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Dihydroergotamine and its metabolite, 8′-hydroxy-dihydroergotamine, as 5-HT_{1A} receptor agonists in the rat brain

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1 In addition to stopping migraine attacks, dihydroergotamine (DHE) is an efficient drug for migraine prophylaxis. Whether 5-HT_{1A} receptors could contribute to the latter action was assessed by investigating the effects of DHE and its metabolite, 8′-OH-DHE, on these receptors in the rat brain.

2 Membrane binding assays with [^{3}H]8-OH-DPAT and [^{3}H]WAY 100635 as radioligands showed that both DHE ($IC_{50} = 28 - 30$ nM) and 8′-OH-DHE ($IC_{50} = 8 - 11$ nM) are high-affinity 5-HT_{1A} receptor ligands.

3 Both DHE and 8′-OH-DHE enhanced the specific binding of [^{35}S]GTP-γ-S to the dorsal raphe nucleus and the hippocampus in brain sections, but to a lower extent than 5-carboxamido-tryptamine (5-CT) in the latter area.

4 Both DHE ($EC_{50} = 10.9 \pm 0.3$ nM) and 8′-OH-DHE ($EC_{50} = 30.4 \pm 0.8$ nM) inhibited the firing of serotoninergic neurons in the dorsal raphe nucleus within brain stem slices.

5 Intracellular recording showed that 8′-OH-DHE was more potent than DHE to hyperpolarize CA1 pyramidal cells in rat hippocampal slices.

6 Both the stimulatory effects of DHE and 8′-OH-DHE on [^{35}S]GTP-γ-S binding and their electrophysiological effects were completely prevented by the selective 5-HT_{1A} receptor antagonist WAY 100635.

7 As expected of 5-HT_{1A} receptor partial agonists, DHE and 8′-OH-DHE prevented any subsequent hyperpolarization of CA1 pyramidal cells by 5-HT or 5-CT.

8 Through their actions at 5-HT_{1A} auto- (in the dorsal raphe nucleus) and hetero-(notably in the hippocampus) receptors, DHE, and even more its metabolite 8′-OH-DHE, can exert both an inhibitory influence on neuronal excitability and anxiolytic effects which might contribute to their antimigraine prophylactic efficiency.

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Abbreviations: ACSF, artificial cerebrospinal fluid; CA1, CA1 area of Ammon's horn in the hippocampus; 5-CT, 5-carboxamido-tryptamine; DHE, dihydroergotamine; DRN, dorsal raphe nucleus; EGTA, ethylene glycol-bis(β-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; GTP-γ-S, guanosine 5′-*O*-(3-thiotriphosphate); HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid); O.D., optical density; 8′-OH-DHE, 8′-hydroxy-dihydroergotamine; 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino) tetralin; R_{in}, input resistance; r.m.p., resting membrane potential; TTX, tetrodotoxin; WAY 100635, *N*-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-*N*-(2-pyridinyl)cyclohexane carboxamide

Introduction

Important progress has been made for the last decade in the knowledge of physiopathological mechanisms underlying migraine attack. This was achieved notably through extensive investigations on the mechanisms of actions of drugs, such as triptans, which efficiently stop headache when they are administered acutely, immediately after the first clinical signs of a migraine attack (Goadsby *et al*., 2002). Like triptans, the classical antimigraine drug, dihydroergotamine (DHE), is also effective in the acute treatment of migraine, probably through its ability to prevent activation of the trigeminovascular pathway, and the associated release of calcitonin gene-related peptide from activated trigeminal fibers (Buzzi *et al*., 1991; Nozaki *et al*., 1992; Hoskin *et al*., 1996). However, in contrast to triptans, DHE is also effective for the prophylactic treatment of migraine (Neumann *et al*., 1986; Scott, 1992; Lantéri-Minet *et al*., 2000; Diener & Limmroth, 2001) and cluster headache (Mather *et al*., 1991), but the mechanisms underlying the latter effect are unknown.

Comparison of the pharmacological profiles of triptans on the one hand and DHE on the other shows that both types of drugs are high affinity agonists at serotonin 5-$HT_{1B/1D}$ receptors, and convergent evidence strongly supports the view

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that this property actually accounts for their efficacy to stop migraine attack under acute conditions (see Goadsby *et al*., 2002). Whereas triptans are indeed rather selective ligands of these receptors, DHE exerts additional effects at other 5-HT receptor types (McCarthy & Peroutka, 1989; Zifa & Fillion, 1992) as well as at dopaminergic and adrenergic receptors (Berde, 1978; Roquebert & Grenie, 1986; Goldstein, 1992), and one can wonder whether at least part of these additional effects might be causally related to the unique prophylactic efficacy of the ergot derivative. Indeed, numerous studies demonstrated that selective dopaminergic and adrenergic receptor agonists and antagonists do not exert antimigraine effects (Hamon & Bourgoin, 2000; Akerman *et al*., 2001), indicating that agonist/antagonist actions of DHE at these receptors do not contribute to its antimigraine properties. In contrast, other data support the idea that 5-HT receptors different from the 5-HT$_{1B/1D}$ types might be implicated in the prophylactic action of DHE (Raskin, 1991; Hamon & Bourgoin, 2000; Terron, 2002). Among them, the 5-HT$_{1A}$ receptor type, which is recognized by nanomolar concentrations of DHE (Newman-Tancredi *et al*., 1997; Pauwels *et al*., 1997; Assié *et al*., 1999), is an interesting target to consider because several other antimigraine drugs, which are efficient under chronic treatment conditions, such as methysergide, cyproheptadine, pizotifen and (-)propranolol also bind with a relatively high affinity to this receptor (Hiner *et al*., 1986). Furthermore, enhanced prolactin secretion in response to 5-HT$_{1A}$ receptor stimulation in patients suffering from migraine without aura led to the proposal that 5-HT$_{1A}$ receptors might be functionally modified (hypersensitive) in migraineurs (Leone *et al*., 1998). Accordingly, 5-HT$_{1A}$ receptor ligands such as tertatolol (Saxena *et al*., 1992) and buspirone (Gozlan *et al*., 1983) have been proposed for migraine prophylaxis, with some positive results with the latter compound (Pascual & Berciano, 1991).

In the light of these data, studies were performed in order to characterize further the actual action of DHE at 5-HT$_{1A}$ receptors in the rat brain. For this purpose, we used membrane-binding assays with selective 5-HT$_{1A}$ receptor radioligands, measurements of [^{35}S]GTP-γ-S binding evoked by 5-HT$_{1A}$ receptor stimulation in brain sections and *in vitro* electrophysiological recordings of neurons endowed with 5-HT$_{1A}$ autoreceptors in the dorsal raphe nucleus (DRN) and postsynaptic 5-HT$_{1A}$ heteroreceptors in the hippocampus (Vergé *et al*., 1986). Using the same approaches, studies were also carried out with 8′-hydroxy-dihydroergotamine (8′-OH-DHE), the main active metabolite of DHE (Chen *et al*., 2002) that accumulates in tissues up to concentrations several folds higher than those of the parent compound under chronic prophylactic treatment conditions (Silberstein, 1997).

Methods

Animals

Experiments were performed using male Sprague – Dawley rats (Centre d'Elevage René Janvier, 53940 Le Genest-Saint-Isle, France) weighing ~150 – 200 g.

