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Effect of the selective 5-HT$_{1A}$ receptor antagonist WAY 100635 on the inhibition of e.p.s.ps produced by 5-HT in the CA1 region of rat hippocampal slices

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1 The actions of N-(2-(-4(2-methoxyphenyl)-1-piperazinyl)ethyl)-N-(2-pyridinyl) cyclohexane carboxamide (WAY 100635), a novel and selective 5-hydroxytryptamine$_{1A}$ (5-HT$_{1A}$) antagonist, on excitatory postsynaptic potentials (e.p.s.ps) were investigated by use of intracellular recordings in pyramidal cells of the CA1 region of rat hippocampal slices.

2 WAY 100635 (10 nM) did not affect any of the investigated parameters of cell excitability such as membrane potential, total input resistance (R_{in}), firing threshold, action potential amplitude, action potential frequency adaptation, and slow afterhyperpolarization (sAHP) which follows repetitive firing of action potentials. WAY 100635 did not have any effect on either the slope or the amplitude of e.p.s.ps evoked by stimulation of the CA1 stratum radiatum.

3 Bath application of either 5-hydroxytryptamine (5-HT, 10–30 μM) or 5-carboxamidotryptamine (5-CT, 300 nM) hyperpolarized the membrane potential ($\Delta V_m = -4.1 \pm 0.9$ and -6.0 ± 0.9 mV, respectively), and reduced R_{in} ($-25 \pm 8\%$ and $-18 \pm 1\%$, respectively). 5-HT blocked the action potential frequency adaptation and significantly reduced the amplitude of the sAHP that follows repetitive firing of action potentials.

4 5-HT significantly decreased the amplitude of evoked e.p.s.ps ($-14 \pm 6\%$). This effect was greater in the presence of the GABA$_A$ receptor antagonist bicuculline (10 μM, $-45 \pm 12\%$) and was mimicked by 5-CT ($-49 \pm 5\%$). Both AMPA and NMDA components of e.p.s.ps were significantly reduced in amplitude by 5–HT ($-38 \pm 8\%$, $n=6$, and $-29 \pm 12\%$, $n=3$, respectively; $P<0.05$).

5 WAY 100635 fully antagonized the hyperpolarization, the reduction of R_{in}, and the decrease in amplitude of e.p.s.ps elicited by 5-HT, while it did not affect the action of 5-HT on the action potential frequency adaptation. In the presence of WAY 100635, 5-HT elicited a depolarization which was blocked by 10–30 μM RS 23597-190, a selective 5-HT$_4$ receptor antagonist.

6 Our data demonstrate that WAY 100635 is devoid of direct effects on CA1 pyramidal cell excitability and on evoked e.p.s.ps, while it fully antagonizes the effects of 5-HT on excitatory synaptic transmission and on hyperpolarization, without affecting the 5-HT$_4$ receptor-mediated response. Since WAY 100635 selectively antagonizes 5-HT$_{1A}$ receptor-mediated actions of 5-HT, our data also demonstrate that the inhibitory action of 5-HT on excitatory synaptic transmission in CA1 is mediated by 5-HT$_{1A}$ receptors.

Keywords: 5-HT; 5-CT; WAY 100635; RS 23597-190; CGS 12066A; 5-HT$_{1A}$ receptor; 5-HT$_4$ receptor; CA1 hippocampus; synaptic potentials

Introduction

The effects of 5-hydroxytryptamine (5-HT) in the central nervous system are mediated through seven classes of receptors (Hoyer *et al*., 1994). At least four classes of 5-HT receptors (5-HT$_{1-4}$) are known to modify neurone excitability and/or neurotransmitter release (Segal, 1976; 1980; Aghajanian & Lakoski, 1984; Segal *et al*., 1989; Andrade & Nicoll, 1987; Araneda & Andrade, 1991; Ropert & Guy, 1991; Beck *et al*., 1992; Torres *et al*., 1994). Although neurones may not express all known 5-HT receptors, 5-HT released by 5-hydroxytryptaminergic terminals projecting to the hippocampus from the raphe nuclei is likely to change synaptic responses in this region (Segal, 1975; Segal & Weinstock, 1983; Segal *et al*., 1989; Klancnik & Phillips, 1991). In the rat hippocampus, 5-HT induces hyperpolarization of CA1 pyramidal cells through 5-HT$_{1A}$ receptor stimulation (Colino & Halliwell, 1987; Ropert, 1988; Beck *et al*., 1992; Passani *et al*., 1994; Corradetti *et al*., 1996). This inhibitory effect masks the concomitant block of a potassium conductance (Colino & Halliwell, 1987), probably exerted by 5-HT$_4$ receptor stimulation, which leads to cell membrane depolarization and to increased excitability of pyramidal cells once the 5-HT$_{1A}$ receptor-mediated response is blocked (Andrade & Nicoll, 1987; Torres *et al*., 1994).

The increase in potassium conductance and consequent hyperpolarization produced by activation of 5-HT$_{1A}$ receptors strongly decrease the excitability of pyramidal cells and may participate in the blocking of long-term potentiation (LTP), as observed in CA1 and CA3 regions of the rat hippocampus (Corradetti *et al*., 1992; Villani & Johnston, 1993).

On the other hand, 5-HT is known to either inhibit or excite GABAergic interneurones in the CA1 region by acting on 5-HT$_{1A}$ (Van den Hooff & Galvan, 1991; Schmitz *et al*., 1995b), and/or 5-HT$_3$ receptors (Ropert & Guy, 1991; Passani *et al*., 1994). These actions may concur to change synaptic excitatory responses in the CA1 region.

