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Modulation of the firing activity of noradrenergic neurones in the rat locus coeruleus by the 5-hydroxtryptamine system

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1 The aim of the present study was to investigate the putative modulation of locus coeruleus (LC) noradrenergic (NA) neurones by the 5-hydroxytryptaminergic (5-HT) system by use of *in vivo* extracellular unitary recordings and microiontophoresis in anaesthetized rats. To this end, the potent and selective 5-HT_{1A} receptor antagonist WAY 100635 (*N*-{2-[4(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl)cyclohexanecarboxamide trihydroxychloride) was used.

2 In the dorsal hippocampus, both local (by microiontophoresis, 20 nA) and systemic (100 μg kg^{-1}, i.v.) administration of WAY 100635 antagonized the suppressant effect of microiontophorectically-applied 5-HT on the firing activity of CA_3 pyramidal neurones, indicating its antagonistic effect on postsynaptic 5-HT_{1A} receptors.

3 WAY 100635 and 5-HT failed to modify the spontaneous firing activity of LC NA neurones when applied by microiontophoresis. However, the intravenous injection of WAY 100635 (100 μg kg^{-1}) readily suppressed the spontaneous firing activity of LC NA neurones.

4 The lesion of 5-HT neurones with the neurotoxin 5,7-dihydroxytryptamine increased the spontaneous firing activity of LC NA neurones and abolished the suppressant effect of WAY 100635 on the firing activity of LC NA neurones.

5 In order to determine the nature of the 5-HT receptor subtypes mediating the suppressant effect of WAY 100635 on NA neurone firing activity, several 5-HT receptor antagonists were used. The selective 5-HT_3 receptor antagonist BRL 46470A (10 and 100 μg kg^{-1}, i.v.), the 5-HT_{1D} receptor antagonist GR 127935 (100 μg kg^{-1}, i.v.) and the 5-$HT_{1A/1B}$ receptor antagonist (−)-pindolol (15 mg kg^{-1}, i.p.) did not prevent the suppressant effect of WAY 100635 on the firing activity of LC NA neurones. However, the suppressant effect of WAY 100635 was prevented by the non-selective 5-HT receptor antagonists spiperone (1 mg kg^{-1}, i.v.) and metergoline (1 mg kg^{-1}, i.v.), by the 5-HT_2 receptor antagonist ritanserin (500 μg kg^{-1}, i.v.). It was also prevented by the 5-HT_{1A} receptor/α_{1D}-adrenoceptor antagonist BMY 7378 (1 mg kg^{-1}, i.v.) and by the α_1-adrenoceptor antagonist prazosin (100 μg kg^{-1}, i.v.).

6 These data support the notion that the 5-HT system tonically modulates NA neurotransmission since the lesion of 5-HT neurones enhanced the LC NA neurones firing activity and the suppressant effect of WAY 100635 on the firing activity of NA neurones was abolished by this lesion. However, the location of the 5-HT_{1A} receptors involved in this complex circuitry remains to be elucidated. It is concluded that the suppressant effect of WAY 100635 on the firing activity of LC NA neurones is due to an enhancement of the function of 5-HT neurones via a presynaptic 5-HT_{1A} receptor. In contrast, the postsynaptic 5-HT receptor mediating this effect of WAY 100635 on NA neurones appears to be of the 5-HT_{2A} subtype.

Keywords: Extracellular unitary recordings; microiontophoresis; presynaptic modulation; 5-HT receptors; dorsal hippocampus; locus coeruleus; WAY 100635

Introduction

It is well established that noradrenergic (NA) neurones modulate the 5-hydroxytryptaminergic (5-HT) system. Dorsal raphe 5-HT neurones receive adrenergic projections from the locus coeruleus (LC) (Loizou, 1969; Anderson *et al*., 1977; Baraban & Aghajanian, 1981; Jones & Yang, 1985; Luppi *et al*., 1995), and pharmacological studies have suggested that the firing activity of 5-HT neurones in the dorsal raphe is dependent on a tonic activation by a noradrenergic input mediated via α_1-adrenoceptors (Svensson *et al*., 1975; Baraban & Aghajanian, 1980; Clement *et al*., 1992). Yoshioka *et al*. (1992) have shown that α_2-adrenoceptor activation reduces 5-HT synthesis in both hippocampus and dorsal raphe nucleus. On the other hand, several lines of evidence support the notion that the 5-HT system also influences brain NA neurones. NA neurones of the LC receive dense 5-HT projections (Pickel *et al*., 1977; Cedarbaum & Aghajanian, 1978; Léger & Descarries, 1978; Segal, 1979; Maeda *et al*., 1991; Vertes & Kocsis, 1994), which do not originate from the dorsal raphe nucleus (Pieribone *et al*., 1989; Aston-Jones *et al*., 1991a). Electrophysiological and biochemical studies have revealed an inhibitory role of 5-HT on the function of LC NA neurones. In particular, although microiontophoretic application of 5-HT agonists does not modify the spontaneous firing activity of LC NA neurones, the activation of 5-HT_1 receptors reduces both glutamate-induced activation and glutamatergic synaptic potentials of these NA neurones (Bobker & Williams, 1989; Aston-Jones *et al*., 1991b). However, in contrast to 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT), partial 5-HT_{1A} agonists, such as buspirone, gepirone, ipsapirone and their common metabolite 1-(2-pyrimidinyl)-piperazine, which is an α_2-adrenoceptor antagonist, have been shown to increase LC neurone firing activity (Sanghera *et al*., 1983; 1990; Engberg, 1992). Systemic but not local administration of selective 5-HT_2 receptor antagonists increase (Rasmussen & Aghajanian, 1986; Gorea & Adrien, 1988; Aghajanian *et al*., 1990) whereas the 5-HT_2 agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-amino-propane (DOI), administered systemically but not locally, de-

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creases the firing activity of LC NA neurones. The latter effect has been proposed to be due to an increased activation of the GABA inhibitory input to the LC (Chiang & Aston-Jones, 1993). Taken together, these data suggest an indirect effect of 5-HT_2 receptor ligands. Raphe nuclei lesions induce an increase of the tyrosine hydroxylase activity in LC (McRae-Degueurce *et al*., 1982) and pretreatment with the 5-HT synthesis inhibitor parachlorophenylalanine increases both tyrosine hydroxylase and the firing activities of LC NA neurones (Crespi 1980; Reader 1986). Furthermore, several studies have demonstrated, *in vivo* and *in vitro*, 5-HT modulations of the release of NA (Blandina *et al*., 1991; Done & Sharp, 1992; Mongeau *et al*., 1994; Matsumoto *et al*., 1995).

