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Antagonist properties of (−)-pindolol and WAY 100635 at somatodendritic and postsynaptic 5-HT_{1A} receptors in the rat brain

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1 The aim of the present work was to characterize the 5-hydroxytryptamine$_{1A}$ (5-HT_{1A}) antagonistic actions of (−)-pindolol and WAY 100635 (N-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-N-(2-pyridinyl) cyclohexane carboxamide). Studies were performed on 5-HT_{1A} receptors located on 5-hydroxytryptaminergic neurones in the dorsal raphe nucleus (DRN) and on pyramidal cells in the CA1 and CA3 regions of the hippocampus in rat brain slices.

2 Intracellular electrophysiological recording of CA1 pyramidal cells and 5-hydroxytryptaminergic DRN neurones showed that the 5-HT_{1A} receptor agonist 5-carboxamidotryptamine (5-CT) evoked in both cell types a concentration-dependent cell membrane hyperpolarization and a decrease in cell input resistance. On its own, (−)-pindolol did not modify the cell membrane potential and resistance at concentrations up to 10 μM, but it antagonized the 5-CT effects in a concentration-dependent manner. Similar antagonism of 5-CT effects was observed in the CA3 hippocampal region. (−)-Pindolol also prevented the 5-HT_{1A} receptor-mediated hyperpolarization of CA1 pyramidal cells due to 5-HT (15 μM). In contrast, the 5-HT-induced depolarization mediated by presumed 5-HT_4 receptors persisted in the presence of 3 μM (−)-pindolol.

3 In the hippocampus, (−)-pindolol completely prevented the hyperpolarization of CA1 pyramidal cells by 100 nM 5-CT (IC_{50} = 92 nM; apparent K_B = 20.1 nM), and of CA3 neurones by 300 nM 5-CT (IC_{50} = 522 nM; apparent K_B = 115.1 nM). The block by (−)-pindolol was surmounted by increasing the concentration of 5-CT, indicating a reversible and competitive antagonistic action.

4 Extracellular recording of the firing rate of 5-hydroxytryptaminergic neurones in the DRN showed that (−)-pindolol blocked, in a concentration-dependent manner, the decrease in firing elicited by 100 nM 5-CT (IC_{50} = 598 nM; apparent K_B = 131.7 nM) or 100 nM ipsapirone (IC_{50} = 132.5 nM; apparent K_B = 124.9 nM). The effect of (−)-pindolol was surmountable by increasing the concentration of the agonist. Intracellular recording experiments showed that 10 μM (−)-pindolol were required to antagonize completely the hyperpolarizing effect of 100 nM 5-CT.

5 *In vivo* labelling of brain 5-HT_{1A} receptors by i.v. administration of [^{3}H]-WAY 100635 ([O-methyl-^{3}H]-N-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl-N-(2-pyridyl)cyclo-hexane-carboxamide) was used to assess their occupancy following *in vivo* treatment with (−)-pindolol. (−)-Pindolol (15 mg kg^{-1}) injected i.p. either subchronically (2 day-treatment before i.v. injection of [^{3}H]-WAY 100635) or acutely (20 min before i.v. injection of [^{3}H]-WAY 100635) markedly reduced [^{3}H]-WAY 100635 accumulation in all 5-HT_{1A} receptor-containing brain areas. In particular, no differences were observed in the capacity of (−)-pindolol to prevent [^{3}H]-WAY 100635 accumulation in the DRN and the CA1 and CA3 hippocampal areas.

6 Intracellular electrophysiological recording of 5-hydroxytryptaminergic DRN neurones showed that WAY 100635 prevented the hyperpolarizing effect of 100 nM 5-CT in a concentration-dependent manner (IC_{50} = 4.9 nM, apparent K_B = 0.25 nM). In CA1 pyramidal cells, hyperpolarization induced by 50 nM 5-CT was also antagonized by WAY 100635 (IC_{50} = 0.80 nM, apparent K_B = 0.28 nM).

Keywords: 5-Hydroxytryptamine; 5-HT_{1A} receptors; (−)-pindolol; antidepressant; dorsal raphe nucleus; hippocampus; hyperpolarization; *in vivo* occupancy

Introduction

The central neurotransmitter 5-hydroxytryptamine (5-HT) is involved in the control of numerous brain functions (for a review, see Frazer & Hensler, 1994), and alterations in the activity of 5-HT-containing neurones have been associated with several neuropsychiatric disorders such as depression, anxiety or dementia. In particular, depression seems to be frequently associated with a decrease in central 5-hydroxytryptaminergic neurotransmission and, conversely, treatments known to increase the 5-hydroxytryptaminergic tone, such as chronic administration of selective 5-HT reuptake inhibitors (SSRIs), have antidepressant properties (De Montigny *et al.*, 1990).

Long-term blockade of 5-HT reuptake by SSRIs such as fluoxetine, citalopram or paroxetine results in the desensitization of somatodendritic 5-HT_{1A} autoreceptors located on 5-hydroxytryptaminergic neurones in the dorsal raphe nucleus (DRN) (Chaput *et al.*, 1986; Blier *et al.*, 1988; Jolas *et al.*, 1994; Le Poul *et al.*, 1995). The principal consequence of this desensitization is a loss of 5-HT_{1A}-mediated inhibitory regulation of cell discharge, leading to a facilitation of 5-hydroxytryptaminergic neurotransmission (see De Montigny *et al.*, 1990). The time lag required for clinical improvement

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during treatment with antidepressants (2–3 weeks) (Montgomery, 1995) overlaps that needed for establishing 5-HT$_{1A}$ autoreceptor desensitization and subsequent changes in 5-hydroxytryptaminergic neurotransmission (Blier *et al*., 1988; De Montigny *et al*., 1990). This suggests that 5-HT$_{1A}$ autoreceptor desensitization might underlie the antidepressant effects of SSRIs (Hjorth & Auerbach, 1995), and that early pharmacological blockade of these receptors might functionally reproduce the desensitization phenomenon, and therefore hasten the clinical improvement of patients.

However, very few selective 5-HT$_{1A}$ receptor antagonists are available to date, and among them, none has been described which selectively blocks somatodendritic 5-HT$_{1A}$ autoreceptors in the DNR, without blocking the other 5-HT$_{1A}$ receptors located on the postsynaptic targets of 5-hydroxytryptaminergic afferents, notably in limbic areas such as the hippocampus (Kia *et al*., 1996). In fact, most of the compounds found to be potent 5-HT$_{1A}$ receptor antagonists have been shown to block preferentially the postsynaptic receptors and to act as partial agonists in the DRN (Glaser & De Vry, 1992; Greuel & Glaser, 1992). The only exception is WAY 100635 (N-(2-(4-(2-methoxyphenyl) - 1 -piperazinyl)ethyl)-N-(2-pyridyl)cyclohexanecarboxamide) which has been demonstrated to act as a potent, selective and full antagonist at all 5-HT$_{1A}$ receptors in relevant animal models (Forster *et al*., 1995; Fletcher *et al*., 1996).

To date no selective 5-HT$_{1A}$ receptor antagonists are available for use in man. However, some β-adrenoceptor antagonists, such as pindolol, have high affinity for 5-HT$_{1A}$ receptors (Hamon *et al*., 1986; Langlois *et al*., 1993) and have been shown to antagonize the presynaptic effects produced by 5-HT$_{1A}$ receptor agonists (Gartside *et al*., 1995; Hjorth & Auerbach, 1995; Romero *et al*., 1996). However, it has also been shown that pindolol is efficient in blocking 5-HT$_{1A}$ receptor-induced responses such as hypothermia (Scott *et al*., 1994), sleep changes (Monti & Jantos, 1994), release of hormones (Levy *et al*., 1995) and discrimination in a standard two-level operant procedure (Ybema *et al*., 1994), which involve postsynaptic receptors.

Clinical studies have been conducted to explore the possibility that concurrent administration of SSRIs and pindolol could reduce the time lag observed in monotherapy with SSRIs alone (Artigas *et al*., 1994; Blier & Bergeron, 1995). These open studies concluded that pindolol hastened the clinical response in depressed patients treated with SSRIs. However, a recent double-blind and placebo controlled trial did not support such a finding (Berman *et al*., 1997). Pindolol efficacy in SSRI-treated patients may be explained through either its 5-hydroxytryptaminergic or β-adrenergic effects. With regard to the 5-hydroxytryptaminergic system, pindolol, for being effective, should act selectively at somato-dendritic 5-HT$_{1A}$ autoreceptors without substantial effects on postsynaptic 5-HT$_{1A}$ receptors. Indeed, activation of the latter receptors is needed for the antidepressant-like action of SSRIs and other drugs in relevant models in rodents (Cesana *et al*., 1995; Detke *et al*., 1995). Recently, electrophysiological data have been obtained in support of the idea that pindolol, and in particular (−)-pindolol, can block the somatodendritic 5-HT$_{1A}$ autoreceptors, without modifying the postsynaptic 5-HT$_{1A}$ response (Artigas *et al*., 1996; Romero *et al*., 1996). Thus (−)-pindolol would represent the first drug able to discriminate between 5-HT$_{1A}$ autoreceptors on DRN-5-hydroxytryptaminergic cells and 5-HT$_{1A}$ receptors on postsynaptic target cells. However, these data are difficult to reconcile with previous results showing that pindolol actually blocks hormonal and behavioural responses to postsynaptic 5-HT$_{1A}$ receptor stimulation (Scott *et al*., 1994; Ybema *et al*., 1994; Levy *et al*., 1995).

In order to assess clearly the respective action of (−)-pindolol at pre- and postsynaptic 5-HT$_{1A}$ receptors, we performed a series of *in vitro* electrophysiological experiments both at the DRN and the hippocampal levels, using similar experimental protocols in both areas. Furthermore, we compared the effects of (−)-pindolol with those induced by WAY 100635 under the same conditions.

Methods

Animals

Experiments were performed on male Sprague Dawley rats (90–100 g body weight, $n=68$, Centre d'Elevage R. Janvier, 53940 Le Genest-St Isle, France). Animals were housed in groups of six and maintained under controlled environmental conditions ($21\pm1^{\circ}$C, 60% relative humidity, 12 h/12 h light/dark cycle, food and water *ad libitum*) for at least 7 days before being used for the experiments. All the procedures involving animals and their care were conducted in accordance with the institutional guidelines that are in compliance with national and international laws and policies (Council directive # 87-848, October 19, 1987, Ministère de l'Agriculture et de la Forêt, Service Vétérinaire de la Santé et de la Protection Animale, permissions # 0299 to M.H. and # 6269 to L.L.).

