis and other testing methods are even more complicated[8,10,13]. It is not surprising, therefore, that since the original suggestion that LSD is a 5-HT antagonist, it was found to block excitatory responses to 5-HT[5,16], to block inhibitory action of dorsal raphe stimulation[8], not to affect responses to 5-HT in lateral geniculate cells[15], to stimulate presumed 5-HT functions[10] and to inhibit activity of serotonin containing cells[1]. Recently, LSD appeared to have 5-HT receptor binding properties, which agrees with our suggestion that it has 5-HT antagonistic functions in the forebrain[2]. The discrepancies among varying results can be attributed to inherent differences among preparations. There might exist at least two types of 5-HT receptors as indicated for some invertebrate neurones[11]. Moreover, differences in densities of 5-HT receptors among different test systems may directly affect the efficacy of antagonism at the receptor by a certain drug. Therefore, the inability to demonstrate antagonism by a certain drug due to potent local anaesthetic effects of the drug may mean that the antagonistic action cannot be tested, and it should not be inferred that the particular drug has or has not an antagonistic activity against 5-HT.

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