An important drawback of the results obtained with various pharmacological agents to study the 5-HT-NA interactions is the lack of selectivity of the drugs used. WAY 100635 (*N*-{2-[4(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl) cyclohexanecarboxamide trihydroxychloride), has been shown to be a potent and selective antagonist of both pre- and postsynaptic 5-HT_{1A} receptors (Fletcher *et al*., 1994; 1996; Fornal *et al*., 1994; Khawaja *et al*., 1994; Gurling *et al*, 1994; Golzan *et al*., 1995; Mundey *et al*., 1994; 1995). The present studies were undertaken to characterize the effects of WAY 100635 by use of *in vivo* electrophysiological paradigms, i.e. extracellular unitary recording and microiontophoresis in anaesthetized male Sprague-Dawley rats on the firing activity of LC NA neurones. The effects of WAY 100635, administered locally or intravenously, on the spontaneous firing activity of LC NA neurones were assessed in an attempt to characterize further the 5-HT modulation of the noradrenergic system.

Methods

The experiments were carried out in male Sprague-Dawley rats weighing 250 to 300 g which were kept under standard laboratory conditions (12:12 light-dark cycle with free access to food and water). They were anaesthetized with chloral hydrate (400 mg kg^{-1}, i.p.) and additional doses were given to maintain constant anaesthesia, monitored by the absence of nociceptive reaction to a tail pinch.

Extracellular unitary recording from CA_3 dorsal hippocampus pyramidal neurones

Recording and microiontophoresis were performed with five-barrelled glass micropipettes broken back to 8–12 μm under microscope control (ASI Instruments, Warren, MI, U.S.A.). The central barrel was filled with a 2 M NaCl solution and used for extracellular unitary recordings. The pyramidal neurones were identified by their large amplitude (0.5–1.2 mV) and long-duration (0.8–1.2 ms) simple spikes alternating with complex spike discharges (Kandel & Spencer, 1961). The side barrels contained the following solutions: 5-HT creatinine sulphate (20 mM in 200 mM NaCl, pH 4), WAY 100635 (10 mM in 200 mM NaCl, pH 3.8), quisqualate (1.5 mM in 200 mM NaCl, pH 8) and 2 M NaCl used for automatic current balancing. The rats were mounted in a stereotaxic apparatus and the micropipettes were lowered at 4.2 mm lateral and 4.2 anterior to lambda into the CA_3 region of the dorsal hippocampus. Since most hippocampus pyramidal neurones are not spontaneously active under chloral hydrate anaesthesia, a leak or a small ejection current of quisqualate (+1 to −6 nA) was used to activate them within their physiological firing range (10–15 Hz; Ranck, 1975). Neuronal responsiveness to the microinotophoretic application of 5-HT was assessed by determining the number of spikes suppressed per nA. The duration of the microiontophoretic applications of the agonist was 50 s. The same ejection current of 5-HT was always used before and during the microiontophoretic application of WAY 100635 for a duration of about 5 min. In order to assess the effectiveness of the lesion of 5-HT neurones with the neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT), the recovery time 50 (RT_{50}) method was used. The RT_{50} value represents the time (in s.) required by the neurone to recover 50% of its initial firing rate from the end of the microiontophoretic application of 5-HT. The RT_{50} value has been shown to be a reliable index of the *in vivo* activity of the 5-HT reuptake process in the rat hippocampus (Piñeyro *et al*., 1994). For instance, in 5,7-DHT-lesioned rats, according to the current of 5-HT used, the RT_{50} value is 2 to 3 times greater than in control rats (Piñeyro *et al*., 1994).

Microiontophoresis and unitary extracellular recordings from LC noradrenergic neurones

The microiontophoresis were performed with five-barrelled micropipettes (R & D Scientific glass CO, Spencerville, MD, U.S.A.) preloaded with fibreglass filaments in order to facilitate filling and the tip was broken back to 4 to 8 μm. The central barrel was used for recording and filled with a 2 M NaCl solution. The side barrels contained the following solutions: 5-HT creatinine sulphate (20 mM in 200 mM NaCl, pH 4), noradrenaline bitartrate (20 mM in 100 mM NaCl, pH 4), WAY 100635 (10 mM in 200 mM NaCl, pH 3.8) and 2 M NaCl used for automatic current balancing. The duration of the microiontophoretic applications of the drugs were 40 s. Locus coeruleus NA neurones were recorded with micropipettes lowered at −0.7 mm interaural and 1.1. to 1.4 mm lateral (Paxinos & Watson, 1982). The NA neurones were identified by their regular firing rate (1–5 Hz), long-duration (0.8–1.2 ms) positive action potentials and their characteristic burst discharge in response to nociceptive pinch of the contralateral hind paw (Aghajanian, 1978).

By unitary extracellular recording with single-barrelled glass micropipettes, the responsiveness of LC NA neurones to the intravenous injection of WAY 100635 (100 μg kg^{-1}) was assessed alone and after the following drugs: the selective 5-HT_3 antagonist BRL 46470A (10 and 100 μg kg^{-1}, i.v.; Newberry *et al*., 1993), the 5-$HT_{1B/1D}$ antagonist GR 127935 (100 μg kg^{-1}, i.v.; Skingle *et al*., 1993), the 5-HT_2 antagonist ritanserin (500 μg kg^{-1}, i.v.; Leysen *et al*., 1985), the 5-HT_{1A} receptor α_{1D}-adrenoceptor antagonist BMY 7378 (1 mg kg^{-1}, i.v.; Chaput & de Montigny, 1988) and the 5-$HT_{1A/1B}$ antagonist (−)-pindolol (15 mg kg^{-1}, i.p.; Romero *et al*., 1996), the non-selective 5-HT antagonists spiperone (1 mg kg^{-1}, i.v.; Griebel, 1995) and metergoline (1 mg kg^{-1}, i.v.; Fuxe *et al*., 1975), and the α_1-adrenoceptor antagonist prazosin (0.1 mg kg^{-1}, i.v.; Marwaha & Aghajanian, 1982). The effect of WAY 100635 (100 μg kg^{-1}, i.v.) on the spontaneous firing rate of NA neurones was also assessed after a pretreatment with 5,7-DHT to lesion 5-HT neurones. The latter was performed under chloral hydrate anaesthesia by injecting 5,7-DHT intracerebroventricularly (200 μg free base in 20 μl of 0.9% NaCl and 0.1% ascorbic acid) 1 h after the injection of desipramine (25 mg kg^{-1}, i.p.) to protect NA neurones from the neurotoxic action of 5,7-DHT. The rats were tested 10 days later. In order to determine possible changes of the spontaneous firing activity of LC NA neurones, four to five electrode descents were carried out through this nucleus in control and 5,7-DHT-pretreated rats.