All the procedures involving animals and their care were conducted in conformity 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 Fôret, Service Vétérinaire de la Santé et de la Protection Animale, permissions #6269 to L.L., #75-116 to M.H. and #6228 to S.B.). Animals were maintained under standard laboratory conditions (22 ± 1°C, 60% relative humidity, 12 h/12 h light – dark cycle with lights on at 7:00 a.m., food and water *ad libitum*) for 5 – 10 days before their use.

Preparation of membranes

Immediately after decapitation, brains were rapidly removed and dissected in the cold (0°C) according to the method of Glowinski & Iversen (1966). Hippocampi from 10 to 15 rats were pooled and homogenized in 40 volumes (v/w) of ice-cold 50 mM Tris-HCl, pH 7.4, using a Polytron disrupter (type PT 10 OD; Touzart-Matignon, Courtaboeuf-Les Ulis Cedex, France). Homogenate was centrifuged at 40,000 × *g* for 20 min at 4°C and the supernatant was discarded. The pellet was washed twice by resuspension in 40 volumes of ice-cold Tris-HCl buffer followed by centrifugation (40,000 × *g*, 20 min, 4°C) and resuspension. The sedimented material was then suspended in 40 volumes of Tris-HCl buffer and incubated at 37°C for 10 min in order to remove endogenous 5-HT (Nelson *et al*., 1978). Membranes were then collected by centrifugation and washed another three times by resuspension/centrifugation as before. The final pellet was suspended in 10 volumes of ice-cold 50 mM Tris-HCl, pH 7.4, and aliquots of the resulting suspension were kept at −80°C until their use for binding assays.

[^{3}H]8-OH-DPAT and [^{3}H]WAY 100635 binding assays

The assay conditions previously described by Hall *et al*. (1985) were used with minor modifications. Briefly, aliquots (50 µl, corresponding to ~0.25 mg of proteins) of membrane suspensions were mixed with 50 mM Tris-HCl, pH 7.4, containing the radiolabeled 5-HT$_{1A}$ receptor agonist [^{3}H]8-OH-DPAT ([^{3}H]8-hydroxy-2-(di-*n*-propylamino) tetralin, 1 nM; Gozlan *et al*., 1983) or antagonist [^{3}H]WAY 100635 ([^{3}H]*N*-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-*N*-(2-pyridinyl)cyclohexane carboxamide, 0.3 nM; Gozlan *et al*., 1995), and various concentrations (0.03 nM – 1.0 µM) of DHE or 8′-OH-DHE (final volume: 0.5 ml), and the mixtures were incubated for 1 h at 25°C. Assays were stopped by adding 3.5 ml of ice-cold 50 mM Tris-HCl, pH 7.4, followed by rapid vacuum filtration (using a Brandel cell harvester, Beckman, Gagny, France) through Whatman GF/B filters that had been presoaked with 50 mM Tris-HCl buffer, pH 7.4. After three additional washes with 3.5 ml of ice-cold Tris buffer, filters were dried and transferred into plastic vials containing 4.5 ml of Aquasol® scintillation fluid (New England Nuclear, Boston, MA, U.S.A.) for radioactivity counting. Nonspecific binding was determined from similar samples supplemented with 10 µM 5-HT.

All assays were performed in triplicate. Data analysis was done by computer-assisted nonlinear regression analysis, using Prism 2.01 (GraphPad). Proteins were determined according to the method of Lowry *et al*. (1951) with bovine serum albumin as standard.

Quantitative autoradiography of 5-HT$_{1A}$ receptor-mediated [^{35}S]GTP-γ-S binding

Rats were decapitated, and their brains were rapidly removed, frozen in isopentane chilled at −30°C with dry ice, and stored at −80°C. Coronal sections (20 μm thick) were cut in a cryostat at −20°C following the landmarks of the stereotaxic atlas of Paxinos & Watson (1998). Sections were then thaw mounted onto gelatin-coated slides, and stored at −80°C for less than 2 weeks, until their use for the autoradiographic measurement of 5-HT$_{1A}$ receptor-stimulated [^{35}S]GTP-γ-S binding according to the protocol of Fabre *et al*. (2000), with minor modifications. Briefly, brain sections were preincubated at room temperature for an initial 15 min period in 50 mM HEPES buffer, pH 7.5, supplemented with 100 mM NaCl, 3 mM $MgCl_2$, 0.2 mM EGTA and 2 mM dithiothreitol, and then for another 15 min in the same (but fresh) buffer with 2 mM GDP and 10 μM 8-cyclopentyl-1,3-dipropylxanthine (an A1 adenosine receptor antagonist) to decrease background labeling (Fabre *et al*., 2000). Thereafter, sections were incubated for 1 h at 30°C in the same (but fresh) buffer with 0.05 nM [^{35}S]GTP-γ-S in either the absence (basal conditions) or presence (stimulated conditions) of 10 μM of 5-carboxamido-tryptamine (5-CT), DHE or 8′-OH-DHE. Nonspecific binding was determined in the presence of each of the latter ligands plus 10 μM WAY 100635 to block 5-HT$_{1A}$ receptors (Fletcher *et al*., 1996). The incubation was stopped by two 2 min washes in ice-cold 50 mM HEPES buffer, pH 7.5, and a brief immersion in ice-cold distilled water. Sections were finally dried and exposed to β-max film (Amersham-Pharmacia Biotech, Little Chalfont, Buckinghamshire, U.K.). Optical density (O.D.) was measured on autoradiographic films, using a computerized image system (Biocom, Les Ulis, France).

Electrophysiological experiments

Immediately after decapitation, the brain was rapidly removed from the skull and immersed in an ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl (126), KCl (3.5), NaH_2PO_4 (1.2), $MgCl_2$ (1.3), $CaCl_2$ (2.0), $NaHCO_3$ (25) and glucose (11). Bubbling the ACSF with an O_2/CO_2 mixture (95/5%) yielded a pH of 7.3 – 7.4. A block of tissue containing the hippocampus or the DRN was cut into coronal sections (400 μm thick) using a vibratome (Corradetti *et al*., 1998). After sectioning, slices were kept in ACSF for at least 1 h at room temperature. They were then transferred to a recording chamber continuously perfused with oxygenated ACSF (2 ml/min at 34°C).

Extracellular recordings

Extracellular recordings were made using a single barrel micropipette (filled with 2 M NaCl, impedance: 12 – 15 MΩ) implanted into the DRN area (Haj-Dahmane *et al*., 1991). In all experiments, the otherwise silent serotoninergic neurons were induced to fire by adding 3 μM of phenylephrine (α_1-adrenoreceptor agonist) into the superfusing ACSF (VanderMaelen & Aghajanian, 1983). When a cell was recorded, it was identified on line as a serotoninergic neuron according to the following criteria: biphasic action potentials of 2 – 3 ms duration, slow (0.5 – 2.0 Hz) and regular pattern of discharge (VanderMaelen & Aghajanian, 1983). Baseline activity was recorded for 5 – 10 min before application of the different drugs *via* a three-way tap system which allowed complete exchange of fluids within 2 min of the arrival of a new solution into the chamber. The electrical signals were fed into a high-input impedance amplifier, an oscilloscope and an electric ratemater triggered by single-action potentials. The integrated firing rate was computed and recorded graphically as consecutive 10 s samples.