It has been suggested that in the CA1 region 5-HT decreases excitatory postsynaptic potentials (e.p.s.ps) by eliciting a 5-HT$_{1A}$ receptor-mediated increase in potassium conductance (see Anwyl, 1990 for a review) and/or presynaptic mechanism(s) involving a decrease in calcium conductance (Schmitz *et al*., 1995a). Indeed, several 5-HT$_{1A}$ receptor agonists such as

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5-HT, 5-carboxamidotryptamine (5-CT), (8-hydroxy-2 di *n*-propylamino) tetralin (8-OH-DPAT), buspirone, and ipsapirone (Jahnsen, 1980; Segal, 1980; Peroutka *et al*., 1987; Rowan & Anwyl, 1987; Ropert, 1988; Sakai & Tanaka, 1993; Schmitz *et al*., 1995a) reduce the electrically evoked e.p.s.p. in this brain region. However, all the compounds tested were not selective enough to insure that the observed decrease in e.p.s.p. was not due to stimulation of other 5-HT receptors. For instance, 8-OH-DPAT and 5-CT, in addition to their agonist action on 5-HT_{1A} receptors, exert $5\text{-HT}_{1B/D}$ receptor-mediated effects and bind to 5-HT_{6-7} receptors (Hoyer *et al*., 1994). Furthermore, 8-OH-DPAT may act as a partial antagonist (Colino & Halliwell, 1987; Beck *et al*., 1992) and decrease fibre volley through still non-elucidated mechanisms (Peroutka *et al*., 1987; Hiner *et al*., 1988). The antagonists available to us until now were either non-selective or they acted as partial or full agonists in the raphe nucleus (Greuel & Glaser, 1992), a phenomenon ascribed to the presence of a reserve of receptors in the 5-hydroxytryptaminergic cells of the raphe (Fletcher *et al*., 1993). In spite of these drawbacks, the evidence obtained with non-selective 5-HT receptor antagonists such as methysergide (Segal, 1980; Klancnik *et al*., 1991) spiperone (Beck *et al*., 1985; Rowan & Anwyl, 1987), methiothepin (Corradetti *et al*., 1992), NAN-190 (Sakai & Tanaka, 1993) converge to indicate a role of 5-HT_{1A} receptors in the inhibitory modulation of e.p.s.ps by 5-HT.

WAY 100635 is a new antagonist devoid of intrinsic activity in several biochemical and behavioural tests (Fletcher *et al*., 1996) having a selective and competitive high-affinity binding for the 5-HT_{1A} receptors. *In vitro* and *in vivo* autoradiographic studies with ^{3}H-labelled WAY 100635 confirmed the regional distribution and receptor density differences previously revealed by binding of agonists (Laporte *et al*., 1994; Gozlan *et al*., 1995; Khawaja *et al*., 1995). In electrophysiological recordings *in vitro*, WAY 100635 acted as a full antagonist of 5-HT_{1A} receptor-mediated responses in the CA1 region of the hippocampus and the dorsal raphe nuclei (Fletcher *et al*., 1996; Mundey *et al*., 1996; Corradetti *et al*., 1996).

The aim of the present work was to study the overall effect of the selective 5-HT_{1A} antagonist WAY 100635 on neurotransmission and to re-evaluate the role of these receptors in the modulation of excitatory neurotransmission in the CA1 region of the hippocampus.

Methods

Preparation of hippocampal slices

Experiments were carried out with rat hippocampal slices *in vitro*, as previously described (Corradetti *et al*., 1996). Male Wistar rats (100–200 g body weight, Charles River, Como, Italy), were killed by decapitation. The hippocampi were rapidly removed and placed on ice-cold oxygenated (95% O_2/5% CO_2) artificial cerebrospinal fluid (aCSF) of the following composition (mM): NaCl 124, KCl 3.0, NaH_2PO_4 1.25, $MgSO_4$ 1.4, $CaCl_2$ 2, $NaHCO_3$ 25, D-glucose 10 (pH 7.4). Hippocampal slices (400 μm thick) were cut with a McIlwain tissue chopper (Gomshall, U.K.) and kept in oxygenated aCSF for at least 1 h at room temperature. A single slice was then placed on a nylon mesh, completely submerged in a small (700 μl) chamber, and superfused with oxygenated aCSF (28–30°C) at a constant flow rate of 2–3 ml min^{-1}. Drugs were administered through a three-way tap and a complete exchange of the chamber volume occurred within 1 min. Antagonists were superfused for at least 20 min before the effects of 5-HT were tested.

Intracellular recording

CA1 pyramidal neurones were recorded in current-clamp mode with 2 M K-methylsulphate- (50–80 MΩ) or 3 M KCl- (35–50 MΩ) filled electrodes. Test pulses (80–110 μs duration; 0.017–0.05 Hz), triggered either by a pulse programmer (Digitimer 4030, Digitimer, Welwyn Garden City, U.K.) or by an IBM-compatible PC, were delivered by stimulus isolation units (DS2, Digitimer) through bipolar nichrome electrodes positioned in the CA1 stratum radiatum. Stimulus-response curves were constructed at the beginning and during the experiment by gradually increasing the stimulus strength. Electrical signals were amplified with an Axoclamp 2A (Axon Instruments, Foster City, U.S.A.) and displayed on an oscilloscope and chart recorder (2800 Gould, Valley View, U.S.A.). Traces were stored on a digital tape (DTR 1200, BioLogic, Claix, France; sampling frequency 48 kHz) and on a computer by use of pClamp software (Axon Instruments) for off-line analysis. Several criteria were used to accept cells for experiments: stable resting membrane potential (r.m.p.) of at least -60 mV and no spontaneous firing of action potentials; no sudden drops in the neuronal input resistance (R_{in}) indicating cell damage; constant amplitude of the spike ($\geqslant 80$ mV) obtained by direct activation of the cell. Modifications of synaptic efficacy by drugs were evaluated by measuring the changes in slope and amplitude of the evoked e.p.s.p. In some experiments the e.p.s.ps were evoked both at r.m.p. and during injection of hyperpolarizing current steps to allow simultanous measurement of cell R_{in} and e.p.s.p. amplitude, maintained below threshold for cell discharge. To study the effects of 5-HT on cell discharge, direct activation of the impaled neurone was obtained by injection of long (400–500 ms, for action potential frequency adaptation) depolarizing current steps through the recording electrode. The slow AHP (sAHP) which follows repetitive firing elicited by injection of depolarizing current ($+500$ pA, 400 ms) through the recording electrode were measured 150 ms after cessation of the depolarizing step. A series of hyperpolarizing current steps (400–500 ms duration, $-500/+500$ pA) were injected repeatedly to monitor cell R_{in} and to construct *I*/V plots. The effects of 5-HT on the N-methyl-D-aspartate (NMDA) receptor-mediated component of the synaptic potential were studied in the presence of 10 μM NBQX (6-nitro-7-sulphamoylbenzo(f)quinoxaline hydrochloride), a selective AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid)-receptor antagonist, and 10 μM bicuculline to block γ-aminobutyric acid $(\text{GABA})_A$ receptor-mediated inhibition. The effects of 5-HT on the AMPA receptor-mediated components of e.p.s.ps were studied in the presence of 50 μM D(-)-2-amino-5-phosphonopentanoic acid (D-AP5), a selective NMDA receptor antagonist and 10 μM bicuculline.