Drugs

WAY 100635 (Wyeth Research, Berkshire, U.K.); clonidine, NA bitartrate, quisqualic acid, 5,7-DHT creatinine sulphate (Sigma Chemical, St. Louis, MO, U.S.A.); BMY 7378 (8-[2-[4-(2-methyoxyphenyl)-1-piperazinyl]ethyl-8-azapirol[4,5]-decane-7.9-dione dihydrochloride; Bristol-Myers Squibb, Wallingford, CT, U.S.A.); metergoline (Farmitalia, Milano, Italia); ritanserin, spiperone, (−)-pindolol and desipramine HCl (Research Biochemicals, Natick, MA, U.S.A.); GR 127935 (*N*-[methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)[1,1,-biphenyl]-4-carboxamide; Glaxo Research, Greenford, U.K.); BRL 46470A ((endo-N-methyl-8-azabicyclo[3,2,1]oct-3yl)-2, 3-dihy-

dro-3, 3-dimethyl-indole-1-carboxamide; Smith Kline Beecham, Harlow, U.K.); (±)-mirtazapine (Organon, Oss, The Netherlands); prazosin HCl (Pfizer, Kirkland, Canada). The concentrations and the doses used for these compounds were chosen on the basis of previous successful experiments carried out in our and other laboratories.

Results

Effect of WAY 100635 on the responsiveness of CA_3 dorsal hippocampus pyramidal neurones to 5-HT

In an initial series of experiments, the capacity of WAY 100635 to block postsynaptic 5-HT_{1A} receptors was verified. It has been previously demonstrated *in vivo* that the microiontophoretic application of 5-HT onto rat dorsal hippocampus pyramidal neurones produces a suppressant effect on their firing activity and that this effect is mediated by postsynaptic 5-HT_{1A} receptors (Blier & de Montigny, 1987; Chaput & Montigny, 1988). When applied on dorsal hippocampus CA_3 pyramidal neurones, 5-HT induced a current-dependent (5–10 nA) reduction of firing activity, generally from 30 to 100% (Figure 1a and b). This suppressant effect occurred in the absence of alteration of the shape of the action potential. In the present study, the systemic administration of WAY 100635 (100 μg kg^{-1}, i.v.) significantly reduced the suppressant effect of 5-HT, applied microiontophoretically, on the firing activity of CA_3 hippocampus pyramidal neurones (spikes suppressed/nA of 5-HT before WAY 100635 = 101 ± 16; after WAY 100635 injection = 48 ± 14, $P<0.05$ by paired Student's t test, $n=8$). As illustrated in Figure 1a, the microiontophoretic application of WAY 100635 (20 nA) did not reduce by itself the firing activity of these neurones, but it attenuated the suppressant effect of 5-HT co-applied through the same micropipette. Figure 1b shows the mean reduction (31%) of the suppressant effect of 5-HT by the concomitant microiontophoretic application of WAY 100635 (20 nA). This confirms the 5-HT_{1A} antagonistic activity of WAY 100635 at postsynaptic 5-HT_{1A} receptors in the dorsal hippocampus.

Effect of WAY 100635 on the firing activity of LC noradrenergic neurones

The effect of WAY 100635 administered locally and systemically was then investigated on electrophysiologically-identified LC NA neurones. As illustrated in Figures 2a and 7a, WAY 100635 (100 μg kg^{-1}, i.v.) reduced the firing activity of LC NA neurones. This suppressant effect of WAY 100635 on the firing activity of LC NA neurones always appeared with a short latency (<60 s).

In order to determine whether the suppressant effect of WAY 100635 on the spontaneous firing rate of the LC NA neurones was due to direct action of this compound on postsynaptic 5-HT receptors in the immediate vicinity of the cell body of NA neurones, this 5-HT_{1A} antagonist was applied by microiontophoresis onto these neurones. As illustrated in Fig-