The effects of each concentration of DHE (0.1 nM – 1 μM) and 8′-OH-DHE (0.1 nM – 1.0 μM) were evaluated by comparing the mean discharge frequency recorded during 2 min immediately prior to the drug application with that recorded at the peak of drug action (usually 2 – 5 min after the beginning of application). When DHE and 8′-OH-DHE were applied in the presence of the 5-HT$_{1A}$ receptor antagonist WAY 100635 (10 nM), their effects were compared with the baseline firing rate and with the discharge frequency recorded during superfusion with WAY 100635 alone. Nonlinear regression fitting of concentration – response curves was carried out using Prism 2.01 (GraphPad) software facilities.

Intracellular recordings

CA1 hippocampal neurons in slices continuously superfused with ACSF at 34°C were recorded in current-clamp mode with 3 M KCl-filled electrodes (35 – 60 MΩ, Corradetti *et al*., 1998). In some experiments, tetrodotoxin (TTX, 1 μM) was added to the superfusing medium in order to prevent any action potentials possibly generated through interneuronal connections impinging onto CA1 neurons (Corradetti *et al*., 1998). 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, Claix, France, 48 kHz sampling frequency) and a computer using pClamp6 software (3 – 10 kHz sampling frequency, Axon Instruments, Foster City, CA, U.S.A.) for off-line measurements. Only neurons with stable resting membrane potential (r.m.p.; range −60/−75 mV) and input resistance (R_{in}; range 35 – 90 MΩ) throughout the recording session were included in the analysis. When cells appeared to have reached a stable membrane potential, pulses of hyperpolarizing current (−900/+400 pA, 400 ms, 0.1 Hz) were delivered through the recording electrode to monitor changes in R_{in} during drug application. The effects of 5-HT (30 μM, 3 – 5 min application), 5-CT (30 and 300 nM, 3 – 5 min application), DHE (100 nM – 30 μM, 5 min application) and 8′-OH-DHE (100 nM – 1 μM, 5 min application) on the membrane excitability were evaluated by comparing the r.m.p. recorded 2 min immediately prior to the drug application with the membrane potential recorded at the peak of drug action.

When DHE was applied in the presence of the 5-HT$_{1A}$ receptor antagonist WAY 100635 (10 nM), its effects on the R_{in} and the membrane potential were compared to those measured during superfusion with WAY 100635 alone.

In experiments aimed at investigating the potential 5-HT$_{1A}$ antagonist properties of DHE and 8′-OH-DHE, the effects of 5-CT and 5-HT on the membrane potential were compared in the presence *versus* the absence of each of the two ergot derivatives.

Statistical analyses

Data were analyzed by one-way ANOVA. *Post hoc* comparisons were made using Newman – Keuls multiple comparison test. Statistical significance was fixed at $P<0.05$.

Chemicals

[^{3}H]8-OH-DPAT (135 Ci mmol^{-1}) was from NEN Life Sciences Product Inc (Boston, MA, U.S.A.). (O-methyl-[^{3}H])-WAY 100635 (83 Ci mmol^{-1}) and [^{35}S]GTP-γ-S (1000 Ci mmol^{-1}) were from Amersham Pharmacia Biotech. Other compounds were 5-HT-creatinine sulfate (Merck, Darmstadt, Germany), GDP dilithium salt (Boehringer Mannheim, Meylan, France), 5-CT and 8-cyclopentyl-1,3-dipropylxanthine (Research Biochemicals International, Natick, MA, U.S.A.) and WAY 100635 (Centre de Recherche Pierre Fabre, Castres, France). DHE was from Sanofi-Synthélabo (Bagneux, France) and 8′-OH-DHE from Schwarz-Pharma (Boulogne, France). DHE was solubilized at 0.1 mM in water, and appropriate dilutions were then made for binding and electrophysiological experiments. 8′-OH-DHE was solubilized at 0.1 mM in a 5% (v/v) aqueous dilution of ethanol, and appropriate dilutions were then made in water. At the maximal residual concentration (1.5%), ethanol exerted no effect in binding and electrophysiological experiments.

Results

Effects of DHE and 8′-OH-DHE on the specific binding of [^{3}H]8-OH-DPAT and [^{3}H]WAY 100635 to rat hippocampal 5-HT$_{1A}$ receptors

As shown in Figure 1a, increasing concentrations of DHE within $10^{-9}-10^{-7}$ M range progressively decreased the specific binding of the two 5-HT$_{1A}$ receptor radioligands. Likewise, its metabolite, 8′-OH-DHE, dose-dependently inhibited the specific binding of the two radioligands onto hippocampal membranes. Indeed, the concentration range of 8′-OH-DHE to decrease [^{3}H]8-OH-DPAT and [^{3}H]WAY 100635 specific binding was three- to four-fold lower than that of DHE (Figure 1a).

The Hill representation (Figure 1b) of the inhibitory curves allowed the calculation of respective IC$_{50}$ values: 28 ± 3 and 30 ± 4 nM for DHE, and 11 ± 3 and 8 ± 3 nM (means$\pm$s.e.m., $n=3$) for 8′-OH-DHE against [^{3}H]WAY 100635 and [^{3}H]8-OH-DPAT specific binding, respectively. Analysis of the straight lines obtained in this representation indicated that the apparent Hill coefficients were significantly ($P<0.05$) higher than unity for both DHE (1.20 ± 0.05 and 1.35 ± 0.04 against [^{3}H]WAY 100635 and [^{3}H]8-OH-DPAT specific binding, respectively; means$\pm$s.e.m., $n=3$) and 8′-OH-DHE (1.34 ± 0.04 and 1.61 ± 0.06, respectively; mean$\pm$s.e.m., $n=3$).

Effects of DHE and 8′-OH-DHE on 5-HT$_{1A}$-mediated [^{35}S]GTP-γ-S binding to rat brain sections

As expected from an effect caused by the stimulation of 5-HT$_{1A}$ receptors, the enhanced binding of [^{35}S]GTP-γ-S in the dentate gyrus and the CA1 area of the hippocampus, the dorsal and median raphe nuclei, and the entorhinal cortex in brain sections exposed to 5-CT completely disappeared in the presence of the selective 5-HT$_{1A}$ receptor antagonist, WAY 100635 (Figure 2). Quantification of the autoradiographic labeling by [^{35}S]GTP-γ-S showed that the 5-HT$_{1A}$ receptor-mediated increase by 10 μM 5-CT was higher in the dentate gyrus, followed, in decreasing order, by the CA1 area and the dorsal raphe nucleus (Table 1), in agreement with previous observations (Sim *et al*., 1997; Fabre *et al*., 2000; Sim-Selley *et al*., 2000). As compared with 5-CT, DHE and 8′-OH-DHE were as efficacious to enhance [^{35}S]GTP-γ-S binding in the DRN, and the effects of these two ergot derivatives were also completely prevented by WAY 100635, indicating their mediation through 5-HT$_{1A}$ receptors (Figure 2, Table 1). At the level of the hippocampal CA1 area, the 5-HT$_{1A}$ receptor-mediated increase in [^{35}S]GTP-γ-S binding by both DHE and 8′-OH-DHE was significantly less ($-14-18$%) than that observed in the presence of 5-CT (Table 1). A similar difference between DHE and 5-CT was observed in the