Drugs

WAY 100635 ((N-(2-(-4(2-methoxyphenyl)-1-piperazinyl) ethyl)-N-(2-pyridinyl) cyclohexane carboxamide) was kindly provided by Dr Michel Hamon; (-)-bicuculline methiodide was from Sigma (St. Louis, U.S.A.); 5-hydroxytryptamine creatinine sulphate, 5-CT (5-carboxamidotryptamine), picrotoxin and CGS 12066A (7-trifluoromethyl-4-(4-methyl-1-piperazinyl)pirrolo[1,2-α]quinoxaline) were from RBI (Natick, U.S.A.); D-AP5, 6-nitro-7-sulphamoylbenzo(f)quinoxaline hydrochloride (NBQX) and RS 23597-190 hydrochloride (3-

(piperidin-1-yl)propyl-4-amino-5-chloro-2-methoxy benzoate) were from Tocris (Bristol, U.K.).

Data analysis

All numerical data are expressed as mean ± s.e.; for statistical analysis, Wilcoxon or Mann-Whitney tests, as appropriate, were employed. A value of $P<0.05$ was considered statistically significant.

Results

CA1 pyramidal cells were recorded from 56 slices taken from 49 rats. Neurones ($n=56$) had a r.m.p. of -65.4 ± 1.5 mV, R_{in} of 46.3 ± 1.7 MΩ, and action potential amplitude greater than 80 mV (88.3 ± 1.9 mV). In most of the experiments, and in particular when neurones were uninhibited, cells were manually clamped at a membrane potential (-72.0 ± 1.6 mV) hyperpolarized relative to their original r.m.p. by injecting a constant negative current of 50–180 pA through the recording electrode. Although this procedure decreased the amplitude of the hyperpolarization produced by 5-HT_{1A} agonists, it allowed better investigation of stimulus-response relationships. When necessary, the current was adjusted during the application of 5-HT_{1A} agonists to keep the membrane potential at the level chosen for a given cell (see e.g. Figures 3a, 4a).

Effects of 5-HT and of 5-CT on e.p.s.ps evoked in control or in bicuculline-containing aCSF

In an initial series of experiments, we tested the effects of 5-HT and 5-CT, a high-affinity 5-HT_{1A} receptor ligand with maximal agonist effects (Beck *et al*., 1992; Corradetti *et al*., 1996), on e.p.s.ps recorded intracellularly from CA1 pyramidal cells in control aCSF. Stimulation of the stratum radiatum evoked e.p.s.ps which increased in amplitude in relation to the stimulus strength and eventually reached the threshold for discharging an action potential. As previously shown, superfusion of 5-HT (30 μM, 5 min application) caused cell membrane hyperpolarization ($\Delta V_m=-6.3\pm1.1$ mV) accompanied by a decrease in R_{in} (-40.9 ± 5.0%). When the stimulation of the stratum radiatum was repeated in the presence of 5-HT the amplitude of the e.p.s.ps decreased from 6.3 ± 1.0 to 5.2 ± 0.8 mV (-15.3 ± 3.8%, $n=6$; $P<0.02$; Figure 1a).

Since 5-HT may affect the stimulus-evoked activity of GABAergic interneurones (see Introduction), unless otherwise stated, the following experiments were carried out in the presence of 10 μM bicuculline, to block the $GABA_A$ receptor-mediated fast inhibition. As expected, under these conditions the e.p.s.ps evoked were longer in duration than in control aCSF (Figure 1b).

Application of 5-HT (10 μM; $n=6$) hyperpolarized pyramidal cells ($\Delta V_m=-4.0\pm0.9$ mV) decreased both the R_{in} (-24.7 ± 6.8%) and the action potential frequency adaptation measured by injecting 400 ms depolarizing (+500 pA) steps (the number of spikes increased from 9.0 ± 1.6 to 13.2 ± 3.4). Also, the amplitude of the slow afterhyperpolarization (sAHP) that develops at the end of the depolarizing step decreased from -3.0 ± 0.7 to -1.5 ± 0.5 mV. 5-HT reduced the amplitude of stimulus-evoked e.p.s.ps from 8.8 ± 1.7 to 4.5 ± 1.0 mV ($P<0.05$). Similar results were obtained with 5-CT (300 nM; $n=4$), which hyperpolarized the cells