Electrophysiological experiments

Preparation of slices of dorsal raphe nucleus and hippocampus Rats were killed by decapitation and the brain was rapidly removed and placed in ice-cold oxygenated (95% O_2; 5% CO_2) artificial cerebrospinal fluid (aCSF) of the following composition (mM): NaCl 126, KCl 3.5, NaH_2PO_4 1.2, $MgCl_2$ 1.3, $CaCl_2$ 2, $NaHCO_3$ 25, D-glucose 11 (pH 7.3). A block of tissue containing the dorsal raphe nucleus, or the dorsal hippocampus, was cut into sections (350–400 μm thick) by use of a vibratome, while immersed in ice-cold aCSF. After being sectioned, slices were kept in oxygenated aCSF for at least 1 h at room temperature (20–23°C). A single slice was then placed on a nylon mesh, completely submerged in a small chamber and superfused with oxygenated aCSF at a constant flow of 2–3 ml min^{-1}. The temperature of superfusing aCSF was adjusted to 33°C with maximal up and down fluctuations of 1°C for which no changes in the activity of the cells were observed. Drugs were administered through a three-way tap system and complete exchange of fluid in the chamber occurred in 1 min. Only one neurone was recorded in each superfused slice.

Extracellular recording of dorsal raphe nucleus neurones Extracellular recordings were made with glass microelectrodes filled with 2 M NaCl (12–15 MΩ). Cells were identified as 5-hydroxytryptaminergic neurones according to the following criteria: biphasic action potentials of 2–3 ms duration, slow (0.5–2 Hz) and regular pattern of discharge. Firing was evoked in the otherwise silent neurones by adding the α_1-adrenoceptor agonist phenylephrine (3 μM) to the superfusing aCSF (VanderMaelen & Aghajanian, 1983). Baseline activity was recorded for at least 10 min before application of the different drugs. The electrical signals were fed into a high-input impedance amplifier (VF 180, BioLogic, France), an oscilloscope and an electronic ratemeter triggered by individual action potentials connected to an A/D converter and a personal computer (Haj-Dahmane *et al*., 1991). By using dedicated software, the integrated firing rate was recorded, computed and displayed on a chart recorder as consecutive

10 s samples. The effects of agonists were evaluated by comparing the mean discharge frequency recorded during 2 min immediately before the drug application with that recorded at the peak of drug action (usually 2–5 min after the beginning of application). When an agonist was applied in the presence of an antagonist, the effect of the agonist was compared with the baseline firing rate and with the discharge frequency recorded during superfusion with the antagonist alone. Antagonists were left to equilibrate for at least 25 min before the effects of the agonists were retested.

Intracellular recordings Neurones were recorded in current-clamp mode with 3 M KCl (35–60 MΩ)-filled electrodes, while brain slices were superfused with aCSF (Corradetti *et al*., 1996). Tetrodotoxin (TTX, 1 μM) was added to the superfusing medium in experiments with hippocampal slices, in order to prevent action potentials possibly generated through interneuronal connections in CA1 and CA3 neurones. In contrast, no TTX was added in experiments with DRN slices so as to preserve the regularity of the discharge of the recorded 5-hydroxytryptaminergic neurones, which allows their identification (see VanderMaelen & Aghajanian, 1983). Electrical signals were amplified with an Axoclamp 2A (Axon Instruments, Foster City, CA, U.S.A.) and displayed on an oscilloscope and a chart recorder. Traces were stored on a digital tape recorder (DTR 1202, Biologic, France, 48 kHz sampling frequency) and in a computer by use of pClamp6 software (3–10 kHz sampling frequency, Axon Instruments) for off-line measurements. Only neurones with stable resting membrane potential (r.m.p.; range −58/−73 mV) and input resistance (R_{in}; range 35–90 MΩ for CA1/CA3 neurones and 120–600 MΩ for DRN cells) throughout the recording session were included in the analysis. When cells appeared to have reached a stable membrane potential, pulses of hyperpolarizing current (200–400 pA, 400 ms, 0.1 Hz) were delivered through the recording electrode to monitor changes in R_{in} during drug application. Alternatively, current-voltage relationships were obtained by measuring the voltage changes in response to hyperpolarizing and depolarizing current pulses of 50–100 pA increments (range −900 to +500 pA), before, during and after the addition of drugs into the superfusing aCSF. During drug application, the cell membrane potential was occasionally restored to the control r.m.p. by injecting a steady current through the recording electrode.

Concentration-response curves and data analysis To generate data for 5-CT concentration-response curves, the membrane potential was recorded while slices were superfused with increasing concentrations of the agonist. Preliminary experiments (not shown) demonstrated that, in a given cell, successive applications of increasing concentrations of 5-CT produced cumulative concentration-response curves, with a maximal response equal to that obtained by application of a single maximal concentration. The magnitude of hyperpolarization, or of agonist-induced change in cell input resistance, was fitted to a hyperbolic function (equation 1): $E = E_{max}/[1 + (EC_{50}/[A])^n]$, where E is the response (ΔV_m) produced by the agonist A at the concentration [A], E_{max} is the maximal response of the cell, and n is the slope index. Non linear regression fitting was carried out with Prism 2.0 (GraphPad) software facilities. To determine the IC_{50} value of the antagonist for a given concentration of agonist, the effect of the agonist was tested in control aCSF and in the presence of various concentrations of the antagonist, gradually increased by using a cumulative protocol. For each slice, a concentration of agonist able to elicit a response of 85–90% of the maximum was chosen to obtain the largest, submaximal control response. This allowed reliable quantification of hyperpolarization in the absence and the presence of several concentrations of antagonist on the same cell. The agonist was superfused until the hyperpolarizing response reached a steady state (typically for 3–5 min). The magnitude of the hyperpolarization obtained in the presence of each concentration of antagonist, expressed as a percentage of that obtained with the agonist alone, was fitted to a logistic function. The K_B value of the antagonist was estimated by use of a general form of the Cheng-Prusoff equation which applies to functional assays (Leff & Dougall, 1993) (equation 2): $K_B = IC_{50}/\{[\![2 + ([A]/EC_{50})^n]\!]^{1/n} - 1\}$ where IC_{50} is the concentration of the antagonist which reduces by half the response to a fixed concentration of the agonist ([A]), EC_{50} is the concentration of the agonist which evokes half of the maximal response in the preparation under study, and n is the slope factor of the concentration-response curve of the agonist. This equation assumes that the antagonism is competitive and that the concentration-response curve of the agonist is adequately described by the logistic function (see equation 1) from which EC_{50} and n are derived. Since WAY 100635 has been proved to act as a 'pseudoirreversible' (see Rang, 1966) antagonist, at least in the CA1 region of the hippocampus (Corradetti *et al*., 1996), the first assumption holds only for (−)-pindolol and we used this equation to estimate its K_B value. EC_{50} and n values of 5-CT in the CA3 region of the hippocampus were taken from Beck *et al*. (1992), whereas corresponding values in the CA1 region and the dorsal raphe nucleus were determined by constructing concentration-response curves (see Results), or from previously published results obtained in similar experiments (Corradetti *et al*., 1996).

In order to analyse the nature of WAY 100635 antagonism and to calculate the apparent K_B value of this compound, concentration-response curves were constructed in experiments with control aCSF and in the presence of the antagonist. Double reciprocal plots of equiactive concentrations of agonist in the presence of antagonist were based on values extracted from the regression curves (Kenakin, 1993; Corradetti *et al*., 1996).

The double reciprocal plot of equiactive concentrations of agonist is based on the assumption that, in a given preparation, equal responses rely on equal fractional occupancy. The fractional occupancy of receptors in the absence of antagonist is given by the mass law equation (equation 3) $[AR]/R_t = [A]/([A] + K_A)$, where AR is the concentration of agonist/receptor complex at agonist concentration [A], R_t is the total number of receptors R and K_A is the affinity constant of the agonist. In the presence of a non-competitive antagonist, this equation can be converted to equation 4: $[AR]/R_t = ([A']/([A'] + K_A))\ (1\text{-}\rho_\beta)$, where ρ_β is the fraction of receptors blocked by the antagonist. With equiactive concentrations of agonist in the absence ([A]) and in the presence ([A']) of antagonist, equations 3 and 4 lead to the equation 5: $[A]/([A] + K_A) = ([A']/([A'] + K_A))\ (1\text{-}\rho_\beta)$ which can be rearranged to equation 6: $1/[A] = [(1/[A'])(1/(1\text{-}\rho_\beta))] + \rho_\beta/(1\text{-}\rho_\beta)K_A$. Regression of 1/[A] versus 1/[A'] yields a straight line of slope $1/(1\text{-}\rho_\beta)$ and intercept $\rho_\beta/(1\text{-}\rho_\beta)K_A$). Then, by relating ρ_β to the K_B value expressed by the equation $\rho_\beta = [B]/([B] + K_B)$, the K_B value of a non-competitive antagonist B can be calculated from equation 7: $K_B = [B]/(\text{slope} - 1)$.

In vivo *binding studies with [^{3}H]-WAY 100635*

Treatments Each rat was gently introduced and maintained in a plexiglas cylinder, with the tail protruding. The tail was dipped in water at 45°C for ~5 s in order to induce vasodilatation, and 250 μl of a [^{3}H]-WAY 100635 solution in

0.154 M NaCl (17 μCi/rat) was slowly injected over 5 s into a caudal vein. Animals were then returned to individual cages until their death (Laporte *et al*., 1994).

Pretreatments with (−)-pindolol (15 mg kg^{-1}) were performed via the intraperitoneal route in a volume of 0.5 ml 100 g^{-1} body weight. (−)-Pindolol was dissolved in distilled water acidified with a few drops of 2 M HCl and injected only once 20 min before [^{3}H]-WAY 100635 (acute treatment), or on three occasions, i.e., 24 h, 16 h and 20 min before the i.v. injection of the tritiated ligand (short-term treatment).

Measurement of ^{3}H accumulation in tissues Rats ($n=15$) were killed by decapitation one hour after the i.v. injection of [^{3}H]-WAY 100635. Blood was collected from trunk vessels in glass tubes containing EDTA, and the brain was removed. Dissection was performed immediately on an ice-cold plate following the method of Glowinski & Iversen (1966). Brain structures (cerebellum, raphe area, brain stem, septum, hippocampus, striatum, anterior and posterior cerebral cortex), and blood were homogenized in 10–20 vol (v/w) of distilled water, and aliquots (50–100 μl) were mixed with 4 ml of Aquasol scintillation liquid (New England Nuclear, Boston, MA, U.S.A.) for radioactivity counting.