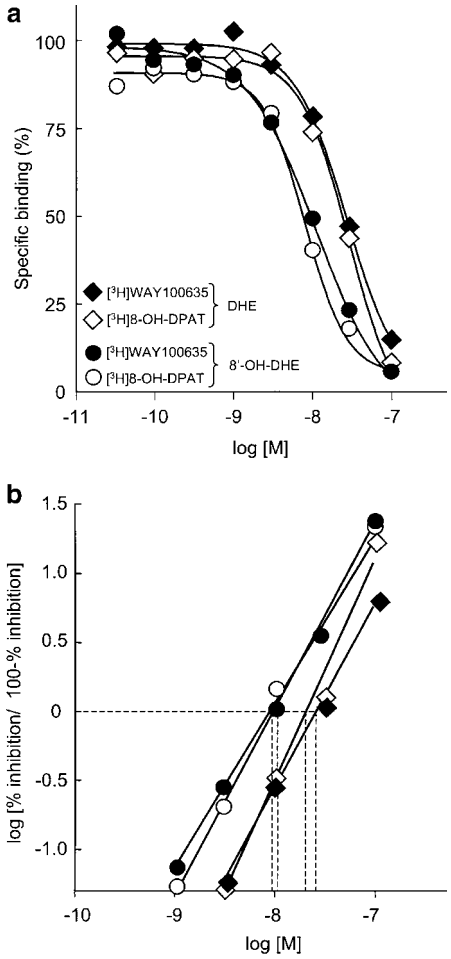

Figure 1 Concentration-dependent inhibition by DHE and 8′-OH-DHE of the specific binding of [^{3}H]8-OH-DPAT and [^{3}H]WAY 100635 to rat hippocampal membranes. (a) Binding assays were carried out with 1.0 nM of [^{3}H]8-OH-DPAT or 0.3 nM of [^{3}H]WAY 100635 using the same membrane preparations. Nonspecific binding (0 on ordinate) was determined in the presence of 10 μM 5-HT. Specific binding is expressed as a percentage of that determined in the absence of DHE or 8′-OH-DHE. Each point is the mean$\pm$s.e.m. of three independent experiments. (b) Hill representations of the curves in (a). The respective intersections (dotted lines) with the abscissa (0 on ordinate) correspond to IC$_{50}$ values and the slopes to apparent Hill coefficients (values are given in the text).

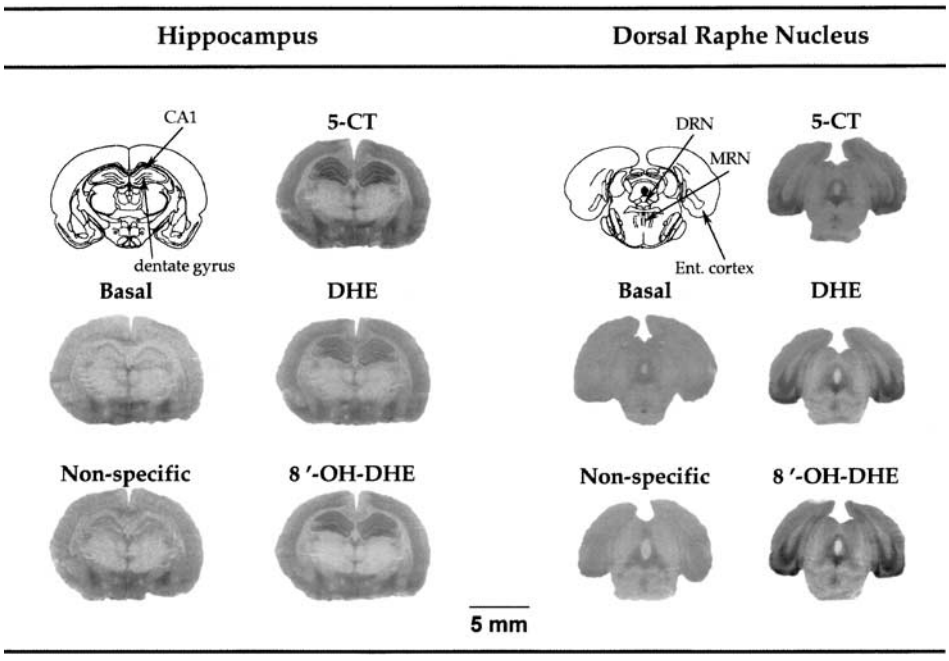

Figure 2 Representative autoradiograms of [^{35}S]GTP-γ-S binding to rat brain sections at the level of the hippocampus and the dorsal raphe nucleus. Effects of 5-CT, DHE and 8′-OH-DHE. Coronal brain sections (20 μm thick) were cut at levels corresponding to plates at −3.3 mm (hippocampus) and −7.8 mm (dorsal raphe nucleus) from bregma in the stereotaxic atlas of Paxinos & Watson (1998). Adjacent sections were incubated with 0.05 nM [^{35}S]GTP-γ-S in the absence of any ligands (Basal) or in the presence of 5-CT (10 μM), DHE (10 μM) or 8′-OH-DHE (10 μM). Nonspecific binding corresponds to sections incubated with any of the latter three compounds plus 10 μM WAY 100635. No differences were found between these three compounds in the presence of WAY 100635. Autoradiograms from five rats were used for the quantitative determinations in Table 1. CA1, CA1 area of Ammon's horn in the hippocampus; DRN, dorsal raphe nucleus; Ent. cortex: entorhinal cortex; MRN, median raphe nucleus.

dentate gyrus (Table 1). In contrast, 8′-OH-DHE-induced increase in [^{35}S]GTP-γ-S binding did not significantly differ from that evoked by 5-CT in this hippocampal area (Table 1).

5-HT$_{1A}$-receptor-mediated electrophysiological effects of DHE and 8′-OH-DHE

In the dorsal raphe nucleus

Superfusion of brain stem slices with DHE (0.1 nM – 1 μM, 3 min application) elicited a concentration-dependent reduction in 5-HT cell firing with an EC_{50} of 10.9 ± 0.3 nM (mean ± s.e.m., $n = 4$) and complete blockade at concentrations ⩾100 nM (Figure 3a and c). Similarly, the application of 8′-OH-DHE (0.1 nM – 1 μM, for 3 min) produced a concentration-dependent inhibition of 5-HT cell firing with an EC_{50} of 30.4 ± 0.8 nM (mean ± s.e.m., $n = 4$) and complete blockade at concentrations ⩾100 nM (Figure 3b and c). As illustrated in Figure 3a and b, the inhibitory effects of both DHE (30 nM) and 8-OH-DHE (30 nM) on the discharge of DRN serotonergic neurons were completely prevented by the 5-HT$_{1A}$ receptor antagonist, WAY 100635 (10 nM).

In the hippocampus

Under control conditions, that is in the absence of drugs, pyramidal neurons in the CA1 area of superfused hippocampal slices were characterized by an r.m.p. value of −61.1 ± 2.0 mV and an R_{in} value of 52.7 ± 8.8 MΩ (mean ± s.e.m., $n = 12$).