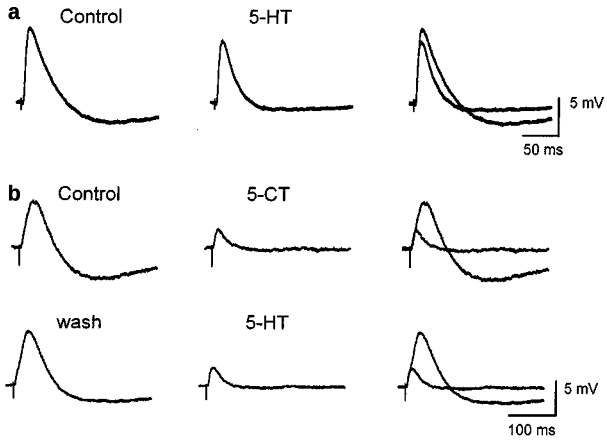

Figure 1 Effect of 5-HT and 5-CT on the amplitude of electrically evoked e.p.s.ps in CA1 pyramidal neurones. (a) Traces are averages of five consecutive responses evoked by stimulation (2.5 V, downward transient) of stratum radiatum in control conditions (left) and after 10 min of superfusion with 30 μM 5-HT (middle). Right panel shows the two responses superimposed. KCl-filled electrode. r.m.p.: −71 mV; R_{in}: 35 MΩ. (b) E.p.s.ps were induced by electrical stimulation (2.7 V, downward transient) of the stratum radiatum in the presence of bicuculline (10 μM). The cell was kept at a V_m of −73 mV by injection of −150 pA to avoid cell firing during synaptic stimulation. Upper panel: left trace: control; middle trace: 300 nM 5-CT (10 min application). Lower panel: left trace: 30 min washout of 5-CT; middle trace: 10 min superfusion of 5-HT (10 μM, 10 min). Note that in the presence of bicuculline, evoked e.p.s.ps were longer in duration than in control. Right panels: corresponding left and right superimposed traces. KCl-filled electrode. r.m.p.: −66 mV; R_{in}: 55 MΩ.

($\Delta V_m=-5.6\pm0.7$ mV; $n=4$) and reduced the R_{in} (-18.1 ± 1.4%). The e.p.s.p. amplitude was reduced from -6.8 ± 1.2 to 3.6 ± 0.9 mV ($P<0.01$). The effects of both 5-HT and 5-CT were observed at all stimulus strengths tested but were greater at stimulus strengths close to the threshold for eliciting an action potential (Figure 2).

Since both 5-HT and 5-CT bind to 5-HT_{1B} receptors, which are known to decrease release of neurotransmitters, including glutamate, in the central nervous system (Maura *et al*., 1991; Maura & Raiteri, 1996), we tested the action of CGS 12066A, endowed with maximal agonist effect on 5-HT_{1B} receptors (Neale *et al*., 1987), on e.p.s.ps. The compound (10 μM) neither affected cell membrane properties ($\Delta V_m=0.7\pm0.8$ mV, $R_{in}\Delta\%=6.3\pm0.8$), nor the amplitude of evoked e.p.s.ps (from 8.7 ± 0.8 in control to 8.2 ± 1.1 mV, $n=3$). Similar results were obtained by with 30 μM CGS 12066A ($n=2$, data not shown).

Effects of WAY 100635 on neurone excitability and on e.p.s.ps

Superfusion of WAY 100635 (10 nM, 30 min) did not change the excitability of CA1 pyramidal cells: neither the membrane potential ($\Delta V_m=0.1\pm0.1$ mV) nor the R_{in} (from 41.9 ± 5.5 to 41.5 ± 5.5 MΩ; $n=9$) were affected. The action potential amplitude (from r.m.p. to peak) was 102.3 ± 2.5 mV in control and 103.9 ± 3.5 mV in WAY 100635-treated cells, and the threshold for firing an action potential upon intracellular current injections was 14.3 ± 1.3 mV in control and 14.6 ± 1.6 mV in the presence of WAY 100635 ($n=5$). In addition, WAY 100635 did not change the action potential frequency adaptation (the number of action potentials before and during application of WAY 100635 were 12.2 ± 2.1 vs 12.3 ± 2.5), nor the amplitude of the sAHP (from -3.6 ± 0.7 to

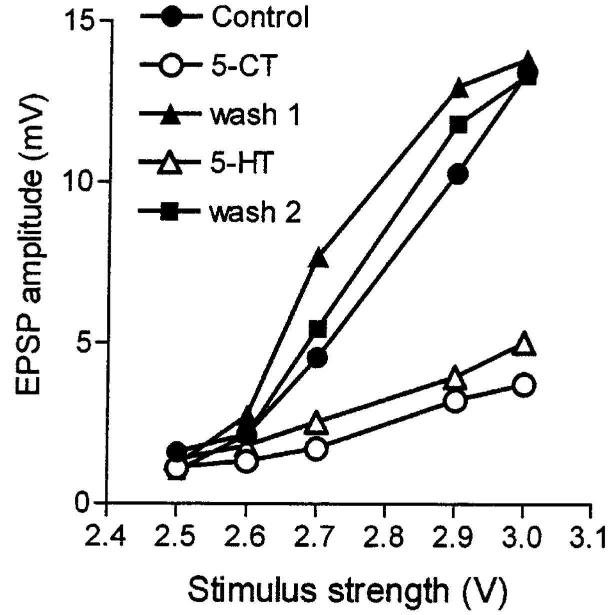

Figure 2 5-HT and 5-CT decreased the amplitude of evoked synaptic potentials in a CA1 pyramidal neurone. Stimulus-response relationships were constructed in control conditions by gradually increasing the stimulus strength. The protocol was repeated after 10 min superfusion of 300 nM 5-CT; after 20 min washout (wash 1), 10 min application of 10 μM 5-HT and 20 min after the second washout (wash 2). Data were obtained from the same cell shown in Figure 1b. Note that the effects of both 5-HT and 5-CT were greater at higher stimulus strengths.