Autoradiography Rats ($n=9$) were killed by decapitation one hour after the i.v. injection of [^{3}H]-WAY 100635, and blood was collected from trunk vessels for counting the radioactivity as above. The brains were immediately removed and frozen at −40°C with isopentane cooled by dry ice. Coronal sections (20 μm) were cut at −15°C in a cryostat, thaw mounted onto gelatin-coated glass slides and dried at 4°C for 30 min on silica gel. Slides were then put in tight contact with a sheet of ^{3}H-Hyperfilm (Amersham), and exposed in the dark at 4°C for one month. Autoradiograms were developed in Kodak Microdol (10 min at 20°C). Optical densities were measured (with a Biocom image analyser) in selected areas of the autoradiographic pictures of brain sections and converted to fmol [^{3}H]-WAY 100635 equivalents mg^{-1} fresh tissue with reference to tritium standards (Amersham).

Statistical calculations

Data were analysed statistically by one-way analysis of variance and, in the case of significance ($P<0.05$), Fisher's test for significant treatment effects was followed by Student's *t* test to compare the treated groups with their respective controls (Snedecor & Cochran, 1967).

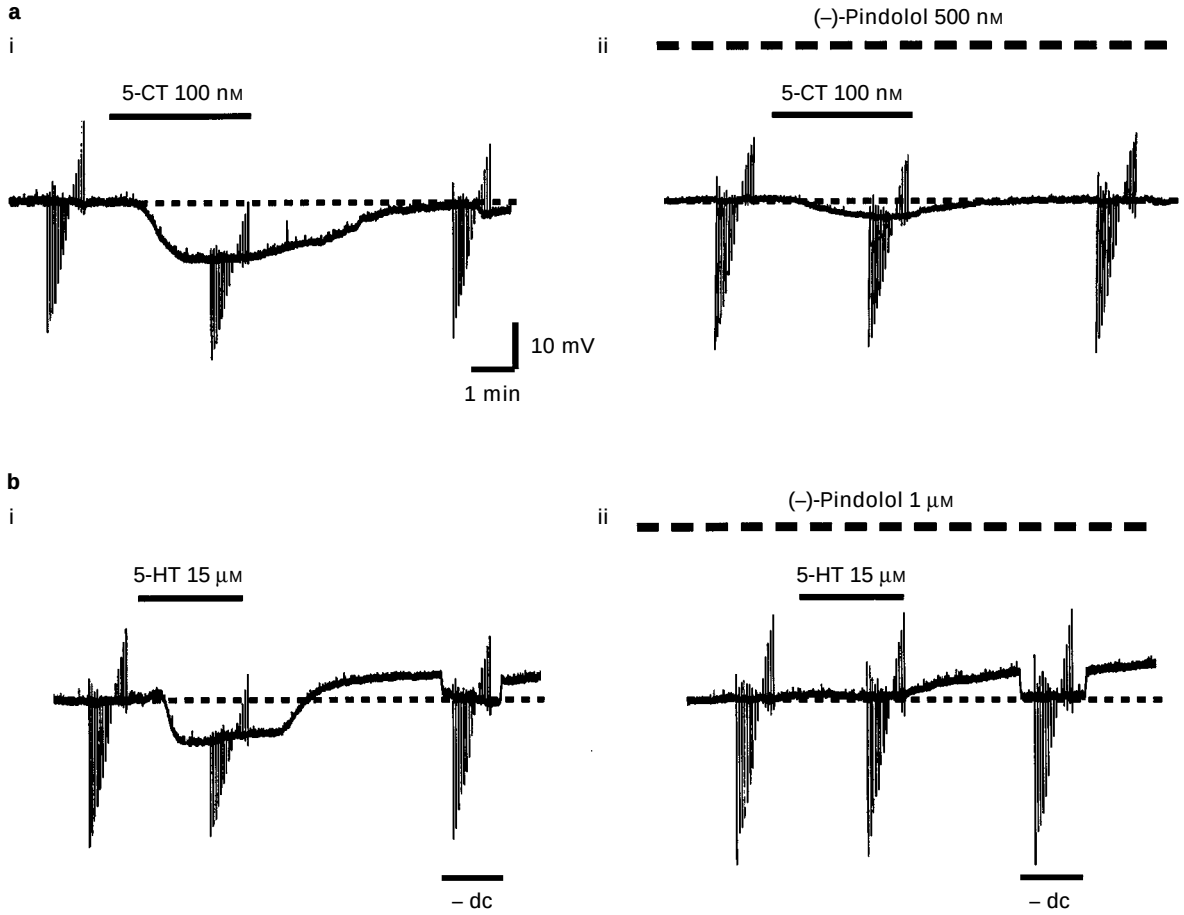

Figure 1 (−)-Pindolol antagonized 5-HT$_{1A}$ receptor-mediated effects of 5-HT and 5-CT on CA1 pyramidal neurones in the hippocampus. (a) Chart recordings of membrane potential of a CA1 pyramidal neurone intracellularly recorded in the presence of tetrodotoxin (1 μM). (i) Application of 5-CT (100 nM, bar) hyperpolarized the cell relative to resting membrane potential (dotted line). Downward and upward rapid deflections shown in this trace, and in the following ones, are electrotonic cell membrane responses to constant current steps (−900 to +500 pA) injected through the recording electrode to construct *I*/V relationships and to monitor R_{in}. (ii) The hyperpolarization and the decrease in R_{in} elicited by 5-CT were antagonized by 500 nM (−)-pindolol (broken line). (bi) In the same cell, 5-HT (15 μM) evoked a rapid hyperpolarization and a slowly developing depolarization, fully revealed upon wash from 5-HT. After a 5 min wash, a steady negative current (−150 pA; −dc) was injected through the electrode to clamp manually the cell to the original V_m. (ii) When a similar protocol was applied in the presence of 1 μM (−)-pindolol, 5-HT elicited only a depolarization. At the same concentration, (−)-pindolol fully prevented the hyperpolarizing effect of 5-CT (not shown). Calibrations in (a) apply to all traces. KCl-filled electrode; r.m.p.: −59 mV, R_{in}: 40 MΩ.

Chemicals

[O-methyl-^{3}H]-WAY 100635 (80 Ci mmol^{-1}) was synthesized by Amersham International (Buckinghamshire, U.K.) for Wyeth-Ayerst Res. Labs (Princeton, NJ, U.S.A.) and generously provided by Dr Lee E. Schechter (Wyeth-Ayerst Research, U.S.A.).

5-Hydroxytryptamine (5-HT) creatinine sulphate or hydrochloride, 5-carboxamidotryptamine (5-CT) maleate, phenylephrine hydrochloride, **R**(+)-baclofen hydrochloride, were from Research Biomedical Inc. (Wayland, U.S.A.); tetrodotoxin was from Sigma; ipsapirone was from Troponwerke (Cologne, Germany) and WAY 100635 was from Wyeth-Ayerst Res. Labs (Princeton, NJ, U.S.A.).

Results

(−)-Pindolol as a 5-HT$_{1A}$ receptor antagonist

Effects of (−)-pindolol on 5-HT$_{1A}$ receptor-mediated responses in hippocampal pyramidal neurones In the first series of experiments, the effects of increasing concentrations of (−)-pindolol were tested on the membrane hyperpolarization produced by application of either 5-CT or 5-HT on CA1 pyramidal cells. These neurones had a resting membrane potential (r.m.p.) of −63.1±2.1 mV and a membrane input resistance (R_{in}) of 62.7±8.8 MΩ (means±s.e.mean, $n=10$ neurones, only one neurone recorded per slice). As shown in Figure 1, (−)-pindolol antagonized the hyperpolarizing effect of both 5-CT (100 nM) and 5-HT (15 μM). When 5-HT was tested ($n=3$) in the presence of (−)-pindolol, a slow depolarization replaced the hyperpolarizing response (Figure 1b). This effect was probably due to stimulation of 5-HT$_4$ receptors, causing a decrease in Ca^{2+}-dependent potassium conductance associated with an increase in R_{in} (Andrade & Chaput, 1991). Thus, the use of 5-HT was not practical for studying the effects of (−)-pindolol on 5-HT$_{1A}$ receptors. For this reason, most of the following experiments were performed with 5-CT, a 5-HT$_{1A}$ receptor agonist that is devoid of 5-HT$_4$ agonist actions at the concentrations used in our study (Beck, 1989). 5-CT (100 nM) elicited a hyperpolarization of 8.4±2.0 mV and a decrease in R_{in} of 47.6±7.3% (means ±s.e.mean, $n=7$ neurones, one neurone recorded per slice). (−)-Pindolol *per se* did not affect either the membrane potential or the R_{in} of recorded cells at concentrations up to 3 μM. In contrast, (−)-pindolol blocked the cell membrane hyperpolarization elicited by 100 nM 5-CT in a concentration-dependent manner, with an IC_{50} value of 92 nM (95% confidence interval: 50.6–166.2 nM; Figure 2). The apparent K_B value of (−)-pindolol calculated with the generalized Cheng-Prusoff equation (see Methods) from these experiments was of 20.1 nM (95% confidence interval: 11.0–36.2 nM). At concentrations up to 3 μM, (−)-pindolol did not affect the hyperpolarizing response (−12.5 mV) to **R**(+)-baclofen (10 μM, $n=2$; not shown), a selective $GABA_B$ receptor agonist (Newberry & Nicoll, 1985).

In CA3 pyramidal cells (r.m.p.: −66.8±1.2 mV; R_{in}: 70.0±8.5 mΩ; means±s.e.mean, $n=5$ neurones, one neurone recorded per slice), 5-CT (300 nM) elicited a hyperpolarization of 13.3±1.2 mV and a decrease in R_{in} of 35.1±4.0% (means±s.e.mean, $n=6$ neurones recorded in 6 slices). (−)-Pindolol did not affect either the membrane potential or the R_{in} of CA3 pyramidal cells at concentrations up to 3 μM, whereas it antagonized the hyperpolarizing response to 5-CT. Figure 3 illustrates a typical experiment where bath application of 300 nM 5-CT induced a hyperpolarization and a reduction of R_{in} of the recorded cell. In the presence of increasing concentrations (100 nM–3 μM, 30 min application) of (−)-pindolol, the 5-CT-induced hyperpolarization and decrease in R_{in} progressively disappeared in a concentration-dependent manner. The antagonism due to 3 μM (−)-pindolol of the hyperpolarization evoked by 5-CT could be surmounted by increasing the concentration of the latter agonist to 3 μM (Figure 3f). The IC_{50} value of (−)-pindolol calculated from this series of experiments was of 522 nM (95% confidence interval: 399–700; Figure 2), and its apparent K_B value was 115.1 nM (95% confidence interval: 85.8–154.4 nM).