Table 1 Specific binding of [^{35}S]GTP-γ-S induced by 5-CT, DHE and 8′-OH-DHE in the dorsal raphe nucleus and the dentate gyrus and CA1 area of the hippocampus in rat brain sections

	[^{35}S]GTP-γ-S specific binding (O.D. × 10^2)		
	5-CT	*DHE*	*8′-OH-DHE*
Hippocampus			
Dentate gyrus	18.41 ± 0.31	14.82 ± 0.53* (−19.5%)	17.07 ± 0.46 (−7.3%)
CA1	16.16 ± 0.48	13.33 ± 0.43* (−17.5%)	13.84 ± 0.57* (−14.4%)
Dorsal raphe nucleus	13.05 ± 0.65	12.64 ± 0.56	12.65 ± 0.58

Brain sections (20 μm) were incubated with 0.05 nM [^{35}S]GTP-γ-S in the presence of 10 μM of 5-CT, DHE or 8′-OH-DHE, with or without 10 μM WAY 100635, as described in Methods. [^{35}S]GTP-γ-S-specific binding (expressed as arbitrary O.D. × 10^2 U) was calculated as the difference between binding in the presence of the agonist alone and that in the presence of the agonist plus WAY 100635. As percentages, the specific binding induced by 10 μM 5-CT corresponded to ∼110%, ∼260% and ∼180% over nonspecific binding in the dorsal raphe nucleus, dentate gyrus and CA1 area, respectively.
Each value is the mean ± s.e.m. of independent determinations in five rats. Percent changes as compared to 5-CT are indicated in parentheses.
*$P < 0.01$ vs [^{35}S]GTP-γ-S specific binding in the presence of 10 μM 5-CT.

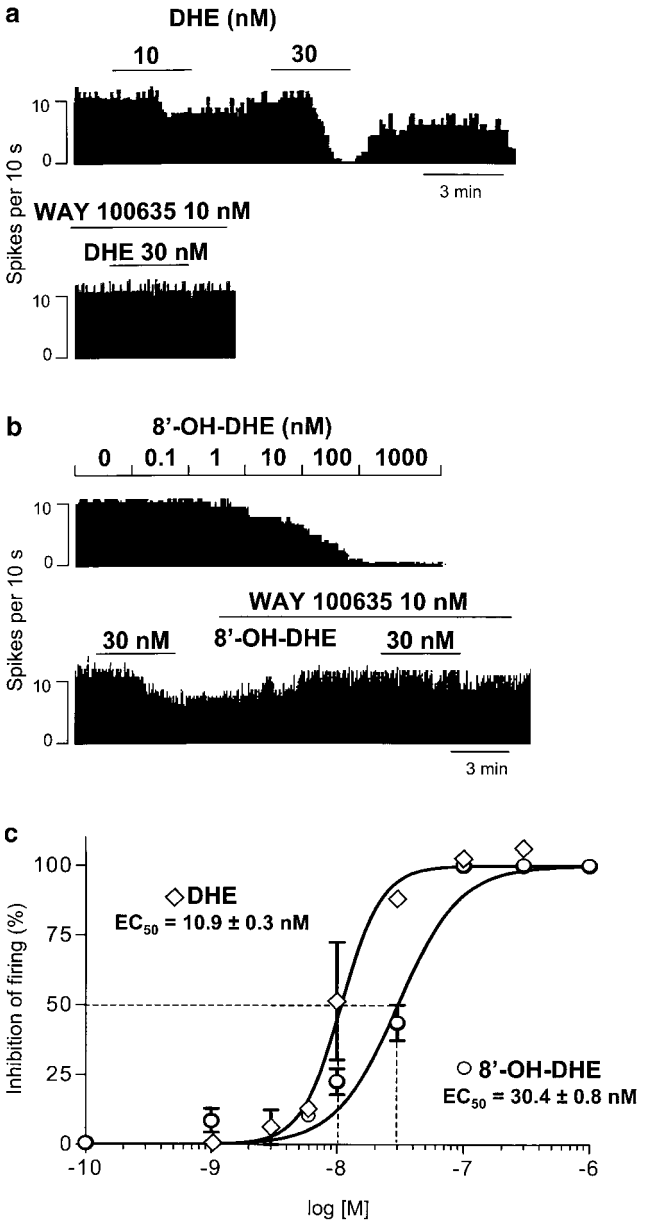

Figure 3 Inhibitory effects of DHE and 8′-OH-DHE on the firing of DRN serotoninergic neurons in rat brain stem slices. Firing rate histograms (in spikes emitted per 10 s) of DRN serotoninergic neurons exposed to increasing concentrations of DHE (a) or 8′-OH-DHE (b). In both cases, the inhibitory effect of these drugs was prevented by the concomitant application of WAY 100635 (10 nM). (c) Concentration curves of the inhibitory effects of DHE and 8′-OH-DHE on the firing of DRN serotoninergic neurons. Data are expressed as percent inhibition with respect to the baseline firing rate (in the absence of DHE and 8′-OH-DHE). Each point is the mean ± s.e.m. of data obtained in at least four individual cells for each concentration ([M] on abscissa) of DHE or 8′-OH-DHE tested. The dotted lines point at the EC_{50} values of the compounds.

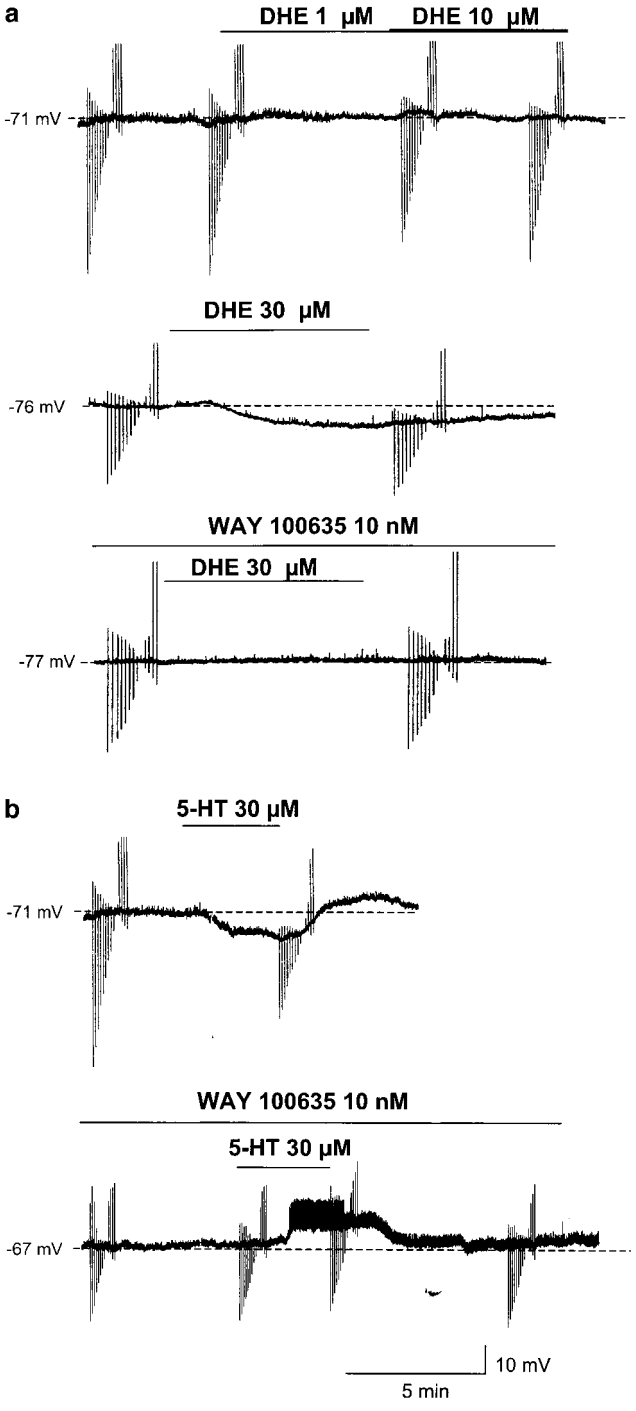

Figure 4 Comparison of the effects of DHE and 5-HT on CA1 pyramidal cells in rat hippocampal slices. (a) Chart recording of the membrane potential of intracellularly recorded CA1 pyramidal neurons exposed to 1 – 30 μM DHE, with or without 10 nM WAY 100635. Downward and upward deflections are electrotonic cell membrane responses to constant current steps (−900/+400 pA) injected through the recording electrode in order to monitor the membrane input resistance (R_{in}). A hyperpolarization, which could be completely prevented by WAY 100635, was observed only in the presence of the highest concentration (30 μM) of DHE tested. (b) Effects of 5-HT (30 μM) in the absence (top trace) or the presence (bottom trace) of 10 nM WAY 100635 on the membrane potential of CA1 pyramidal cells. Whereas 5-HT alone produced a hyperpolarization and a decrease in R_{in}, in the presence of WAY 100635, the indoleamine depolarized the plasma membrane. Similar data were obtained in three to four individual cells for each condition (a,b).