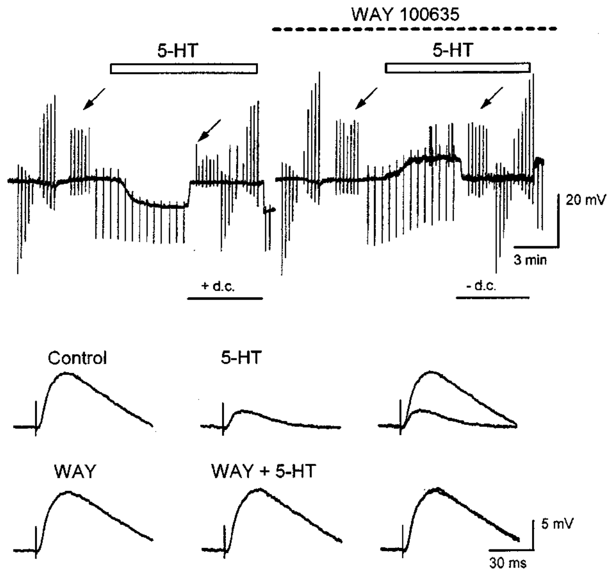

Figure 3 WAY 100635 (WAY) selectively antagonizes the membrane hyperpolarization and the decrease in e.p.s.p. amplitude induced by application of 5-HT in a CA1 hippocampal neurone. (a) Chart record of the membrane potential recorded from a CA1 pyramidal cell in which 5-HT (10 μM) was applied alone or in the presence of the 5-HT$_{1A}$ antagonist WAY 100635 (10 nM). The sequence of downward/upward deflections are cell membrane responses to negative/positive current pulses ($-500/+500$ pA, varied by 100 pA, 400 ms, 1/7 s) injected through the recording electrode to construct *I*/V curves and to monitor action potential frequency adaptation (accommodation). The following downward deflections are electrotonic cell membrane responses to constant current pulses (-300 pA, 400 ms, 1/30 s) to monitor R_{in}. Upward deflections indicated by arrows are e.p.s.ps evoked by electrical stimulation (1.7 V) of stratum radiatum. During 5-HT application the membrane potential was manually clamped at the control value (-69 mV) by injecting $+250$ pA (+d.c.) through the recording electrode (left panel). Note that the effects of 5-HT were completely blocked by WAY 100635 (right panel) and that in the presence of the antagonist, 5-HT elicited a small depolarization. Membrane potential was clamped at the control value by injecting negative current (−d.c.: -90 pA). KCl-filled electrode. r.m.p.: -69 mV. R_{in}: 43 MΩ. (b) E.p.s.ps evoked by stimulation (1 V, fast transient) of stratum radiatum. Upper: control (left trace), 10 min after 10 μM 5-HT application (middle trace). Lower panel: WAY 100635 (10 nM) alone (left trace) or in combination with 10 μM 5-HT (middle trace). In the right panel corresponding traces are superimposed. KCl-filled electrode. r.m.p.: -68 mV. R_{in}: 30 MΩ.

3.5 ± 1.0 mV; $n=6$). In the presence of WAY 100635, the e.p.s.p. amplitude did not change (from 7.7 ± 1.0 to 7.3 ± 1.0 mV). WAY 100635 completely antagonized the hyperpolarization and the decrease in R_{in} caused by application of 5-HT (10–30 μM; $n=7$; Figure 3). In the presence of WAY 100635, 5-HT elicited a depolarization ($\Delta V_m = +1.7 \pm 1.5$ mV; $n=7$, Figure 3a), probably due to activation of 5-HT$_4$ receptors (Andrade & Nicoll, 1987; Torres *et al.*, 1994), since this effect was completely antagonized by 10–30 μM RS 23597-190 hydrochloride, a selective (Eglen *et al.*, 1993) 5-HT$_4$ antagonist ($\Delta V_m = 0.1 \pm 0.1$ mV; $n=7$).

The presence of 10 nM WAY 100635 abolished the decrease in e.p.s.p. amplitude caused by 5-HT, both in control aCSF (from 6.2 ± 0.7 to 6.1 ± 0.8 mV; $n=4$) and in the presence of bicuculline (from 7.8 ± 1.0 to 7.5 ± 1.0 mV; $n=4$; Figure 3b). The effects of WAY 100635 persisted after wash (up to 2 hours), confirming the very slow dissociation of the antagonist from the receptor (Corradetti *et al.*, 1996).

Since the effect of 5-HT was greater at higher stimulus strengths (see Figure 2), i.e. when the evoked (disinhibited) e.p.s.p. was likely to involve the activation of NMDA receptors (Collingridge *et al.*, 1988), we carried out a series of experiments on the AMPA and NMDA components of e.p.s.p. isolated pharmacologically. The AMPA component of the e.p.s.p. was isolated by the presence of 50 μM D-AP5, superfused for at least 25 min. 5-HT (10 μM) decreased the amplitude of the e.p.s.ps from 9.2 ± 1.1 to 5.5 ± 0.7 mV ($n=6$; Figure 4). This effect was fully antagonized by 10 nM WAY 100635 ($n=6$; Figure 4).

In 3 experiments the NMDA component of the e.p.s.p. was isolated by the addition of 10 μM NBQX in the superfusing solution (Figure 5). The demonstration that in these conditions the evoked synaptic response was NMDA-mediated, was two fold. Firstly, when afferent fibres were stimulated, the voltage-dependence of a pure NMDA receptor-mediated response was different from the voltage dependence of an NMDA/AMPA e.p.s.p. (compare a and b in Figure 5). In addition, D-AP5 (50 μM) completely blocked the synaptic response obtained in the presence of NBQX (not shown). In these neurones 5-HT significantly decreased the amplitude of the NMDA component of the e.p.s.p. from 4.9 ± 0.6 to 3.6 ± 0.9 mV at resting membrane potential. This effect was completely antagonized by WAY 100635 (Figure 5b).