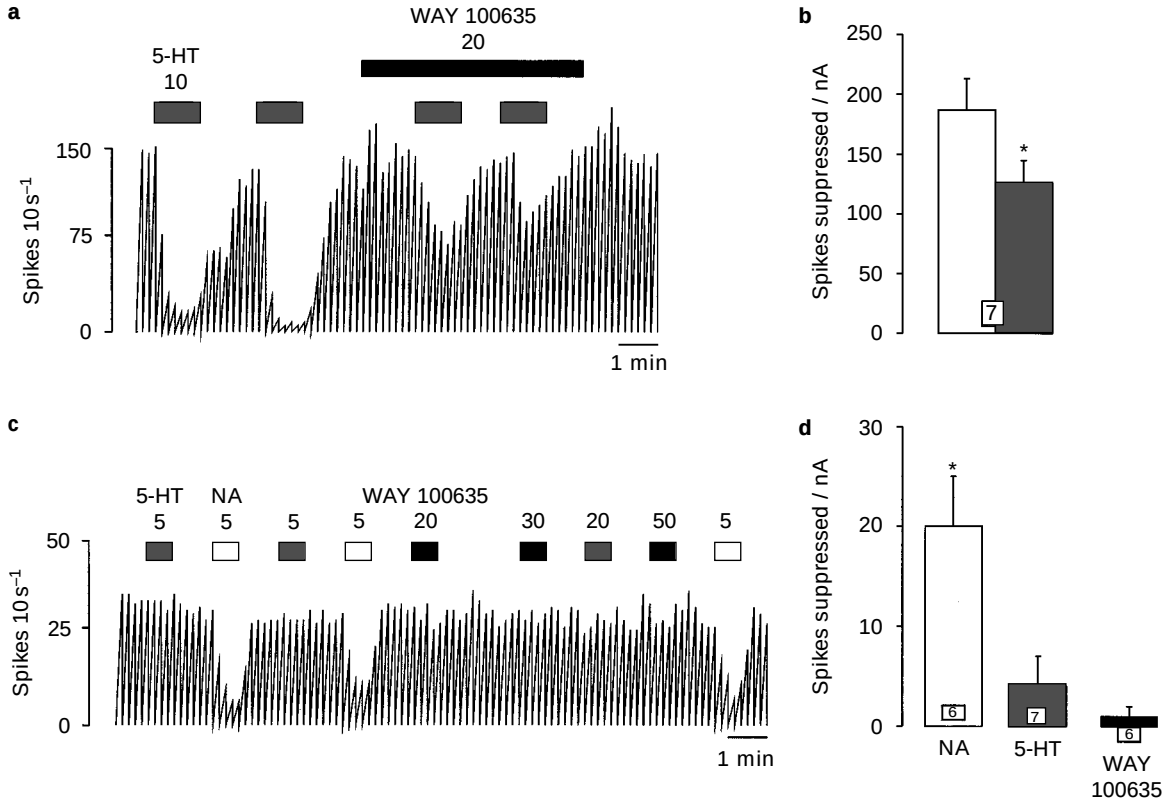

Figure 1 (a) Integrated firing rate histograms of the dorsal hippocampus CA_3 pyramidal neurone showing its responsiveness to microiontophoretic application of 5-HT before and during the local administration of WAY 100635. This neurone was activated with quisqualate ejection current of −2 nA. Horizontal bars indicate the duration of the applications for which the current is given in nA. Corresponding results (mean ± s.e.mean) are presented in (b); responses to 5-HT (open column) before and (stippled column) after the application of WAY 100635. The numbers at the bottom of the columns indicate the number of neurones tested. *$P<0.05$, by paired Student's t test. (c) Integrated firing rate histograms of LC noradrenergic neurone showing its responsiveness to microiontophoretic application of NA, 5-HT and WAY 100635. The firing activity of this neurone was spontaneous. Horizontal bars indicate the duration of the applications for which the current is given in nA. Corresponding results (means ± s.e.mean) are presented in (d). Note that only the microiontophoretic application of NA significantly modified the firing activity of LC neurones. *$P<0.05$, by unpaired Student's t test (Comparing the number of spikes in the 40 s period immediately preceding the microiontophoretic application and the number of spikes during the application).

ures 1c and d), the spontaneous firing activity of LC NA neurones was not altered by the microiontophoretic application of WAY 100635. Furthermore, 5-HT applied microiontophoretically did not modify the firing activity of LC NA neurones whereas NA reduced it (Figure 1c and d).

In order to determine a possible involvement of 5-HT neurones in the suppressant effect of WAY 100635 on the firing activity of LC NA neurones, the lesioning of 5-HT neurones was performed with the neurotoxin 5,7-DHT. As illustrated in Figure 2, the suppressant effect of WAY 100635 was markedly attenuated by the 5,7-DHT pretreatment. In 5,7-DHT-lesioned rats, the suppressant effect of WAY 100635 on the spontaneous firing activity of LC NA neurones was reduced by 63% (Figure 2c). Moreover, the increased firing activity observed after 5,7-DHT pretreatment could not account for the reduced suppressant effect of WAY 100635 ($P>0.05$, by ANCOVA).

In each 5,7-DHT-lesioned rat, the effectiveness of the 5,7-DHT pretreatment was verified by use of the RT_{50} method in the dorsal hippocampus. As illustrated in Figure 3, 5-HT denervation markedly prolonged the effect of 5-HT microiontophorectically-applied onto dorsal hippocampus CA_3 pyramidal neurones: the RT_{50} value was increased by 488% in 5,7-DHT-lesioned rats, thus confirming a thorough destruction of 5-HT neurones.

In order to determine the spontaneous firing activity of LC NA neurones, four to five electrode descents through the LC were carried out in each of the control and 5,7-DHT-pretreated rats. As illustrated in Figure 4, the lesioning of 5-HT neurones produced a 67% increase in the firing activity of LC NA neurones.

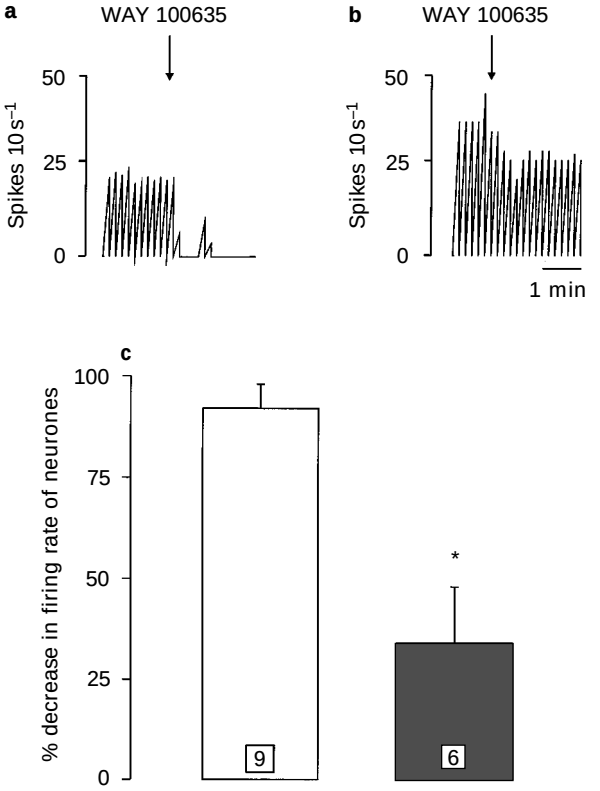

Figure 2 Integrated firing rate histogram of noradrenergic neurones recorded in the LC showing their responses to WAY 100635 (100 μg kg^{-1}, i.v.) in a sham-operated rat (a) and in a 5,7-DHT pretreated rat (b). (c) Responsiveness of noradrenergic neurones to WAY 100635 (100 μg kg^{-1}, i.v.) in control (open columns) and 5,7-DHT-pretreated (stippled column) rats (means ± s.e.mean). The numbers at the bottom of the columns indicate the number of neurones tested. *$P<0.05$, by unpaired Student's t test.