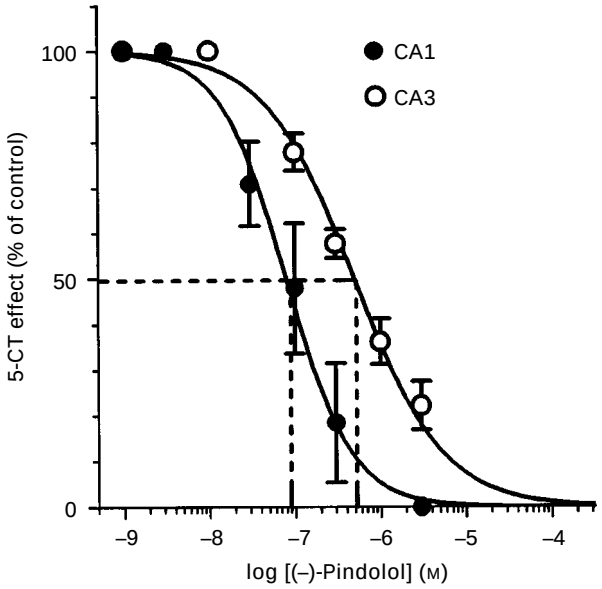

Figure 2 Concentration-response curves for the antagonism by (−)-pindolol of hyperpolarizing responses evoked by 5-CT, in the CA1 and CA3 regions of the rat hippocampus. Data are expressed as % of the response in the presence of 5-CT alone. Each point is the means of data obtained in 4–7 cells for each concentration of (−)-pindolol; vertical lines show s.e.mean. When not shown, the s.e.mean was smaller than the symbol. The curves show fittings of the experimental data with non-linear regression. The dotted lines indicate the IC_{50} values of (−)-pindolol derived from experiments in the CA1 or the CA3 area.

Effects of (−)-pindolol on 5-HT$_{1A}$ receptor-mediated responses in 5-hydroxytryptaminergic neurones of the dorsal raphe nucleus To compensate for the loss of noradrenergic tonus due to deafferentation in slices, the firing of 5-hydroxytryptaminergic neurones was evoked by continuous superfusion with the α_1-adrenoceptor agonist phenylephrine at 3 μM (see Methods). Under these conditions, 5-HT-containing neurones can be identified due to their regular discharge at a frequency of 0.5–2 Hz, and stable recording of firing rate can be obtained for up to 2 h (see Haj-Dahmane *et al.*, 1991).

In the first series of extracellular recordings, we estimated the EC_{50} values of two 5-HT$_{1A}$ receptor agonists, ipsapirone and 5-CT. Ipsapirone (10–300 nM, 3 min application) elicited a concentration-dependent inhibition of cell firing with an EC_{50} value of 66.7 nM (95% confidence interval: 55.3–80.4 nM; $n=7$ neurones, one neurone recorded per slice). Complete blockade of firing was observed in the presence of 300 nM ipsapirone in all cells examined (see Figure 4a). Application of 5-CT (3–150 nM) also elicited a concentration-dependent inhibition of cell firing with an EC_{50} value of 18.3 nM (95% confidence interval: 17.4–19.2 nM; $n=7$

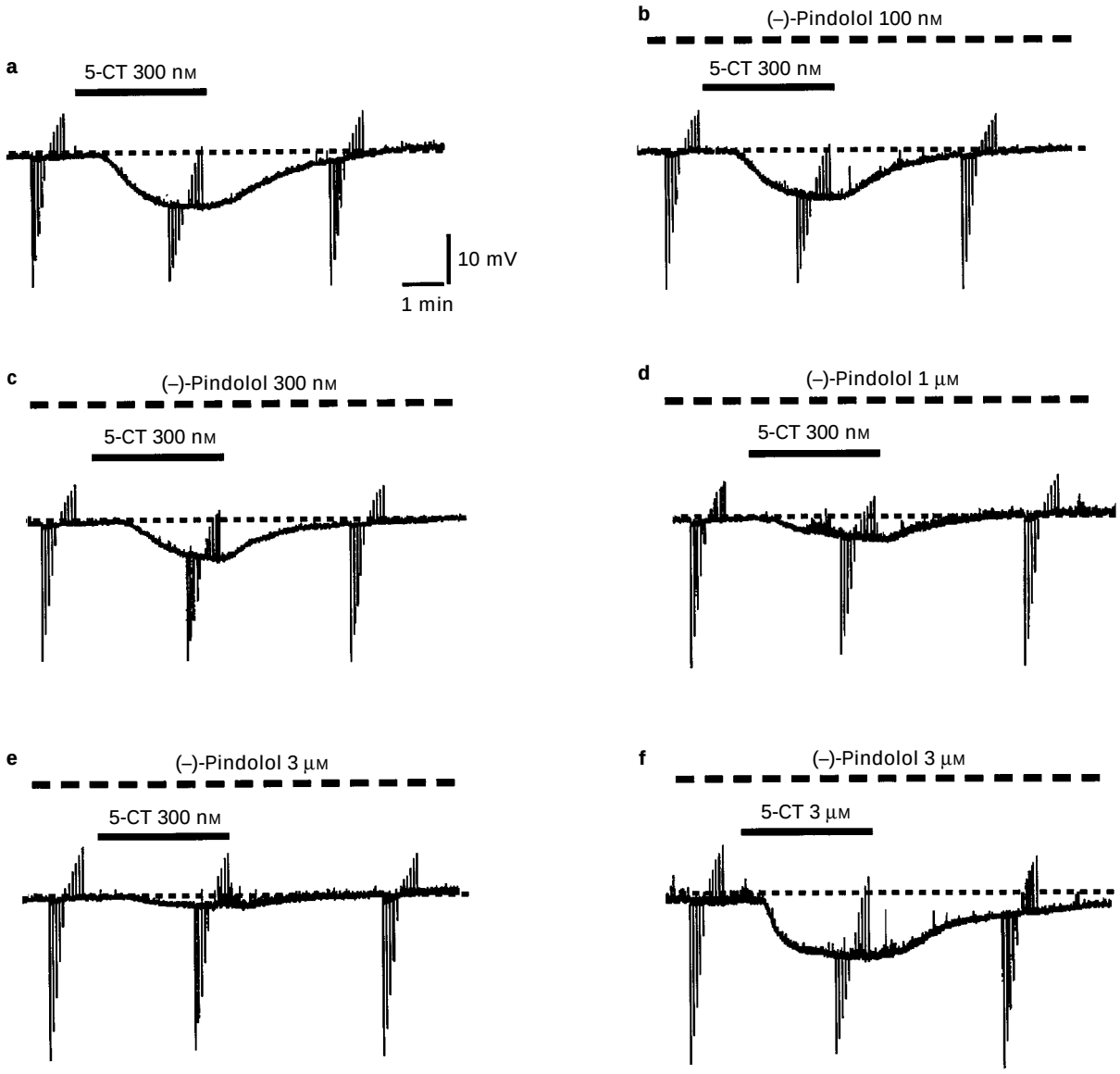

Figure 3 Concentration-dependent and surmountable antagonism by (−)-pindolol of 5-CT-induced hyperpolarization of pyramidal cells in the hippocampal CA3 area. Chart recordings of membrane potential of a CA3 pyramidal cell intracellularly recorded in the presence of tetrodotoxin (1 μM). (a) Application of 300 nM 5-CT (bar) hyperpolarized the cell relative to r.m.p. (dotted line) and decreased the R_{in}. Increasing concentrations of (−)-pindolol (100 nM – 3 μM, broken lines: traces b – e) antagonized the response to 5-CT in a concentration-dependent manner. (f) The antagonism exerted by 3 μM (−)-pindolol was surmounted by 3 μM 5-CT. Downward and upward rapid deflections shown in all traces are electrotonic cell membrane responses to constant current steps (−500 to +500 pA) injected through the recording electrode to construct I/V relationships and to monitor R_{in}. Calibrations in (a) apply to all traces. KCl-filled electrode; r.m.p.: −70 mV, R_{in}: 55 MΩ.

neurones, one neurone recorded per slice). Complete blockade of discharge was obtained with 100 nM 5-CT in all cells examined (see Figure 4c).

Figure 4a shows the effects of a fully effective concentration of ipsapirone on the firing of a presumed 5-hydroxytryptaminergic neurone in the DRN. Ipsapirone (300 nM for 3 min) reversibly blocked the cell discharge. Superfusion with (−)-pindolol (30 nM – 1 μM) antagonized the action of ipsapirone in a concentration-dependent manner. This effect of (−)-pindolol was surmountable as illustrated by the experiment shown in Figure 4b where the effects of increasing concentrations of ipsapirone (100 nM – 1 μM) were tested in the presence of 1 μM (−)-pindolol. Under these conditions, ipsapirone up to 300 nM (see also Figure 4a) hardly affected the cell discharge. However, application of a higher concentration of ipsapirone, 1 μM, resulted in a marked decrease in firing rate. Similarly, (−)-pindolol antagonized the inhibitory effect of 100 nM 5-CT in a concentration-dependent manner (Figure 4c).

Figure 5 shows the inhibition curves resulting from non-linear regression analysis of data obtained in experiments similar to those illustrated in Figure 4. The IC_{50} value of (−)-pindolol calculated from this series of experiments with ipsapirone (100 nM) was of 132.5 nM (95% confidence interval: 104.6 – 167.7 nM). Calculations from experiments with 100 nM 5-CT gave an IC_{50} value of 597.9 nM (95% confidence interval: 549.2 – 650.1 nM) for (−)-pindolol. The apparent K_B value of the latter antagonist derived from these data was of 124.9 nM (95% confidence interval: 102.3 – 150 nM) and 131.7 nM (95% confidence interval: 124.3 – 139.4 nM) from experiments with ipsapirone and 5-CT, respectively.