In a separate series of experiments, we tested the possibility that the first application of 5-HT, acting through one of its several non 5-HT$_{1A}$ receptors, might have changed the responsiveness of pyramidal cells to a second application of the agonist. In the presence of WAY 100635 applied from the beginning of the recording, 5-HT (10–30 μM) neither hyperpolarized pyramidal cells nor affected the amplitude of e.p.s.ps ($n=7$) or of their AMPA ($n=3$) and NMDA ($n=4$) components.

The decrease in e.p.s.p. amplitude elicited by 5-HT and its antagonism by WAY 100635 in all the conditions tested are summarized in Figure 6, where the statistical significance of the changes is also indicated.

Discussion

The major results of our investigation were that the 5-HT_{1A} selective antagonist WAY 100635 blocked the 5-HT_{1A} receptor-mediated responses and the decrease in excitatory neurotransmission elicited by 5-HT, whereas it did not affect either neurone excitability or synaptic transmission in the CA1 region of the rat hippocampus. We used 10 nM WAY 100635, a concentration that afforded full antagonism of 5-HT_{1A} responses in CA1 pyramidal cells without affecting the selectivity of the drug (Fletcher *et al*., 1996; Corradetti *et al*., 1996). At this concentration, WAY 100635 did not change either the amplitude or the duration of e.p.s.ps evoked by the stimulation of the stratum radiatum. The antagonist did not modify any of the parameters of cell excitability including r.m.p., R_{in}, or action potential amplitude. In particular, it did not change either the action potential frequency adaptation or the slow AHP which follows the repetitive cell discharge caused by a long (400 ms) cell depolarization. In addition, the presence of WAY 100635 unmasked a depolarization elicited by 5-HT, that was blocked by the selective 5-HT_4 antagonist RS 23597-190 hydrochloride, confirming that this response is indeed mediated by 5-HT_4 receptors (Torres *et al*., 1995).

This work confirms and extends to excitatory synaptic potentials previous results which investigated the selectivity of WAY 100635 on 5-HT_{1A} receptors in CA1 pyramidal neurones