Effects of DHE

As illustrated in Figure 4a, no changes in the membrane potential of CA1 neurons were observed upon the addition of 1 or 10 μM DHE into the superfusing fluid of hippocampal slices. However, some decrease in R_{in} (−20%) occurred in the presence of 10 μM DHE. At a higher concentration, 30 μM,

DHE produced a clearcut hyperpolarization of CA1 neurons (by -7.2 ± 1.6 mV, mean$\pm$s.e.m., $n=4$) and a decrease in R_{in} (by 20%), which could be completely prevented by WAY 100635 (10 nM), as expected from their mediation through 5-HT$_{1A}$ receptor stimulation. Similarly, 30 μM 5-HT produced both a hyperpolarization (by -12.4 ± 2.1 mV, mean$\pm$s.e.m., $n=4$) and a decrease in input resistance (by 48%) of the plasma membrane of CA1 neurons, but these effects were of larger amplitude than those evoked by the same concentration of DHE (cf. Figure 4b and a). In the presence of 10 nM WAY 100635, the 5-HT-evoked decrease in R_{in} was completely prevented, and the hyperpolarization was converted into a depolarization (Figure 4b), probably in response to the stimulation of 5-HT$_4$ receptors (Pugliese *et al*., 1998).

In order to determine whether the apparent low potency of DHE to trigger a 5-HT$_{1A}$ receptor-mediated hyperpolarization of CA1 pyramidal neurons could be caused by a concomitant depolarization in response to the activation (through other receptor types) of excitatory interneurons inpinging onto CA1 cells, experiments were also performed in the presence of TTX (1 μM) to prevent the occurrence of such indirect mechanisms within the slices. However, like that observed in the absence of TTX (Figure 4a), no changes in the membrane potential of CA1 neurons were noted upon superfusion of hippocampal slices with DHE at 0.1, 1.0 and 10 μM (Figure 5), and a limited hyperpolarization (by -8.2 ± 1.5 mV, mean$\pm$s.e.m., $n=3$) was recorded only with 30 μM DHE in the presence of 1 μM TTX.

Finally, we tested whether DHE, at concentrations apparently inactive on their own, could affect the response to 5-HT and 5-CT. As shown in Figure 6a, tissue exposure to DHE at 1 then 10 μM completely prevented the hyperpolarization expected from the subsequent application of 30 μM 5-HT. Similarly, pretreatment of hippocampal slices with 10 μM DHE also completely abolished any subsequent hyperpolarization by 30 nM 5-CT (not shown). With both 5-HT and 5-CT, we observed that a washout period of 60 min after tissue pretreatment with 10 μM DHE for only 5 min was not enough to restore the hyperpolarizing response to these 5-HT$_{1A}$ receptor agonists (not shown).

Effects of 8′-OH-DHE

The addition of 8′-OH-DHE (100 nM – 1 μM) to the ACSF superfusing hippocampal slices produced a concentration-dependent hyperpolarization of CA1 pyramidal cells and a decrease in their input resistance, which reached their maximal amplitude at 300 nM of the drug (Figure 7a). At this concentration, the resulting decrease in membrane potential (-6.5 ± 1.2 mV, $n=4$) was only half of that produced by 30 μM 5-HT (see Figure 4b). As expected from their mediation

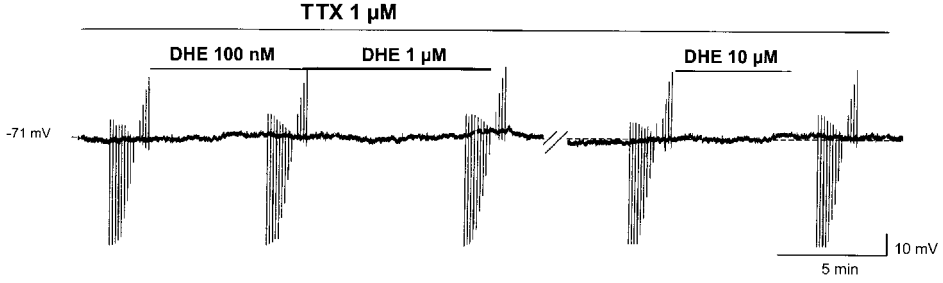

Figure 5 Effects of DHE (100 nM, 1 and 10 μM) on CA1 pyramidal cells in hippocampal slices continuously superfused with 1 μM TTX. No modification of the membrane potential was produced by DHE under this condition. Similar data were obtained in three to four individual cells for each concentration of DHE.

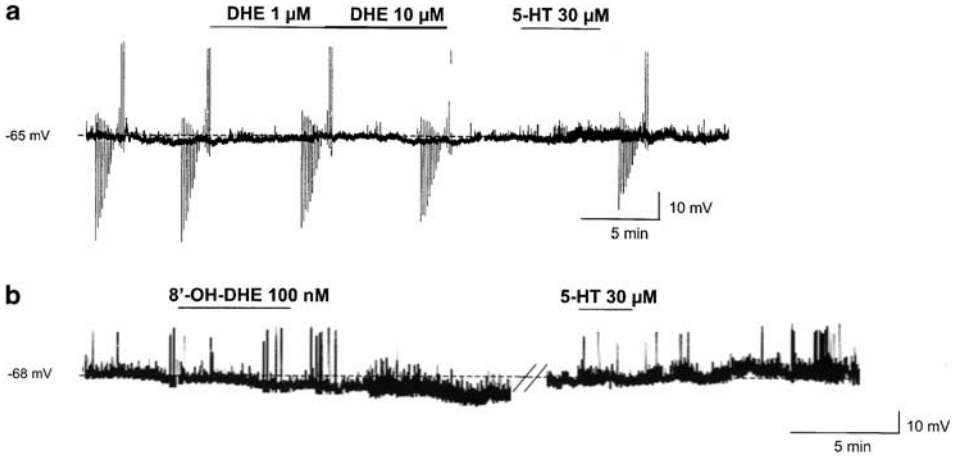

Figure 6 Blockade by DHE and 8′-OH-DHE of 5-HT-evoked responses in CA1 pyramidal cells. (a) Both membrane hyperpolarization and decrease in R_{in} normally observed in response to the application of 30 μM 5-HT (see Figure 4b) were completely prevented by prior application of 1 and 10 μM DHE, which were inactive on their own. (b) At 30 min after a 5 min application of 100 nM 8′-OH-DHE, which produced a limited hyperpolarization of the plasma membrane, 5-HT (30 μM) no longer hyperpolarized the cell. Instead, a limited depolarization was noted in response to the indoleamine. Similar data have been obtained in four to five individual cells for each condition (a,b).