Intracellular recording of 5-hydroxytryptaminergic neurones (r.m.p.: −63.3 ± 2.0 mV; R_{in}: 320 ± 33 MΩ; means ± s.e.mean, n = 15 neurones recorded in 15 slices) showed that 5-

CT (1 nM – 1 μM) caused a concentration-dependent hyperpolarization of cell membrane, with a maximal response of -16.7 ± 0.3 mV, associated with a decrease in R_{in} ($-50.7 \pm 4.6\%$; means $\pm$ s.e.mean, $n = 10$ neurones, one neurone recorded per slice). The EC_{50} value of 5-CT and the slope of the concentration-response curve were equal to 14.2 nM

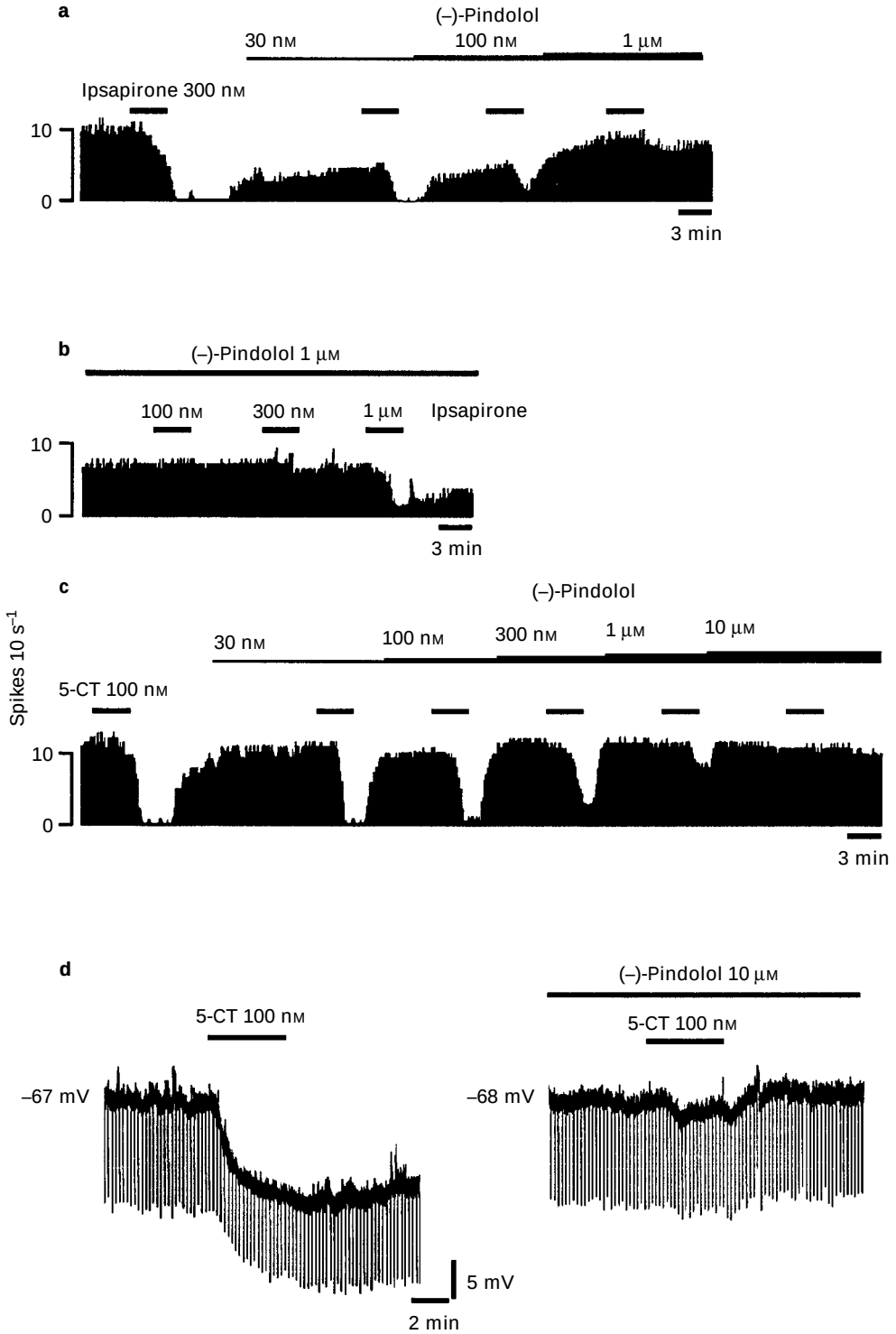

Figure 4 (–)-Pindolol antagonized the decrease in firing rate and the hyperpolarization evoked by 5-HT$_{1A}$ receptor agonists on 5-hydroxytryptaminergic cells in the dorsal raphe nucleus. (a) Superfusion with increasing concentrations of (–)-pindolol (30 nM – 1 μM, staircase line) prevented, in a concentration-dependent manner, the reduction of the firing rate produced by 300 nM ipsapirone (bars). (b) In a different cell, the antagonism by (–)-pindolol (1 μM) was surmounted by increasing the concentration of ipsapirone. (c) Concentration-dependent antagonism by (–)-pindolol (30 nM – 10 μM, staircase bar) of the decrease in firing rate elicited by 100 nM 5-CT (bars). (d) Intracellular recordings of the membrane potential in a 5-hydroxytryptaminergic neurone in the DRN. Left trace: superfusion of 5-CT (100 nM) caused a hyperpolarization, accompanied by a decrease in R_{in}. Both effects were reversible after prolonged wash (15 min, not shown). Right trace: in the presence of 10 μM (–)-pindolol (30 min), the effects of 5-CT were prevented. Downward deflections are electrotonic cell membrane responses to constant current pulses (–100 nA, 400 ms) injected through the recording electrode to monitor R_{in}. The r.m.p. of the recorded cell is indicated on the left of each trace. R_{in}: 155 MΩ; KCl-filled electrode.

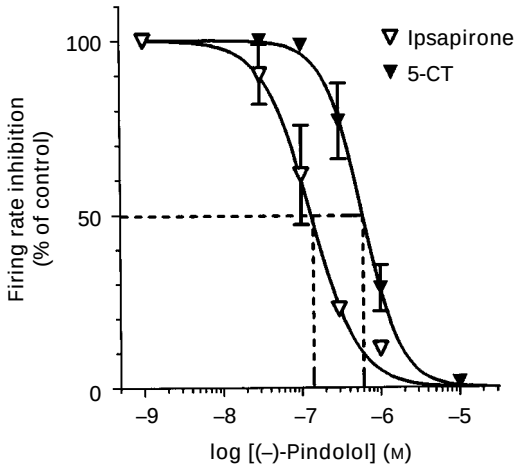

Figure 5 Concentration-response curves for the antagonism by (−)-pindolol of the inhibitory effects of 5-CT and ipsapirone on the firing of DRN 5-hydroxytryptaminergic neurones. Data are expressed as % of the responses to 100 nM ipsapirone (100% on the ordinate scale corresponds to a 86±8% decrease in cell firing) or to 100 nM 5-CT (100% is for 92±5% decrease in cell firing) in the absence of (−)-pindolol. Each point is the mean of data obtained in 5–6 cells for each concentration; vertical lines show s.e.mean. When not shown, the s.e.mean was smaller than the symbol. The curves show fittings of the experimental data with non-linear regression. The dotted lines indicate the IC_{50} values of (−)-pindolol (abscissa scale) against ipsapirone- or 5-CT-induced effects.

(95% confidence interval: 11.5–17.4 nM) and 1.1±0.1 (mean±s.e.mean, $n=10$), respectively. This EC_{50} value is close to that derived from extracellular recording experiments (see above).

In four 5-hydroxytryptaminergic neurones of the DRN from which intracellular recordings were obtained, we tested the effects of increasing concentrations of (−)-pindolol on the response to 100 nM 5-CT. The concentration-dependent effect of the antagonist was similar to that observed in extracellular recording experiments. As shown in Figure 4d, (−)-pindolol prevented the hyperpolarization due to 5-CT.

In vivo *binding of (−)-pindolol to 5-HT$_{1A}$ receptors in the rat brain* Previous studies (Laporte *et al*., 1994) have shown that the ratio of total ^{3}H accumulation in 5-HT$_{1A}$ receptor rich areas such as the hippocampus and the septum over non-specific ^{3}H accumulation in the cerebellum was maximal one hour after i.v. injection of [^{3}H]-WAY 100635 in control animals. Indeed, this ratio was equal to 13.7 and 10.9, respectively, in the present series of experiments (Table 1). In addition, the anterior raphe area, which comprises the median and dorsal raphe nuclei (Figure 6) and the cerebral cortex, especially the entorhinal cortex (Figure 6), also accumulated relatively high levels of ^{3}H in rats injected with [^{3}H]-WAY 100635 (Table 1). Only low to moderate levels of ^{3}H were found in the cerebellum, the striatum and the brain stem (Table 1).

The autoradiograms in Figure 6 showed that (−)-pindolol administered either acutely or on three occasions within 24 h (short-term treatment) markedly reduced ^{3}H accumulation throughout the brain of [^{3}H]-WAY 100635 injected rats. Direct measurement of ^{3}H in tissue homogenates indicated that pretreatment with a single 15 mg kg^{-1} dose i.p. of (−)-pindolol resulted in a ~70% reduction in ^{3}H accumulation in all brain areas except the cerebellum and the striatum (Table 1). After repeated short-term administration of the same dose of (−)-pindolol, the reduction in ^{3}H accumulation was as pronounced as that noted after acute (−)-pindolol treatment in the brain stem, and the anterior and posterior cortex, but it was significantly larger in the anterior raphe area, the septum and the hippocampus. Thus, assuming that ^{3}H accumulation in the cerebellum was non-specific (Laporte *et al*., 1994), it could be estimated that acute (−)-pindolol administration reduced by 73–82% the specific labelling in the anterior raphe area, the septum and the hippocampus, and that this percentage reached 91–94% after short-term treatment with this drug (Table 1).

Table 1 Effects of acute or short-term treatment with (−)-pindolol on the regional distribution of ^{3}H in rats injected with [^{3}H]-WAY 100635 (i.v.)