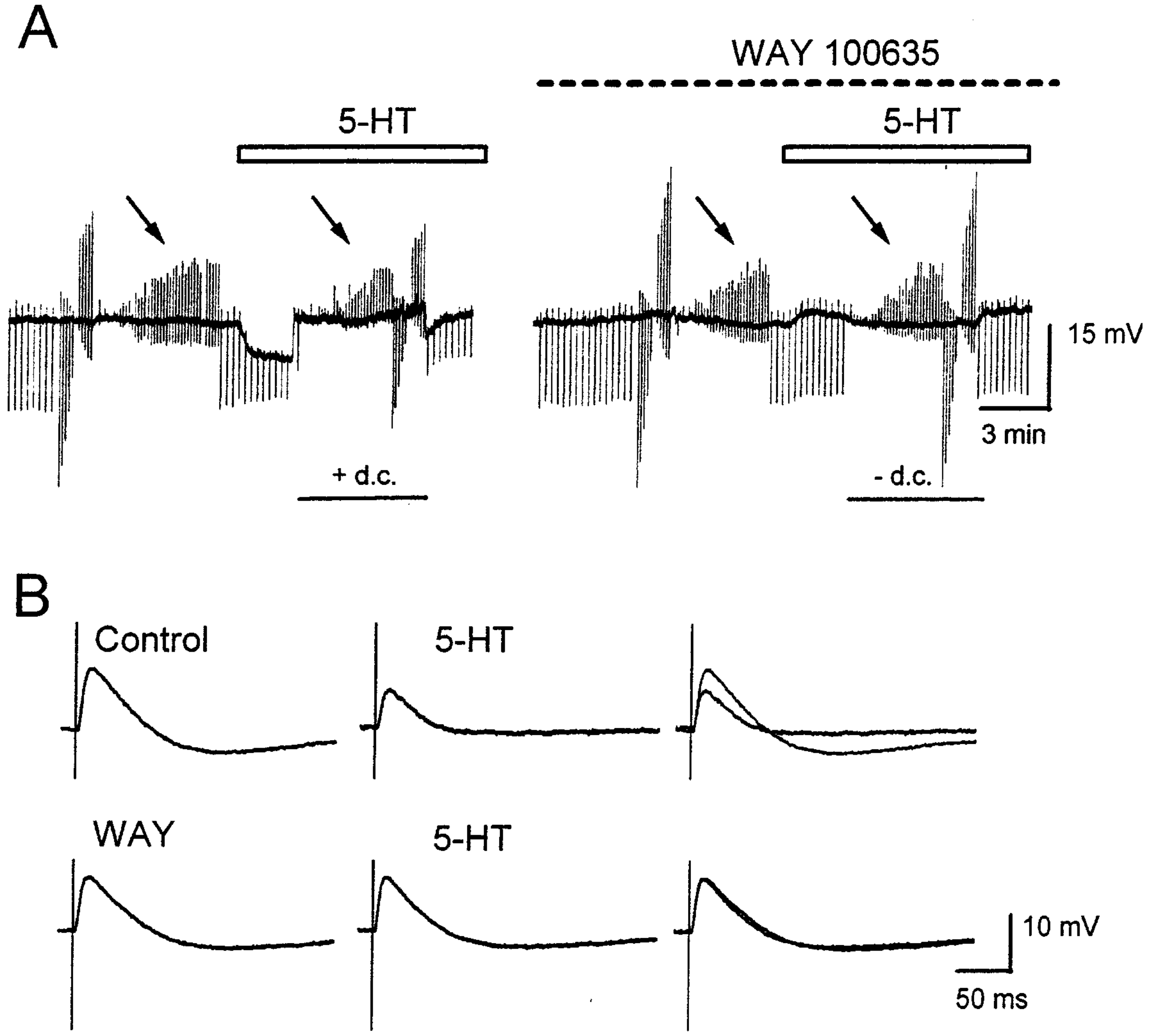

Figure 4 WAY 100635 blocked the decrease in the amplitude of AMPA receptor-mediated e.p.s.p. elicited by 5-HT. The responses were obtained in the presence of bicuculline (10 μM) and D-AP5 (50 μM). (a) Continuous chart record of the resting membrane potential of a CA1 pyramidal cell in which 5-HT (10 μM) was applied alone or in combination with 10 nM WAY 100635. The sequence of downward/upward deflections are cell membrane responses to negative/positive current pulses ($-500/+500$ pA, varied by 100 pA, 400 ms, 1/7 s) injected through the recording electrode to construct *I*/V curves and to monitor action potential frequency adaptation (accommodation). The following downward deflections are electrotonic cell membrane responses to constant current pulses (-300 pA, 400 ms, 1/30 s) to monitor R_{in}. Upward deflections indicated by arrows are stimulus-response curves constructed by gradually increasing the stimulus strength of the electrical stimulation of stratum radiatum. During 5-HT application the membrane potential was clamped at the control value (-66 mV) by injecting (+d.c.; +190 pA) through the recording electrode (left panel). In the right panel membrane potential was clamped at the control value by injecting negative current ($-$d.c.: -30 pA). KCl-filled electrode, r.m.p.: -66 mV. R_{in}: 60 MΩ. (b) E.p.s.ps evoked by stimulation (17 V, fast transient) of stratum radiatum. Upper panel: control (left trace), 10 min after 10 μM 5-HT application (middle trace). Lower panel: WAY 100635 (10 nM) alone (left trace) or in combination with 10 μM 5-HT (lower, middle trace). In the right panel the corresponding traces are superimposed. KCl-filled electrode, r.m.p.: -66 mV. R_{in}: 60 MΩ.

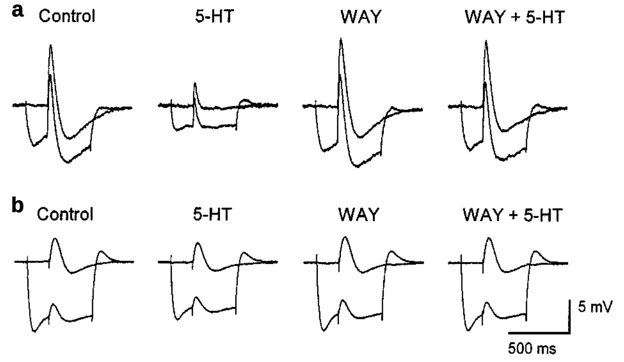

Figure 5 WAY 100635 antagonized the decrease in amplitude of the NMDA receptor-mediated component of e.p.s.ps induced by 5-HT. (a) E.p.s.ps evoked by stimulation (3.3 V) of stratum radiatum in the presence of bicuculline (10 μM). Synaptic potentials were recorded in the CA1 pyramidal neurone at two different membrane potentials: r.m.p. (-70 mV) and during a hyperpolarizing step (-200 pA, 400 ms). Note that superfusion of 10 nM WAY 100635 *per se* did not affect membrane resistance and e.p.s.p. amplitude, while it completely antagonized the effects induced by 10 μM 5-HT. KCl-filled electrode. r.m.p.: -70 mV. R_{in}: 40 MΩ. (b) NMDA receptor-mediated e.p.s.ps, isolated by the presence of NBQX (10 μM), were evoked (6 V, fast transient) at r.m.p. (-74 mV) and during a hyperpolarizing step (-500 pA, 400 ms). Note the voltage-dependent amplitude of responses. KCl-filled electrode. R_{in}: 52 MΩ.

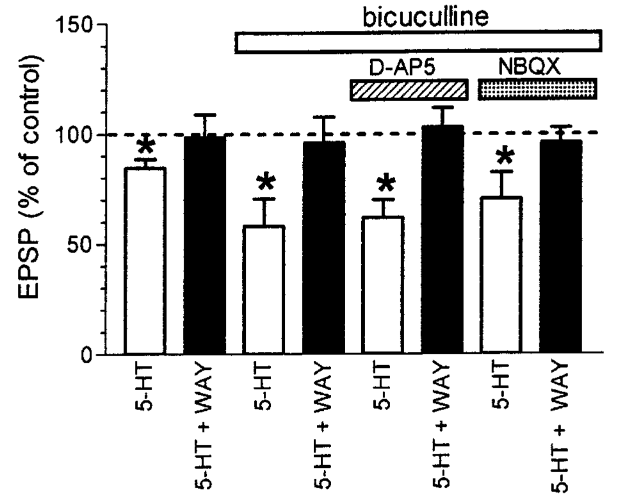

Figure 6 Comparison of the effects of 5-HT, alone or in combination with WAY 100635, on excitatory synaptic potentials evoked under different pharmacological treatments. Effects of 5-HT (30–10 μM, 5–7 min application) in the absence and in the presence of 10 nM WAY 100365 in different experimental protocols. Each column is the mean $\pm$ s.e. of the values expressed as the percentage of control. Asterisks indicate a statistically significant difference ($P<0.05$) from controls and from the corresponding treatment with WAY 100635.

and 5-hydroxytryptaminergic cells of the dorsal raphe nuclei (Gozlan *et al*., 1995; Fletcher *et al*., 1996; Corradetti *et al*., 1996). The fact that WAY 100635 antagonized the decrease in e.p.s.p. amplitude elicited by 5-HT and the 5-HT$_{1A}$ agonist 5-CT demonstrates that activation of 5-HT$_{1A}$ receptors strongly inhibits excitatory neurotransmission in the CA1 region of rat hippocampus.