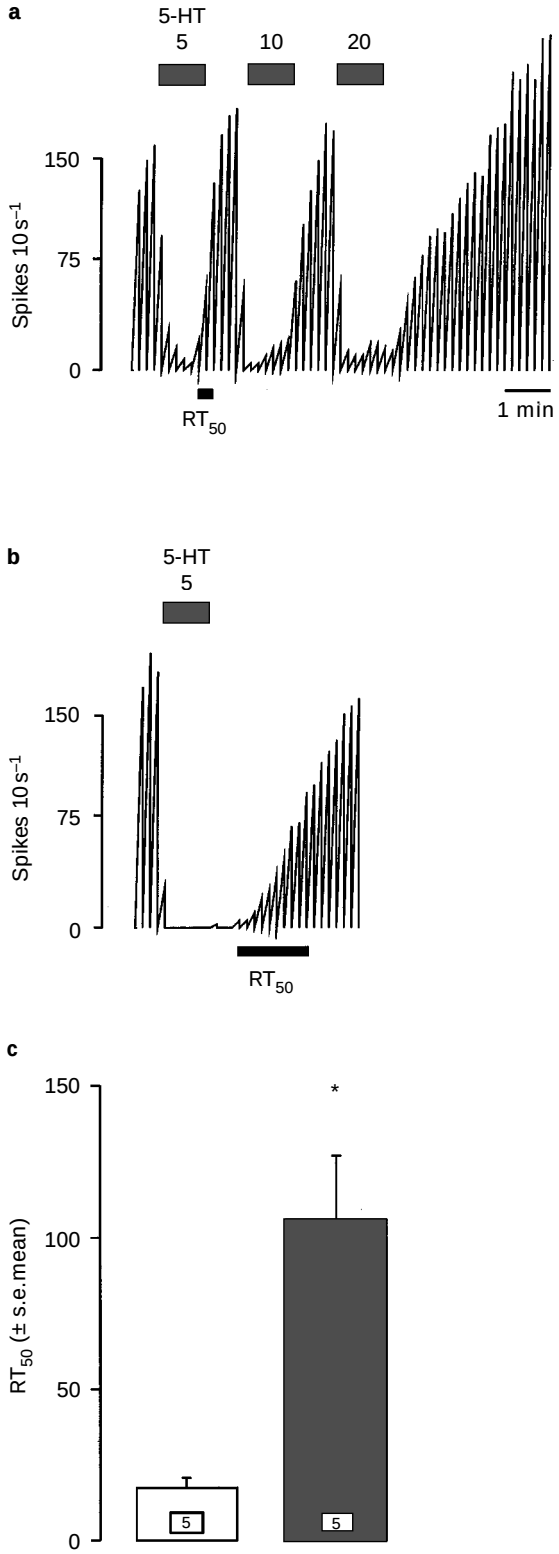

Figure 3 Integrated firing rate histograms of two dorsal hippocampus CA_3 pyramidal neurones in sham operated (a) and 5,7-DHT pretreated (b) rats showing their responsiveness to microiontophoretic application of 5-HT. These neurones were activated with quisqualate ejection current of −1 and −3 nA, respectively. The solid horizontal bars indicate the duration of the applications for which the current is given in nA and the hatched bars indicated RT_{50} (see Methods section). For 5 nA applications of 5-HT, corresponding results (mean ± s.e.mean) are presented (c); (open column) sham operated rats, (stippled column) 5,7-DHT pretreated rats. The number at the bottom of the columns indicate the number of neurones tested, one neurone in each rat. *$P<0.05$, by unpaired Student's t test.

Effects of 5-HT antagonists on the suppression of the firing activity of LC noradrenergic neurones by WAY 100635

Given that the suppressant effect of WAY 100635 on the firing activity of LC NA neurones was prevented by lesioning 5-HT neurones, the nature of the presynaptic 5-HT receptor involved was then investigated with 5-HT antagonists. As illustrated in Figure 5(a, b and c), the selective 5-HT_3 receptor antagonist BRL 46470A (10 and 100 μg kg^{-1}, i.v.) and the 5-$HT_{1B/1D}$ receptor antagonist GR 127935 (100 μg kg^{-1}, i.v.) did not modify by themselves the spontaneous firing activity of LC NA neurones and failed to alter the suppressant effect of WAY 100635 (100 μg kg^{-1}, i.v.) on the firing activity of LC NA neurones (Figure 5d). Figure 6 illustrates the effects of pretreatment, with the 5-HT receptor antagonists ritanserin, metergoline and spiperone. As shown by Rasmussen and Aghajanian (1986), the 5-HT_2 receptor antagonist ritanserin (500 μg kg^{-1}, i.v.) increased the firing activity of LC NA neurones by $20 \pm 6\%$, ($P < 0.05$, $n = 7$), although not all neurones were affected (see Figure 6a). Ritanserin blocked the suppressant effect of WAY 100635 on the firing activity of these neurones (Figure 6a and d). The 5-$HT_{1/2}$ antagonist metergoline (1 mg kg^{-1}, i.v.) by itself, but not the 5-$HT_{1A/2A}$ antagonist spiperone (1 mg kg^{-1}, i.v.), increased the firing activity of LC NA neurones, by $26 \pm 8\%$ ($P < 0.05$, $n = 7$, data not shown), and both drugs reduced the suppressant effect of WAY 100635 on the firing activity of these neurones. The mean antagonistic effects of ritanserin, metergoline and spiperone are presented in Figure 6d. The 5-HT_{1A} antagonist BMY 7378 increased the firing activity of LC NA neurones and prevents the suppressant effect of (−)-mirtazapine, an α_2-heteroreceptor antagonist, on these neurones (Haddjeri *et al*., 1996). In addition, BMY 7378 inhibits the firing activity of dorsal raphe 5-HT neurones with an ED_{50} of about 20 μg kg^{-1}, i.v. (Chaput & de Montigny, 1988; Cox *et al*., 1993) and reduces the release of 5-HT in the ventral hippocampus (Hjorth *et al*., 1995). In the present study, the suppressant effect of WAY 100635 was both prevented (Figure 7c and d) and reversed (Figure 5b) by BMY 7378 (1 mg kg^{-1}, i.v.). Pretreatment with either WAY 100635 or BMY 7378 did not modify the suppressant effect of the α_2-adrenoceptor agonist clonidine, showing that these two 5-HT antagonists did not block the somatodendritic α_2-autoreceptor (Figure 7c). In order to determine whether the suppressant effect of WAY 100635 on the firing activity of LC NA neurones was due to an antagonism of the cell body 5-HT_{1A} autoreceptors of 5-HT neurones, (−)-pindolol, an effective antagonist of these receptors (Romero *et al*., 1996), was used. (−)-Pindolol (15 mg kg^{-1}, i.p., administered 20 min before the recording from LC NA neurones) appeared to reduce by itself the firing activity of LC NA neurones; 4 of the 6 neurones recorded had a firing activity below 1 Hz (1.2 ± 0.2 Hz, $n = 6$) (Figure 7b). However, (−)-pindolol failed to alter the suppressant effect of WAY 100635 on the firing activity of these neurones (Figure 7b and d). WAY 100635 has also moderate (0.23 μM) affinity for α_1-adrenoceptor binding sites (Fletcher *et al*., 1996). In order to assess the possible involvement of α_1-adrenoceptors in the suppressant effect of WAY 100635, the α_1-adrenoceptor antagonist prazosin was used. For 7 rats, prazosin (0.1 mg kg^{-1}, i.v.) by itself did not modify significantly the spontaneous firing activity of LC NA neurones (data not shown). However, this dose of prazosin prevented the suppressant effect of WAY 100635 on the firing activity of LC NA neurones (decrease of firing in control: $92 \pm 6\%$; decrease in firing after prazosin: $11 \pm 7\%$, $P < 0.01$).