	^3H (d.p.m. mg^{-1} tissue)		
Structure	*Controls*	*Acute treatment*	*Short-term treatment*
Cerebellum	37.6±1.6	40.2±2.6	57.0±4.0
Striatum	61.3±8.3	49.7±2.5	64.0±3.4
Brain stem	187.7±11.1	62.0±3.0*	65.7±2.1*
Anterior cortex	250.8±17.4	80.7±7.7*	74.4±1.0*
Posterior cortex	272.1±11.6	82.2±6.4*	80.0±2.7*
Anterior raphe area	329.3±29.9	92.1±5.9* (−82%)	73.2±4.7*† (−94%)
Septum	410.8±28.1	127.1±15.1* (−77%)	78.9±1.2*† (−94%)
Hippocampus	515.4±32.7	167.6±17.4* (−73%)	98.5±3.0* (−91%)

Acute or short-term treatment with (−)-pindolol (15 mg kg^{-1}, i.p.) was as described in Methods. [^{3}H]-WAY 100635 (17 μCi 0.25 ml^{-1}) was injected i.v. 20 min after the last injection of (−)-pindolol or vehicle (Controls), and rats were killed 60 min later. Measurement of ^{3}H in blood yielded similar values in the thre groups of rats (3798±306 d.p.m. 0.1 ml^{-1}, mean±s.e.mean for all rats, $n=15$). Eight brain areas were dissected and their ^{3}H content is expressed as d.p.m. mg^{-1} tissue. Each value is the mean±s.e.mean of 5 independent determinations (in 5 rats). The percentages in parentheses are the reductions in ^{3}H tissue accumulation as compared to control values, when ^{3}H contents in cerebellum (non-specific accumulation) were subtracted from total ^{3}H contents in each treated group. *$P<0.05$ as compared with control rats; †$P<0.05$ as compared with rats treated only once with (−)-pindolol (acute treatment).

Further quantitative assessments of the inhibitory effects of *in vivo* treatment with (−)-pindolol on ^{3}H accumulation in the brain of [^{3}H]-WAY 100635 injected rats were made on autoradiographic films. Measurements limited to well defined subareas in the regions that were considered in their entirety in the previous quantitative study confirmed that acute treatment with (−)-pindolol reduced ^{3}H accumulation to similar extents in CA1 and CA3 hippocampal layers, layer IV of the cerebral (parietal) cortex and dorsal raphe nucleus (Table 2). Furthermore, in all the latter structures, short-term administration of (−)-pindolol produced a larger inhibition of ^{3}H accumulation than acute treatment with this drug (Table 2).

WAY 100635 as a 5-HT$_{1A}$ receptor antagonist

Effects of WAY 100635 on raphe 5-hydroxytryptaminergic and hippocampal CA1 pyramidal neurones In a second series of intracellular recording experiments, we tested the action of WAY 100635 on the hyperpolarizing response to 5-CT of 5-hydroxytryptaminergic neurones in the DRN and of CA1 pyramidal cells in the hippocampus.

As already mentioned, superfusion of 5-CT onto 5-hydroxytryptaminergic neurones in the DRN caused hyperpolarization of the cell membrane which, in regularly firing cells,

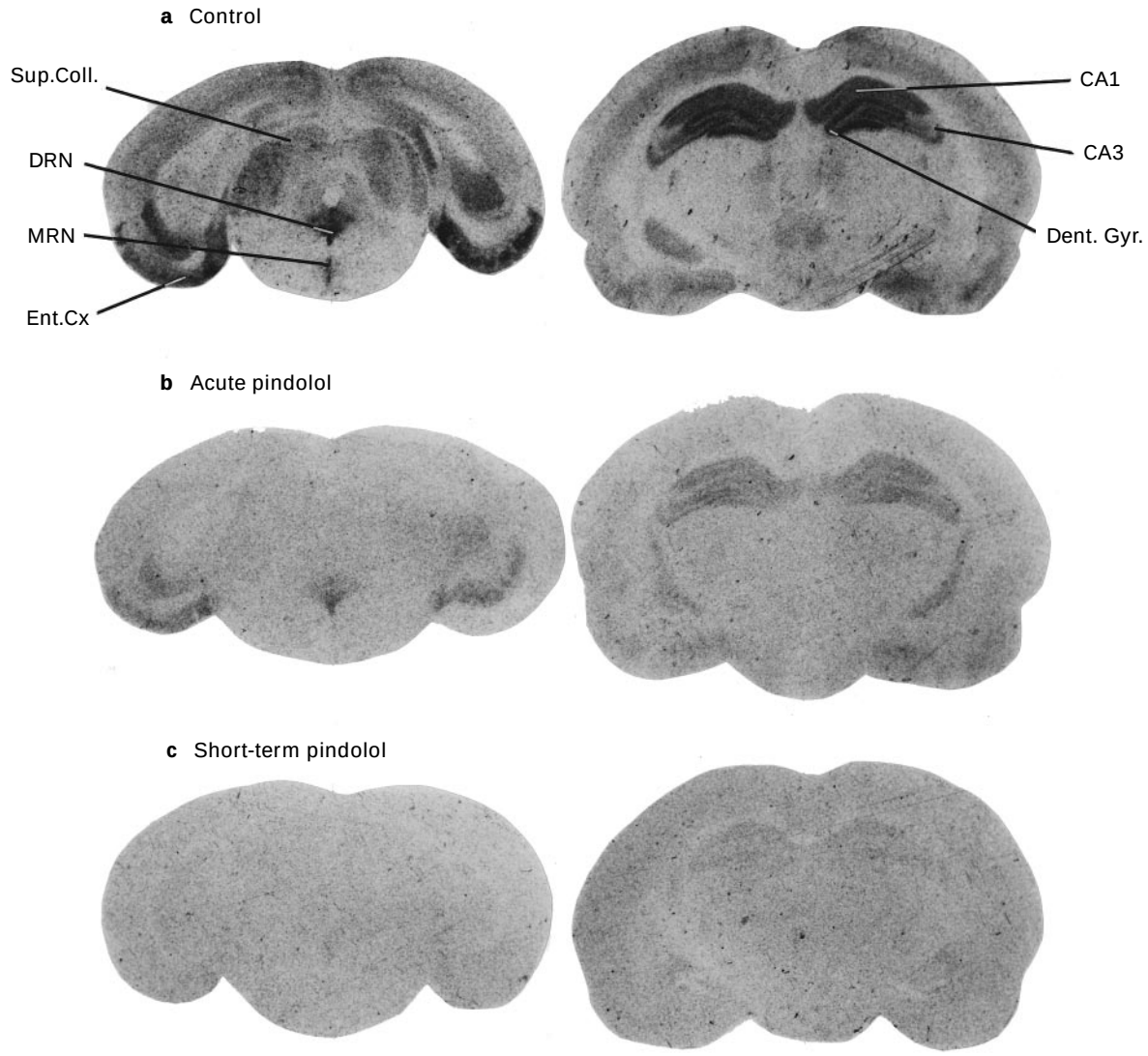

Figure 6 Autoradiograms of brain coronal sections, at the level of the dorsal raphe nucleus (left) or the dorsal hippocampus (right), from rats pretreated with (a) vehicle (control), or (b) one (acute treatment) or (c) three (short-term treatment) doses of (−)-pindolol, before [^{3}H]-WAY 100635 i.v. injection. (−)-Pindolol (15 mg kg, i.p.) was administered 20 min (acute treatment), or 24 h, 16 h and 20 min (short-term treatment) before [^{3}H]-WAY 100635 (17 μCi in 0.25 ml of 0.9% NaCl, i.v.) and rats were killed 1 h after the last injection. Brain sections were exposed to ^{3}H-hyperfilms for one month. Sup.coll.: superior colliculi; DRN: dorsal raphe nucleus; MRN: median raphe nucleus; Ent.Cx: entorhinal cortex; CA1 and CA3: specific areas of Ammon's horn: Dent.Gyr.: dentate gyrus.

Table 2 Quantitative autoradiographic distribution of ^{3}H in the brain of rats injected with [^{3}H]-WAY 100635 after acute or short-term pretreatment with (−)-pindolol

	[^{3}H]-WAY 100635 equivalents (fmol mg^{-1} tissue)			
	Dorsal raphe nucleus	*CA1 area*	*CA3 area*	*Parietal cortex (layer IV)*
Controls	24.63±0.57	18.36±0.34	6.06±0.28	12.47±0.25
Pindolol				
Acute treatment	6.00±0.49* (−76%)	5.64±0.22* (−69%)	1.29±0.18* (−79%)	2.28±0.13* (−82%)
Short-term treatment	1.57±0.18*,† (−94%)	2.11±0.15*,† (−89%)	0.87±0.17* (−86%)	1.29±0.23*,† (−90%)

Rats were treated only once (acute treatment) or three times (short-term treatment over 24 h) with (−)-pindolol or vehicle (Controls) as described in Methods. Twenty min after the last injection, all rats received 17 μCi of [^{3}H]-WAY 100635 i.v. and were killed 60 min later. Blood levels of ^{3}H were not significantly different in the three groups of rats, and corresponded to 3570±327 d.p.m. 0.1 ml^{-1} (mean±s.e.mean for all rats, $n=9$). Coronal sections of each brain were put directly in tight contact with ^{3}H-hyperfilm, and autoradiograms were developed after exposure for one-month. Optical densities measured on autoradiograms were converted to fmol of [^{3}H]-WAY 100635 equivalents mg^{-1} fresh tissue. Each value is the mean±s.e.mean of 31–72 measurements on autoradiograms from 3 rats in each group. *$P<0.05$ as compared with control rats; †$P<0.05$ as compared with rats treated acutely with (−)-pindolol.