Previous studies showed that 5-HT$_{1A}$ antagonists (methysergide, spiperone, and NAN 190) which block 5-HT$_{1A}$ receptor-mediated hyperpolarization, also antagonize the reduction of excitatory synaptic transmission caused by 5-HT in the CA1 region of the hippocampus (Beck *et al*., 1985; Klancnik & Phillips, 1991; Sakai & Tanaka, 1993). Accordingly, 5-HT$_{1A}$ agonists (8-OH-DPAT, ipsapirone, buspirone) mimicked the effects of 5-HT on the e.p.s.ps (Rowan & Anwyl, 1987; Hiner *et al*., 1988; Sakai & Tanaka, 1993). However, these early results were obtained with non-selective agonists and antagonists and extracellular recordings. Our results are the first conclusive evidence that activation of 5-HT$_{1A}$ receptors modulate synaptic transmission in this brain region. Indeed, we used WAY 100635 which selectively binds to 5-HT$_{1A}$ receptors, both *in vitro* (Gozlan *et al*., 1995; Forster *et al*., 1995) and *in vivo* (Laporte *et al*., 1994; Fletcher *et al*., 1993), and blocks 5-HT$_{1A}$ receptor-mediated responses in the CA1 region, without affecting pyramidal cell excitability, or the responses due to activation of GABA$_B$ and 5-HT$_4$ receptors (Corradetti *et al*., 1996 and present results).

As previously found by Segal (1980), we observed that the action of 5-HT on e.p.s.ps in control aCSF was small (-15%) in the presence of the GABA$_A$ receptor antagonist, though, 5-HT reduced the amplitude of the e.p.s.p. by 45%. This may be explained by the fact that two opposing factors contribute to the determination of the amplitude of the early e.p.s.ps: glutamatergic excitation and GABAergic inhibition via activation of GABA$_A$ receptors (Dingledine & Gjerstad, 1979). 5-HT decreases not only the excitatory but also the inhibitory neurotransmission that impinges upon pyramidal cells, through, presumably, 5-HT$_{1A}$ receptors (Schmitz *et al*., 1995b). Therefore, the modulatory effect of 5-HT on excitatory neurotransmission is counterbalanced by the reduced inhibitory effect of GABAergic interneurones. This apparently led to an overall larger reduction of the e.p.s.p. amplitude when GABA$_A$ neurotransmission was blocked, than when it was not.

5-HT decreased both the AMPA and NMDA components of the e.p.s.ps. Using extracellular recordings of dendritic potentials, Staubli & Otaky (1994) showed that 5-HT decreases the NMDA component of excitatory neurotransmission during conditioning trains aimed at inducing long term potentiation (LTP). This effect may contribute to the block of LTP exerted by 5-HT in the rat hippocampus (see also: Corradetti *et al*., 1992; Villani & Johnston, 1993). Our results directly demonstrate that 5-HT decreases the NMDA component of e.p.s.ps through a 5-HT$_{1A}$ receptor-mediated action. The fact that both components of e.p.s.ps were affected by 5-HT may suggest that 5-HT decreases glutamate release from presynaptic terminals. However, the reduction of the e.p.s.p. amplitude is more probably due to a postsynaptic mechanism, where the synaptic currents are shunted through the potassium conductance activated by the 5-HT$_{1A}$ receptors. At the moment, the location of the 5-HT$_{1A}$ receptors involved in the modulation of e.p.s.ps remains to be elucidated. Biochemical data suggest that 5-HT$_{1A}$ receptors have a postsynaptic location in the hippocampus (Pompeiano *et al*., 1992; Mengod *et al*., 1996), although Schmitz *et al*. (1995a) observed that the 5-HT$_{1A}$ agonist 8-OH-DPAT decreases e.p.s. currents recorded in CA1 pyramidal cells and suggested that 5-HT exerts its effects partially through presynaptic 5-HT$_{1A}$ receptors. In brain regions other than the hippocampus the release of neurotransmitters is inhibited by presynaptic 5-HT$_{1B/D}$ and 5-HT$_2$ receptors (Maura *et al*., 1991; Tanaka & North, 1993; Maura & Raiteri, 1996). So far, though, there is no direct evidence of heterosynaptic 5-HT$_{1B/D}$ receptors modulating the release of excitatory aminoacids in the hippocampus. In addition, our experiments conducted with CGS 12066A, a 5-HT$_{1B}$ agonist (Neale *et al*., 1987), showed that the inhibitory action of 5-HT on synaptic potentials was not elicited by the interaction with 5-HT$_{1B}$ receptors. This finding is in agreement with results obtained in the CA1 region of the hippocampus, with CP 93129, another 5-HT_{1B} agonist (Dr H.W.G.M. Boddeke, personal communication). Regardless of the location of the effectors, though, our results clearly demonstrate that the effects of 5-HT on e.p.s.ps are exerted through 5-HT_{1A} receptors and that WAY 100635 blocks them. The CA1 region of the hippocampus appears to be involved in several functions of the CNS, including memory processing, anxiety and mood control. 5-HT released by terminals originating in the dorsal raphe nuclei regulates CA1 pyramidal cell discharge through activation of several types of receptors. It is conceivable that at least part of the anxiolytic and antidepressant effects observed with 5-HT_{1A} agonists *in vivo* (reviewed by De Vry, 1995) result from inhibition of CA1 pyramidal cells.

In conclusion, our results demonstrate that WAY 100635 *per se* is devoid of any noticeable effects on e.p.s.ps, while it selectively blocks the decrease in neurotransmission produced by 5-HT. This latter finding, obtained by using the most potent and selective 5-HT_{1A} receptor antagonist currently available, also demonstrates that 5-HT inhibits e.p.s.ps through stimulation of 5-HT_{1A} receptors. WAY 100635 has been recently used in healthy people to delineate the regional distribution of 5-HT_{1A} receptors (Pike *et al.*, 1995; Osman *et al.*, 1996) and the knowledge of the functional consequences of the blockade of 5-HT_{1A} receptors may become necessary in order to interpret these investigations. The lack of aspecific effects of WAY 100635 on synaptic transmission further supports the potential usefulness of this compound in studies on central 5-HT_{1A} receptor location and function *in vivo*.

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