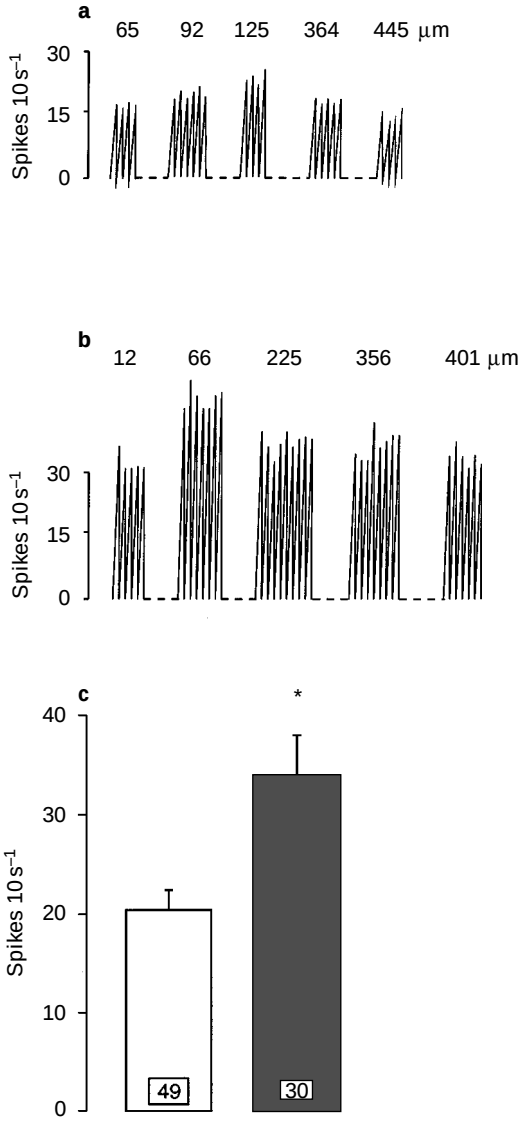

Figure 4 Integrated firing rate histograms of LC noradrenergic neurones, recorded in one electrode descent in the LC, showing their spontaneous firing activity in control (a) and 5,7-DHT-pretreated rats (b). The number above each neurone indicates the depth from the floor of the fourth ventricle at which it was recorded. Corresponding results (means ± s.e.mean) are presented on (c); (open column) control and (stippled column) 5,7-DHT pretreated rats. The numbers at the bottom of the columns indicate the number of neurones tested. *$P < 0.05$ (unpaired Student's t test).

Discussion

The microiontophoretic application of WAY 100635, as well as its intravenous administration, significantly attenuated the suppressant effect of microiontophoretically-applied 5-HT on the firing activity of CA_3 dorsal hippocampus pyramidal neurones, thus demonstrating its antagonistic action at postsynaptic 5-HT_{1A} receptors (Figure 1a and b). In contrast to NA, WAY 100635 and 5-HT, applied directly by microiontophoresis onto NA neurones, failed to modify their spontaneous firing activity (Figure 1b and d). However, the systemic administration of WAY 100635 (100 μg kg^{-1}, i.v.) suppressed the spontaneous firing activity of LC NA neurones (Figure 2a). This suppressant effect of WAY 100635 on the firing activity of LC NA neurones was prevented by a 5,7-DHT pretreatment (Figure 2b and c). Furthermore, after the 5-HT system had been lesioned, the firing activity of LC NA neurones was increased, suggesting the existence of a tonic inhibition of LC NA neurones by their 5-HT afferents (Figure 4). In order to determine the nature of the presynaptic 5-HT receptor involved in this suppressant effect of WAY 100635, several 5-HT receptor antagonists were used. The 5-HT_3 receptor antagonist BRL 46470A, the 5-$HT_{1B/1D}$ receptor antagonist GR 127935 and the 5-$HT_{1A/1B}$ receptor antagonist (−)-pindolol failed to prevent the effect of WAY 100635 (Figures 5; 7b and d). However, the suppressant effect of WAY