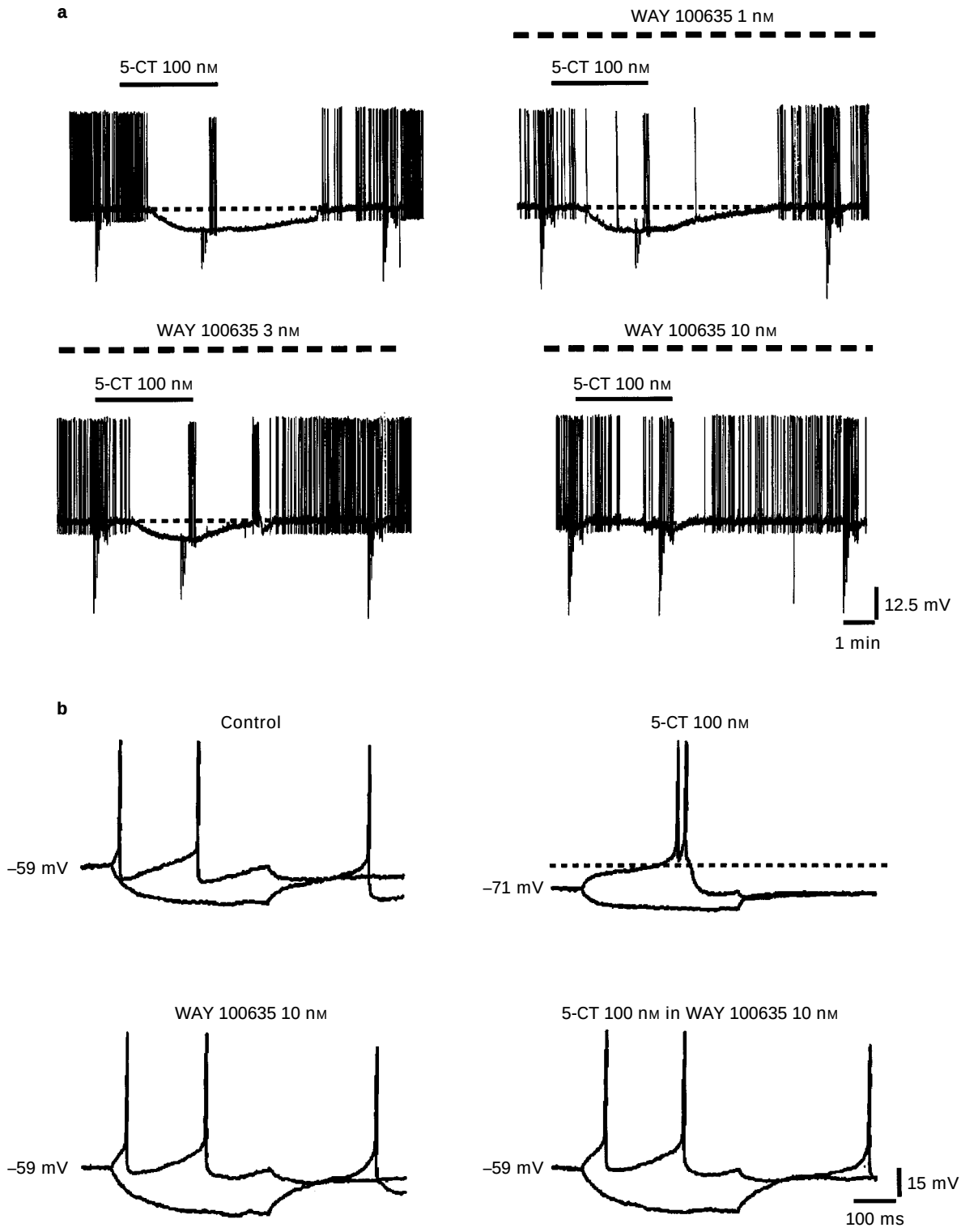

Figure 7 WAY 100635 antagonized the hyperpolarization and the decrease in R_{in} elicited by 5-CT in intracellularly recorded 5-hydroxytryptaminergic cells of the DRN. (a) Chart recording of membrane potential of a regularly firing 5-hydroxytryptaminergic neurone. Upward fast deflections are truncated action potentials. Superfusion of 100 nM 5-CT (bar) hyperpolarized the cell membrane, decreased the R_{in} (see also b), and blocked the firing of the neurone. In the same cell, superfusion of increasing concentrations of WAY 100635 (1–10 nM, 30 min each) antagonized the effects of 5-CT. Fast downward deflections are electrotonic cell membrane responses to constant current steps (−200 to +250 pA, 500 ms) injected through the recording electrode to construct *I*/V relationships and to monitor R_{in} (see also b). The regular firing of the cell partially masked the cell membrane responses to current injections, so that the entire set of responses can be visualized at this chart speed only during the effect of 5-CT. r.m.p.: −59 mV; R_{in}: 300 MΩ. (b) Responses of the cell to injection of constant current steps (±50 pA, 500 ms) through the recording electrode shown at expanded timebase. 5-CT (100 nM: upper right traces) hyperpolarized the cell and decreased R_{in} in comparison to control (left traces). WAY 100635 (10 nM, 30 min: lower left traces) changed neither r.m.p. nor R_{in}, but it prevented the effects of 5-CT (lower right traces).

led to an extinction of detectable firing by use of either intracellular or extracellular recording techniques (Figures 4c and 7a). In ten intracellularly recorded 5-hydroxytryptaminergic DRN cells (r.m.p.: -64.2 ± 2.3 mV; R_{in}: 361 ± 50 MΩ, means ± s.e.mean, one cell recorded per slice), 100 nM 5-CT elicited a hyperpolarization of 14.3 ± 1.4 mV and a decrease in R_{in} of $48.1\pm6.2\%$. WAY 100635 (3–30 nM) blocked the effects of 5-CT in a concentration-dependent manner (Figures 7–9) with an IC_{50} value of 4.89 nM (95% confidence interval: 3.02–7.92 nM). A similar action of WAY 100635 was observed on five CA1 pyramidal cells (r.m.p.: -64.8 ± 2.0 mV, R_{in}: 40.0 ± 6.7 MΩ, means ± s.e.mean, one cell recorded per slice), in which the hyperpolarization caused by 50 nM 5-CT (-6.8 ± 0.6 mV) was blocked by the antagonist in a concentration-dependent manner (Figure 8). The IC_{50} value of WAY 100635 on these cells was 0.83 nM (95% confidence interval: 0.64–1.08 nM).

To evaluate the K_B value of WAY 100635 in the DRN, we constructed concentration-response curves for 5-CT in the absence and the presence of WAY 100635 at 10 or 30 nM (Figure 9a). As shown in Figure 9a, 30 nM WAY 100635 ($n=2$) decreased the maximal response to 5-CT and allowed accurate determination of K_B value from the double reciprocal plot of equiactive concentrations (Figure 9b; $K_B=0.25$ nM, 95% confidence interval: 0.24–0.26 nM). A similar value of K_B (0.19 nM), but with a broader confidence interval (0.15–0.27 nM), probably due to the less pronounced depression of the maximal response (see Kenakin, 1993) was calculated from data obtained with 10 nM WAY 100635 ($n=4$).

Discussion

The data presented here demonstrate that (−)-pindolol antagonizes 5-HT$_{1A}$ receptor-mediated electrophysiological responses of both 5-hydroxytryptaminergic cells in the DRN and pyramidal neurones in the hippocampus. The antagonism exerted by (−)-pindolol on 5-HT$_{1A}$ agonist-induced hyperpolarization and/or cell firing was surmountable and reversible both in hippocampal pyramidal cells and in 5-hydroxytryptaminergic neurones of the DRN. In contrast, (−)-pindolol did not affect the 5-HT-induced depolarization that was probably mediated by 5-HT$_4$ receptors on pyramidal cells (Andrade & Chaput, 1991). In addition, the responses to baclofen in both the hippocampus and the DRN were not modified by (−)-pindolol, thus excluding the possibility that the drug non-specifically blocked G-proteins or potassium channels to which

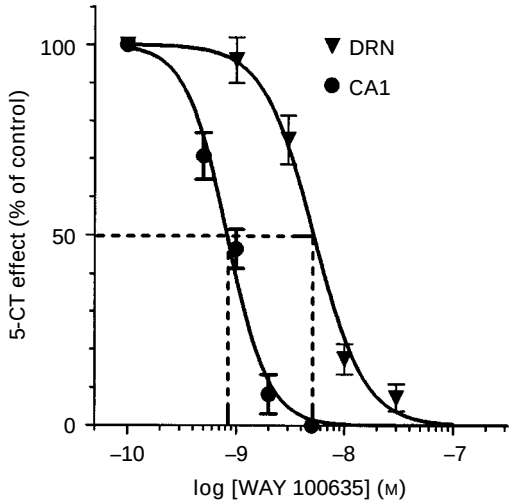

Figure 8 Concentration-response curves for the antagonism by WAY 100635 of 50 nM 5-CT-evoked hyperpolarizing responses in hippocampal CA1 pyramidal cells and of 100 nM 5-CT-evoked hyperpolarizing responses in 5-hydroxytryptaminergic neurones in the dorsal raphe nucleus (DRN). Data are expressed as % of the response in the absence of WAY 100635. Each point is the mean of data obtained in 4–7 cells for each concentration of WAY 100635; vertical lines show s.e.mean. When not shown, the s.e.mean was smaller than the symbol. The curves show fittings of the experimental data with non-linear regression. The dotted lines indicate the IC_{50} values of WAY 100635 (abscissa scale) against 5-CT-induced effect in the CA1 area and the dorsal raphe nucleus.

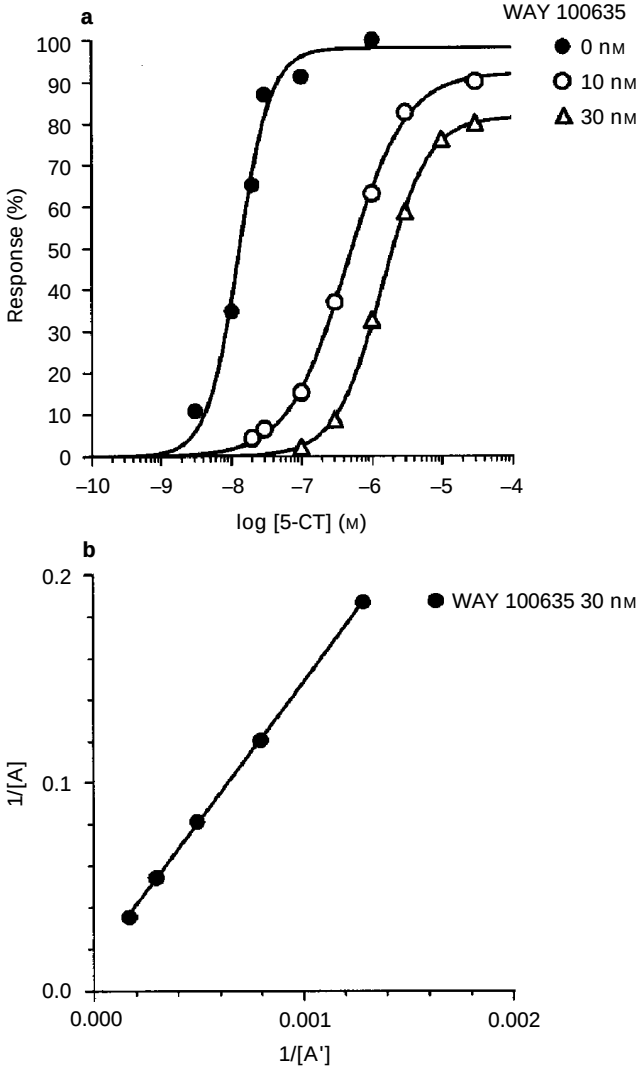

Figure 9 Concentration-response curves of 5-CT-induced hyperpolarization of a DRN 5-hydroxytryptaminergic cell in the presence of two different concentrations of WAY 100635. (a) Cumulative concentration-response curves of 5-CT (3 nM–30 μM) were constructed in the absence (0 nM) or the presence of 10 nM or 30 nM of WAY 100635. Each concentration of 5-CT was applied for 5 min, and WAY 100635 was left to equilibrate for 30 min before the agonist was retested. WAY 100635 shifted the curves to the right in a concentration-dependent manner, and depressed the maximal response at 30 nM. Curves were fitted to experimental points by nonlinear regression. KCl-filled electrode; r.m.p.: −62 mV, R_{in}: 260 MΩ. (b) Linear regression ($r^2=0.99$, slope 120.2 ± 1.4) of the double reciprocal plot of equally effective concentrations of 5-CT in the absence ([A]) and in the presence ([A']) of 30 nM WAY 100635. Equally effective concentrations were calculated from curves shown in (a).

both 5-HT$_{1A}$ receptors and GABA$_B$ receptors are functionally coupled (Andrade *et al*., 1986).