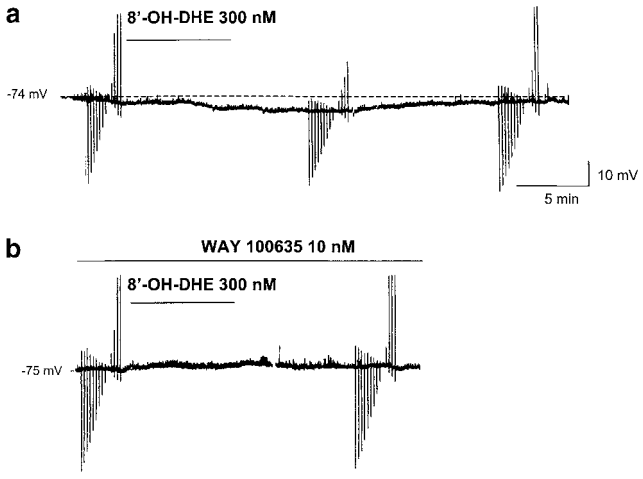

Figure 7 Prevention by WAY 100635 of the hyperpolarizing effect of 8′-OH-DHE on CA1 pyramidal cells. (a) Application of 300 nM 8′-OH-DHE produced a long-lasting hyperpolarization of a CA1 hippocampal cell. The decrease in downward deflections (corresponding to cell membrane responses to constant current steps injected through the recording electrode) after the application of 8′-OH-DHE indicates a decrease in R_{in}. (b) Prevention by continuous superfusion with 10 nM WAY 100635 of the hyperpolarizing effect of 300 nM 8′-OH-DHE on a CA1 pyramidal neuron. Similar data have been obtained in four individual cells for each condition (a,b).

through 5-HT$_{1A}$ receptor stimulation, 8′-OH-DHE-induced hyperpolarization and decrease in R_{in} (−20%) were completely prevented by concomitant tissue superfusion with WAY 100635 (10 nM) (Figure 7b).

In another series of experiments, we investigated whether pretreatment of hippocampal slices with the lowest efficient concentration of 8′-OH-DHE, 100 nM, could affect the response to 5-HT (30 μM). As shown in Figure 6b, such a pretreatment actually abolished the hyperpolarizing effect of the indoleamine. Like that already noted with DHE itself, the blocking action of the DHE metabolite against 5-HT was long lasting since it could still be observed 30 min after cessation of only a 5 min application of 8′-OH-DHE onto the slices.

Discussion

In agreement with previous studies (McCarthy & Peroutka, 1989; Newman-Tancredi *et al*., 1997; Pauwels *et al*., 1997; Assié *et al*., 1999), *in vitro* binding experiments confirmed that DHE is a high-affinity ligand at 5-HT$_{1A}$ receptors in the rat brain. In addition, we demonstrated that its main metabolite, 8′-OH-DHE, also displays this property, and indeed, in binding assays with rat hippocampal membranes, 8′-OH-DHE had a higher affinity than DHE for 5-HT$_{1A}$ receptors identified by the specific binding of both the radiolabeled agonist [^{3}H]8-OH-DPAT (Gozlan *et al*., 1983) and the radiolabeled antagonist [^{3}H]WAY 100635 (Gozlan *et al*., 1995). Interestingly, concentration-dependent inhibition of the specific binding of each radioligand by both DHE and 8′-OH-DHE yielded apparent Hill coefficients higher than unity, indicating more complex interactions than only a competition between these compounds and each radioligand for the same 5-HT$_{1A}$ receptor binding sites. Indeed, previous investigations by Assié *et al*. (1999) already led to the conclusion that DHE interacts in an allosteric manner with 5-HT$_{1A}$ receptors. Also, Sundaram *et al*. (1995) noted that another ergot derivative with 5-HT$_{1A}$ agonist properties and efficacy in the prophylactic treatment of migraine (Somerville & Hermann, 1978), lisuride, binds to 5-HT$_{1A}$ receptors in a different manner compared to classical agonists such as 8-OH-DPAT and 5-HT itself. Interestingly, the same apparent affinity (IC_{50} value) was noted for DHE as well as 8′-OH-DHE whether binding assays were performed with the agonist radioligand [^{3}H]8-OH-DPAT or the antagonist radioligand [^{3}H]WAY 100635, indicating that both compounds probably interacted equally with the 'agonist' and the 'antagonist' form of the 5-HT$_{1A}$ receptor, like that already noted for lisuride (Sundaram *et al*., 1995).

Extending previous biochemical investigations on 5-HT$_{1A}$ receptors expressed in transfected cells (Newman-Tancredi *et al*., 1997; Pauwels *et al*., 1997; Assié *et al*., 1999), autoradiographic quantification of [^{35}S]GTP-γ-S binding to rat brain sections showed that DHE acts as a 5-HT$_{1A}$ receptor agonist. Similarly, its metabolite, 8′-OH-DHE, was also found to increase [^{35}S]GTP-γ-S binding in specific brain regions as expected from the stimulation of local 5-HT$_{1A}$ rceceptors. However, as compared with that caused by the full 5-HT$_{1A}$ receptor agonist, 5-CT (Fabre *et al*., 2000), the maximal enhancement of [^{35}S]GTP-γ-S binding produced by a saturating concentration (10 μM) of DHE and 8′-OH-DHE was significantly less in the CA1 area of the hippocampus, indicating that DHE and its metabolite acted as partial 5-HT$_{1A}$ receptor agonists in this area. Within the dentate gyrus, the evoked [^{35}S]GTP-γ-S binding was significanly less than that caused by 5-CT only in the case of DHE, thereby suggesting the following order of agonist efficacy at postsynaptic 5-HT$_{1A}$ receptors in the hippocampus: 5-CT > 8′-OH-DHE > DHE. In contrast, in the DRN, the three agonists enhanced [^{35}S]GTP-γ-S binding to the same extent, as expected for full agonists at 5-HT$_{1A}$ autoreceptors. However, this characteristic might be more apparent than actual because of the presence of a large 5-HT$_{1A}$ receptor reserve in the DRN (Meller *et al*., 1990) (but not in the hippocampus, Yocca *et al*., 1992), which allows partial agonists to induce the same maximal response as full agonists.