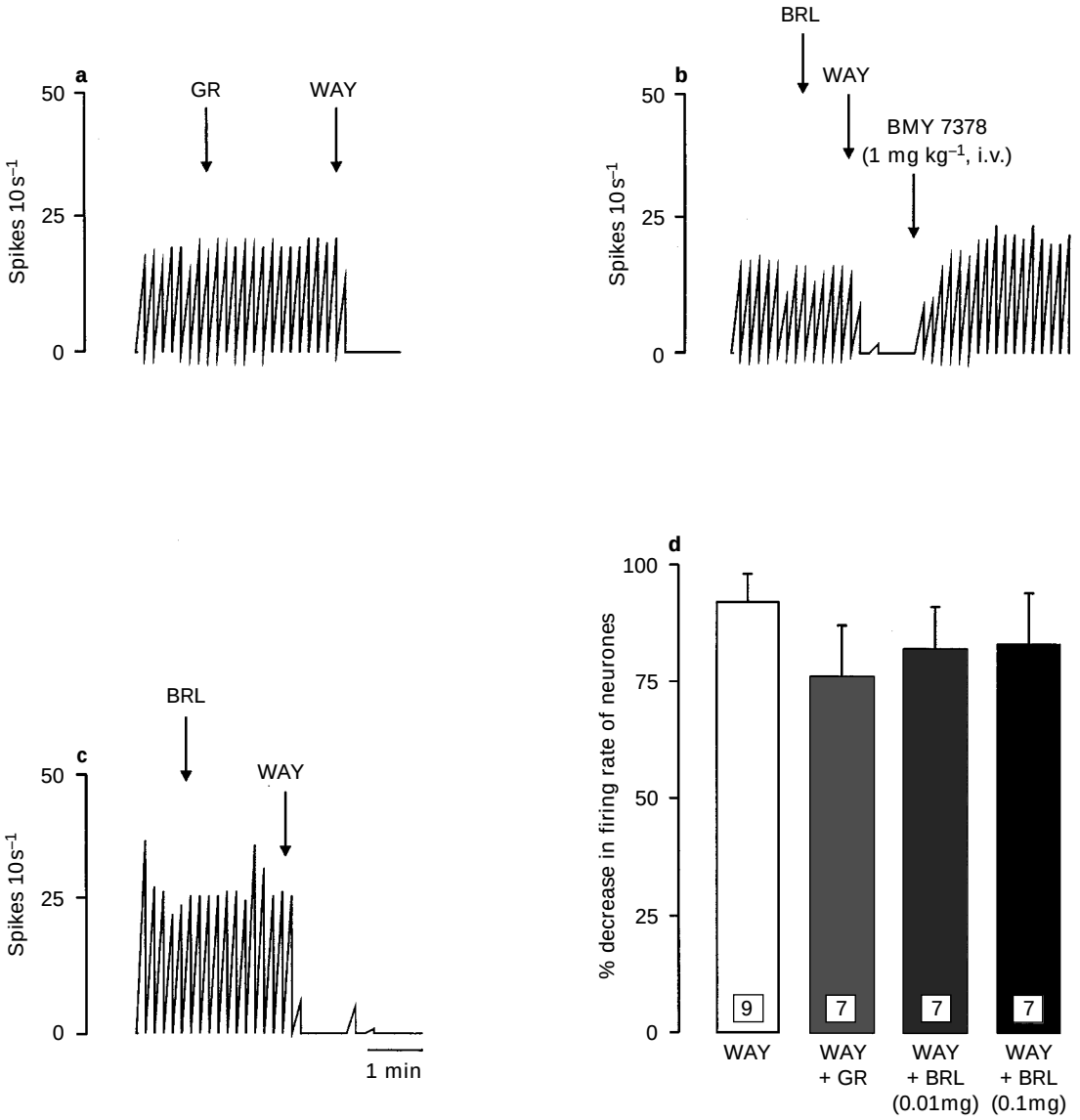

Figure 5 Integrated firing rate histograms of noradrenergic neurones recorded in the LC showing their responses to WAY 100635 (WAY, 100 μg kg^{-1}, i.v.) after the administration of GR 127935 (GR, 100 μg kg^{-1}, i.v.) (a), in another rat after the injection of BRL 46470A, (BRL, 10 μg kg^{-1}, i.v.) in (b) and 100 μg kg^{-1}, i.v., in (c). (d) Responsiveness of NA neurones to WAY 100635 (100 μg kg^{-1}, i.v.) in control, in GR 127935 and BRL 46470A-pretreated rats (means $\pm$ s.e.mean).

100635 was prevented by the 5-HT$_{1A/2A}$ receptor antagonist spiperone, by the non-selective 5-HT receptor antagonist metergoline, by the 5-HT$_2$ receptor antagonist ritanserin (Figure 6 and 7), by the 5-HT$_{1A}$ receptor α_{1D}-adrenoceptor antagonist BMY 7378 and by the α_1-adrenoceptor antagonist prazosin.

WAY 100635 is a potent and selective antagonist at both pre- and postsynaptic 5-HT$_{1A}$ receptors (Fletcher *et al*., 1996). Khawaja *et al*. (1994) have shown, by use of [^{3}H]-WAY 100635, that this ligand has an affinity of 0.37 nM and a maximal binding capacity of 312 fmol mg^{-1} protein at 5-HT$_{1A}$ binding sites in rat hippocampal membranes. Moreover, the maximal number of binding sites labelled with [^{3}H]-WAY 100635 was 36% higher than those labelled by [^{3}H]-8-OH-DPAT. This is probably due to the fact that WAY 100635 is a high affinity ligand of both G-protein-coupled and free 5-HT$_{1A}$ receptors binding subunits, whereas 8-OH-DPAT only binds to G-protein-coupled 5-HT$_{1A}$ receptors (Golzan *et al*., 1995). In rat cortical membranes, WAY 100635 can also bind to α_1-adrenoceptors labelled with [^{3}H]-prazosin, but with an affinity 300 times lower than that for 5-HT$_{1A}$ receptors (Fletcher *et al*., 1996). It is unlikely that the low α_1-adrenoceptor affinity of WAY 100635 could contribute to the suppressant effect on LC NA neurones firing activity since Marwaha & Aghajanian (1982) have previously shown that α_1-adrenoceptor antagonists, including prazosin, do not affect the spontaneous firing activity of LC NA neurones. Mundey *et al*. (1994) in the guinea-pig and Gartside *et al*. (1995) in the rat have observed that intravenous administration of WAY 100635 increases the firing activity of dorsal raphe 5-HT neurones, whereas Fletcher *et al*. (1994) found no change in the rat. WAY 100635, applied microiontophoretically, prevented the suppressant effect of the 5-HT$_{1A}$ agonist 8-OH-DPAT on the firing activity of dorsal raphe 5-HT neurones in guinea-pigs (Mundey *et al*., 1995) and WAY 100635 (applied by microiontophoresis and administered intravenously) antagonized the suppressant effect of microiontophoretically-applied 5-HT on the rat dorsal raphe 5-HT neurones (Author's unpublished observations). Furthermore, several studies have shown that systemic administration of WAY 100635 can antagonize the suppressant effects of 5-HT and of 8-OH-DPAT (Fletcher *et al*., 1994; Mundey *et al*., 1994; Craven *et al*., 1994), and that of the selective 5-HT reuptake inhibitor (SSRI), paroxetine (Gartside *et al*., 1995) on 5-HT neurone firing activity. Furthermore, the increase of extracellular 5-HT levels in the rat ventral hippocampus induced by the SSRI, citalopram, was enhanced by WAY 100635 (Hjorth & Milano, 1995) and WAY 100635 prevented the