The activation of 5-HT$_{1A}$ autoreceptors in the DRN is well known to produce hyperpolarization and decrease of 5-hydroxytryptaminergic cell firing (VanderMaelen & Aghajanian, 1983; Sprouse & Aghajanian, 1987; Haj-Dahmane *et al*., 1991; present results). Intracellular and extracellular recordings clearly showed that (−)-pindolol blocked in a concentration-dependent manner the hyperpolarization and the inhibition of cell discharge due to the 5-HT$_{1A}$ agonist 5-CT. In addition, extracellular recordings confirmed that (−)-pindolol also antagonized the inhibition of 5-hydroxytryptaminergic cell firing caused by the selective 5-HT$_{1A}$ agonist ipsapirone. We used a general Cheng-Prusoff equation (see Methods) to calculate the apparent K_B value of (−)-pindolol, from EC_{50} and IC_{50} values of agonist and (−)-pindolol, derived from non linear fitting of respective concentration-response curves. The similarity of the K_B values of (−)-pindolol, calculated from experiments with two different 5-HT$_{1A}$ receptor agonists: 132 nM (5-CT) and 125 nM (ipsapirone), indicates that, for preventing the agonist-induced inhibition of 5-hydroxytryptaminergic cell firing, (−)-pindolol binds to 5-HT$_{1A}$ receptors. The application of a similar approach to estimate the K_B value of (−)-pindolol against 5-CT-induced hyperpolarization of pyramidal cells in hippocampus gave a value of 115 nM for experiments performed in the CA3 area. This value is close to that calculated with the same agonist in the DRN, suggesting that the 5-HT$_{1A}$ receptors on 5-hydroxytryptaminergic cells in the DRN and on CA3 pyramidal cells in Ammon's horn are pharmacologically similar. In contrast, the lower K_B value of (−)-pindolol, 20 nM, calculated from experiments on CA1 pyramidal cells might indicate that 5-HT$_{1A}$ receptors in this region differ to some extent from those expressed in CA3 and DRN. A similar conclusion has been proposed by Beck *et al*. (1992). However, such differences do not concern all antagonists, since, in contrast, the characteristics presently found for 5-HT$_{1A}$ receptor blockade by WAY 100635 in the DRN were closely similar to those previously obtained in the CA1 hippocampal area (Corradetti *et al*., 1996).

Intracellular recording techniques applied to 5-hydroxytryptaminergic neurones of the DRN showed that WAY 100635, on its own, affected neither r.m.p. nor R_{in}, as previously shown on CA1 pyramidal cells (Corradetti *et al*., 1996). This demonstrates that, in contrast with other 5-HT$_{1A}$ receptor ligands that act as antagonists in the hippocampus and as partial agonists in the DRN (Greuel & Glaser, 1992), WAY 100635 is devoid of any 5-HT$_{1A}$ receptor agonist properties in either region. Indeed, WAY 100635 only antagonized the hyperpolarization and the decrease in R_{in} caused by 5-CT application onto both cell types. The antagonism exerted by WAY 100635 at concentrations up to 10 nM was surmountable, whereas, at 30 nM, a decrease of the maximal response to 5-CT was observed. Since *in vitro* binding studies (Laporte *et al*., 1994; Gozlan *et al*., 1995) demonstrated that WAY 100635 inhibits competitively the specific binding of agonists to 5-HT$_{1A}$ receptors, we interpret the effect with 30 nM WAY 100635 as reflecting a 'pseudoirreversible'-antagonism (see Rang, 1996), probably due to peculiarities in the kinetics of the drug binding to 5-HT$_{1A}$ receptors. Although obtained with concentrations of WAY 100635 ten fold higher than those needed to depress the maximal response to 5-CT in CA1 pyramidal cells, this effect in the DRN is similar to that previously observed in the hippocampus (Corradetti *et al*., 1996). Such a difference in sensitivity regarding the 'pseudo irreversible' antagonism by WAY 100635 might be explained by the existence of a larger reserve of functional 5-HT$_{1A}$ receptors on 5-hydroxytryptaminergic neurones in the DRN than on pyramidal cells in the hippocampus (Yocca *et al*., 1992; Cox *et al*., 1993), or by a possible difference in signal transduction in these two cell types.

Since the antagonism by WAY 100635 of 5-CT responses in the DRN was non competitive, we could not calculate the apparent K_B with the Cheng-Prusoff equation (Leff & Dougall, 1993). Thus, we used the double reciprocal plot of equiactive agonist concentrations in the absence and the presence of a concentration of WAY 100635 which depressed the maximal response to 5-CT (Kenakin, 1993). The K_B value obtained, 0.25 nM, was very close to that found in previous experiments performed with this drug on CA1 pyramidal cells: 0.23 nM (Corradetti *et al*., 1996).

Comparison of the IC_{50} values of (−)-pindolol and WAY 100635 showed that the former drug is a much weaker 5-HT$_{1A}$ receptor antagonist than the latter. However, like that found for WAY 100635, (−)-pindolol was apparently more potent on CA1 hippocampal cells than on 5-hydroxytryptaminergic neurones in the DRN, probably because of the existence of a large reserve of functional 5-HT$_{1A}$ receptors on the latter cells (Cox *et al*., 1993).

Therefore, our *in vitro* data do not support the conclusion of Romero *et al*. (1996). Using *in vivo* extracellular recordings, these authors found that pretreatment with (−)-pindolol for two days antagonized the 5-HT-induced inhibition of DRN 5-hydroxytryptaminergic cell discharge due to acute administration of the selective 5-HT reuptake blocker paroxetine, but did not affect the responses of CA3 pyramidal cells to 5-HT or 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT). These observations led Romero *et al*. (1996) to conclude that (−)-pindolol, at doses that antagonize 5-HT action on 5-HT$_{1A}$ autoreceptors in the DRN, exerts no antagonism of 5-HT$_{1A}$ receptor activation in the CA3 region. One possible explanation of the discrepancy between the data of Romero *et al*. (1996) and those presented here could be that our experiments were performed *in vitro* as opposed to *in vivo*. Indeed, although (−)-pindolol efficiently blocked hippocampal 5-HT$_{1A}$ receptors in brain slices, it might be inactive at these receptors *in vivo* because the drug does not reach the hippocampus when injected systemically. In fact, experiments with [^{3}H]-WAY 100635 for the *in vivo* labelling of brain 5-HT$_{1A}$ receptors (Laporte *et al*., 1994) clearly demonstrated that (−)-pindolol bound to these receptors throughout the brain, including the CA1 and CA3 areas in the hippocampus. Thus, at the same dose as that used by Romero *et al*. (1996) (15 mg kg^{-1} i.p.) and with a closely related short-term administration protocol similar to that used by these authors, (−)-pindolol was found to reduce dramatically the subsequent *in vivo* binding of [^{3}H]-WAY 100635 to 5-HT$_{1A}$ receptors in the dorsal raphe nucleus as well as in the CA1 and CA3 areas of the hippocampus. Therefore, it can be concluded that, *in vivo*, (−)-pindolol reaches equally well these two brain regions, where our *in vitro* data indicated that it acts as a potent 5-HT$_{1A}$ receptor antagonist.

In contrast to our studies where the same intracellular recording procedures were used to assess the effects of known concentrations of (−)-pindolol at 5-HT$_{1A}$ receptors in the DRN and the hippocampus, different procedures were used by Romero *et al*. (1996) in their own studies in these two regions. The stimulation of 5-HT$_{1A}$ autoreceptors was achieved by endogenous 5-HT the extracellular concentration of which was raised due to 5-HT reuptake blockade by paroxetine, whereas the stimulation of presumed postsynaptic 5-HT$_{1A}$ receptors in the hippocampal CA3 area was obtained by direct micro-

iontophoretic application of agonists (Romero *et al*., 1996). Because the antagonistic action of (−)-pindolol at 5-HT$_{1A}$ receptors is competitive and weak, it is conceivable that local application of unknown, possibly too high, concentrations of agonists displaced the drug and elicited inhibition of firing. Such a possibility would explain the discrepancy between the interpretation of Romero *et al*. (1996) and our studies which did not show a greater sensitivity to (−)-pindolol of 5-hydroxytryptaminergic cells in comparison to hippocampal CA3 pyramidal neurones.

Other groups have also described the blockade of postsynaptic 5-HT$_{1A}$ receptors by pindolol in various models (Scott *et al*., 1994; Ybema *et al*., 1994; Levy *et al*., 1995), supporting the fact that the antagonists presently available, being either β-adrenoceptor or more selective 5-HT$_{1A}$ receptor ligands, do not exert a preferential action at the presynaptic level in the DRN. Because such a preferential presynaptic action is thought to underly the ability of pindolol to hasten the clinical response in depressed patients treated with 5-HT reuptake inhibitors (Artigas *et al*., 1994; 1996; Blier & Bergeron, 1995), an important question is whether its 5-HT$_{1A}$ and/or β-adrenoceptor blocking properties actually contribute to this effect.

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