Electrophysiological experiments also showed that DHE and 8′-OH-DHE are agonists at 5-HT$_{1A}$ autoreceptors in the DRN. Thus, as expected from such ligands (Haj-Dahmane *et al*., 1991), both DHE and 8′-OH-DHE produced a concentration-dependent inhibition of the firing of DRN serotoninergic neurons, and this effect could be completely prevented by the selective 5-HT$_{1A}$ receptor antagonist WAY 100635. At the hippocampal level, the changes caused by both DHE and 8′-OH-DHE to membrane potential and input resistance in CA1 pyramidal neurons indicated that these two compounds act only as partial agonists at 5-HT$_{1A}$ receptors on these cells. Interestingly, a clearcut hyperpolarization was noted with only 0.1 μM of 8′-OH- DHE whereas 30 μM of DHE was needed to trigger this response. This difference further supported the relative order of agonist potency/efficacy derived from both radioligand binding studies (Figure 1) and quantitative autoradiographic measurements of [^{35}S]GTP-γ-S-specific binding (Figure 2, Table 1): 5-CT > 8′-OH-DHE > DHE. The lower efficacy of DHE could not be caused by some concomitant depolarization (through possible actions of the ergot derivative at other, non-5-HT$_{1A}$, receptors), which

would have masked the expected hyperpolarization at concentrations $<30\,\mu$M because the blockade of 5-HT$_{1A}$ receptors by WAY 100635 did not reveal any DHE-induced depolarization of CA1 hippocampal neurons. In contrast, the blockade of 5-HT$_{1A}$ receptors allowed 5-HT to exert a depolarizing influence (probably through 5-HT$_4$ receptor stimulation; Pugliese *et al*., 1998) on these neurons, showing that if a similar mechanism would have existed in case of DHE, the protocol used should have allowed its demonstration. Indeed, DHE is not recognized by 5-HT$_4$ receptors (Zifa & Fillion, 1992), which explains its failure to mimic the depolarizing effect of 5-HT in the presence of WAY 100635. On the other hand, DHE, at concentrations up to $10\,\mu$M, was also unable to hyperpolarize CA1 hippocampal neurons in the presence of TTX to prevent possible interference by excitatory interneurons, which would have been activated by the ergot derivative. Accordingly, it can be concluded that both DHE and 8′-OH-DHE act as partial agonists at hippocampal postsynaptic 5-HT$_{1A}$ receptors, with a higher potency/efficacy for the metabolite compared to the parent compound.

As expected of partial agonists, 5-HT$_{1A}$ receptor occupancy by these drugs prevented any further stimulation by 5-HT or 5-CT, and indeed, the latter two agonists completely lost their 5-HT$_{1A}$-mediated hyperpolarizing effect on CA1 hippocampal neurons in slices that had been pretreated with either DHE or 8′-OH-DHE. Previous studies with ergotamine also showed that this compound exerts a 5-HT$_{1A}$-mediated inhibitory influence on the electrical activity of CA3 hippocampal neurons and prevents the inhibitory action of 5-HT on these neurons, leading to the conclusion that ergotamine, like DHE and 8′-OH-DHE, acts as a partial 5-HT$_{1A}$ receptor agonist on these cells (Haddjeri *et al*., 1998). Interestingly, the effects of DHE and 8′-OH-DHE were long lasting, since they persisted after a washout of at least 30 – 60 min after their addition for only 5 min into the superfusing ACSF. These data corroborate the well-established capacity of DHE and its metabolites to accumulate for several hours in tissues after acute treatment (Silberstein, 1997).

With regard to the functional consequences of the effects of DHE and 8′-OH-DHE at DRN 5-HT$_{1A}$ autoreceptors, it can be inferred that these compounds might exert anxiolytic-like properties through their capacity to inhibit the firing of 5-HT neurons, thereby reducing the tone on postsynaptic 5-HT receptors. Indeed, other partial 5-HT$_{1A}$ receptor agonists such as buspirone and ipsapirone are well known to decrease anxiety-driven behaviors in relevant animal paradigms through their action specifically at these autoreceptors to inhibit the firing of DRN 5-HT neurons (Higgins *et al*., 1988; Jolas *et al*., 1995). As migraine attacks are often triggered by stress and/or anxiogenic events, it can be reasonably inferred that the prophylactic action of DHE may be underlain, at least partly, through its own effects, and those of its main metabolite 8′-OH-DHE, at DRN 5-HT$_{1A}$ autoreceptors.

At the cellular level, the stimulation of 5-HT$_{1A}$ receptors always triggers a hyperpolarization, thereby reducing the excitability of neurons endowed with these receptors (Araneda & Andrade, 1991; Beck *et al*., 1992; Pugliese *et al*., 1998). Comparison between DHE and 8′-OH-DHE showed that the metabolite, much more than the parent compound, exerted at least a partial agonist action at both DRN and hippocampal 5-HT$_{1A}$ receptors, suggesting that it probably stimulates 5-HT$_{1A}$ receptors throughout the CNS. A general decrease in the excitability of 5-HT$_{1A}$ receptor expressing neurons can thus be expected especially under chronic treatment conditions such as those for migraine prophylaxis, because of the long-lasting tissue accumulation of 8′-OH-DHE (Wyss *et al*., 1991; Silberstein, 1997; De Hoon *et al*., 2001). This effect should notably concern serotoninergic neurons in anterior raphe nuclei whose activation has been proposed as a first event in the hypothetical mechanisms triggering a migraine attack (see Goadsby *et al*., 2002). Accordingly, 5-HT$_{1A}$ receptor (partial) stimulation by 8′-OH-DHE and, to a lower extent, DHE, might both reduce neuron hyperexcitability, which has been proposed to be causally related to migraine attack (Palmer *et al*., 2000), and prevent stress-induced activation of serotoninergic neurons, including those in the DRN which send fibers around blood vessels in the meninges (Edvinsson *et al*., 1983, Moreno *et al*., 1994, but this is still a matter of controversy, see Cohen *et al*., 1996, for a review). Through the latter action, 8′-OH-DHE (and its parent compound) might therefore prevent local 5-HT release, and subsequent activation of pial 5-HT receptor types (5-HT$_{2B}$, 5-HT$_7$) possibly at the origin of migraine-triggering vasodilation (see Schmuck *et al*., 1996; Terron, 2002).

Previous studies have shown that 5-HT$_{1A}$ receptors adapt to chronic treatment with direct or indirect agonists (Chaput *et al*., 1991; Li *et al*., 1996; Le Poul *et al*., 2000; Sim-Selley *et al*., 2000). However, in the case of partial agonists such as DHE and 8′-OH-DHE, which act at 5-HT$_{1A}$ receptors through molecular mechanisms different from those of other 'classical' 5-HT$_{1A}$ agonists (see above), the question of 5-HT$_{1A}$ receptor adaptation under chronic treatment conditions has still to be addressed. In any case, it can already be emphasized that the anxiolytic-like effects of other 5-HT$_{1A}$ receptor partial agonists such as the azapirone derivatives buspirone and gepirone are actually more pronounced after chronic compared to acute treatment (Cole & Rodgers, 1994; Yamashita *et al*., 1995), and one can wonder whether the same potentiation may also develop regarding the potential anxiolytic-like action of 8′-OH-DHE and DHE during prophylactic treatment of migraine.

In conclusion, the probable widespread decrease in neuronal excitability in the CNS associated with potential anxiolytic effects and indirect vasomotor effects on pial vessels that are expected from the stimulation of 5-HT$_{1A}$ receptors might contribute to the well-established therapeutic efficacy of chronic treatment with DHE to prevent migraine attacks. In this respect, its metabolite, 8′-OH-DHE, probably plays a key role because it accumulates in tissues and its potency/efficacy at postsynaptic 5-HT$_{1A}$ receptors is higher than that of the parent compound. However, these 5-HT$_{1A}$-mediated actions have to be considered as only one component of the pharmacological profile responsible for the prophylactic efficacy of DHE (and its metabolite) because its concomitant ability to stimulate 5-HT$_{1B/1D}$ receptors (Buzzi *et al*., 1991; Nozaki *et al*., 1992; Hoskin *et al.,* 1996), to desensitize 5-HT$_{2B}$ receptors (Hamon *et al*., 2002) and to block 5-HT$_7$ receptors (Ruat *et al*., 1993) also probably accounts for the prevention of migraine under chronic treatment conditions (Schmuck *et al*., 1996; Terron, 2002).

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