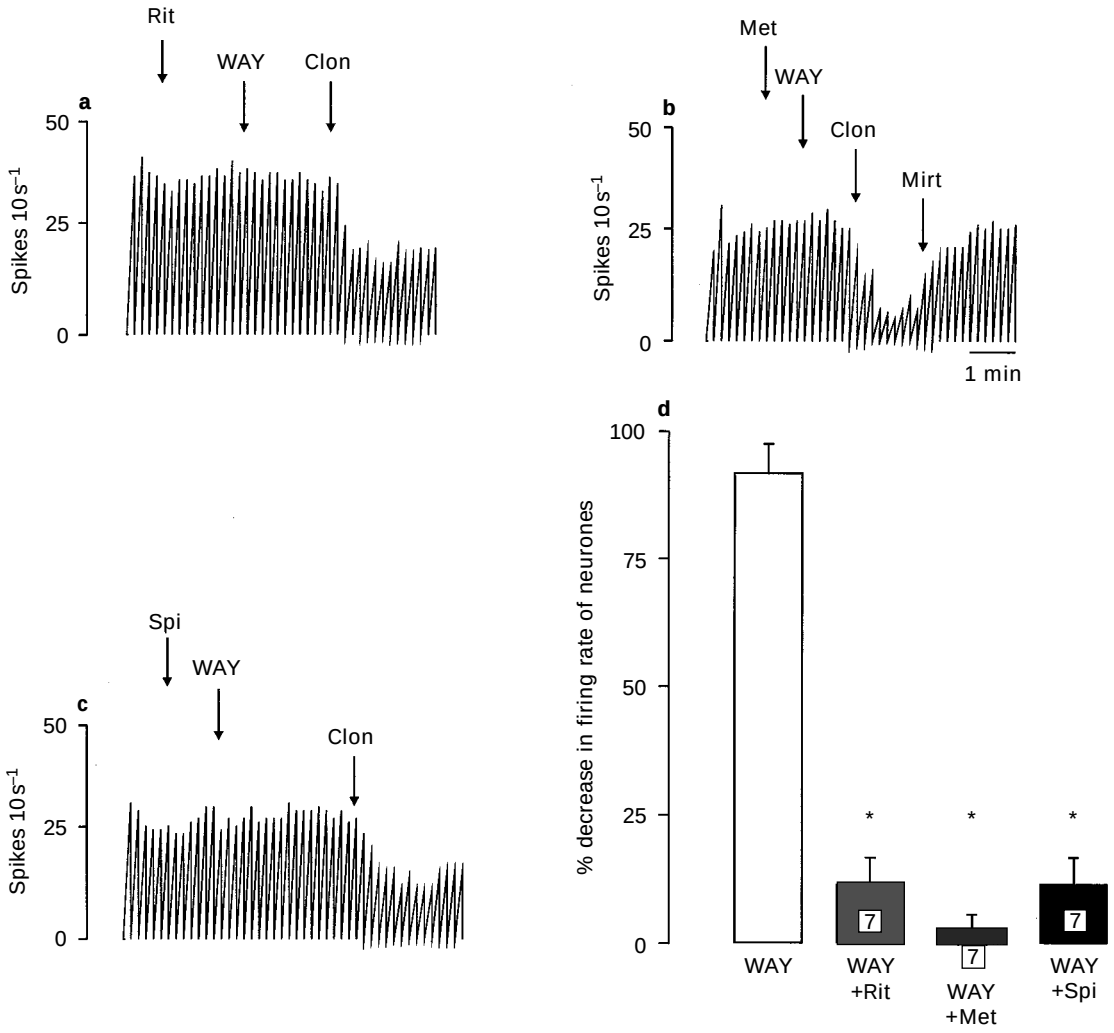

Figure 6 Integrated firing rate histogram of noradrenergic neurones recorded in the LC showing their responses to WAY 100635 (WAY, 100 μg kg^{-1}, i.v.) after the administration of ritanserin (Rit, 0.5 mg kg^{-1}, i.v.) (a), after the injection of metergoline (Met, 1 mg kg^{-1}, i.v.) (b) and spiperone (Spi, 1 mg kg^{-1}, i.v.) in (c). Responses to clonidine (Clon, 3 μg kg^{-1}, i.v.) are also shown (in (b) 5 μg kg^{-1}, i.v.) and in (b) to (±)-mirtazapine (Mirt, 250 μg kg^{-1}, i.v.). (d) Responsiveness of NA neurones to WAY 100635 (100 μg kg^{-1}, i.v.) in control, ritanserin, metergoline and spiperone-pretreated rats (means ± s.e.mean). *$P<0.05$ (unpaired Student's t test).

decrease of 5-HT release induced by systemic administration of 8-OH-DPAT in the rat ventral hippocampus (Gurling *et al*., 1994) and in the dorsal raphe (Davidson & Stamford, 1995). Taken together, these results indicate that WAY 100635 is indeed an effective antagonist of both pre- and postsynaptic 5-HT$_{1A}$ receptors.

In the present study, the intravenous administration, but not the microiontophoretic application, of WAY 100635 suppressed the firing activity of LC NA neurones. It is important to emphasize that the lack of effect observed with local application of WAY 100635 on the soma of LC NA neurones does not exclude a possible involvement of receptors on the dendritic tree, as is the case with the suppressant effect of apomorphine on the firing activity of nigral dopamine neurones (Akaoka *et al*., 1992). However, the suppressant effect of WAY 100635 was prevented by 5,7-DHT pretreatment, implying the involvement of a presynaptic action of WAY 100635 on the 5-HT system. The LC receives a dense 5-HT innervation and electrophysiological and biochemical studies have suggested that the 5-HT system exerts a tonic inhibition of LC NA neurones (see Introduction). Consistent with this notion, the lesioning 5-HT neurones with 5,7-DHT increased the spontaneous firing activity of LC NA neurones (Figure 4b and c).

In order to determine the nature of the 5-HT receptor mediating the suppressant effect of WAY 100635 on the firing activity of LC NA neurones, 5-HT receptor antagonists were used. The selective 5-HT$_3$ receptor antagonist BRL 46470A, the 5-HT$_{1B/1D}$ receptor antagonist GR 127935, and the 5-HT$_{1A/1B}$ receptor antagonist (−)-pindolol did not modify the suppressant effect of WAY 100635. On the basis of the latter results, one may assume that the 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$ and 5-HT$_3$ receptor subtypes are not involved in the mediation of the effect of WAY 100635 on LC NA neurones. Hence, it then appears paradoxical that the 5-HT$_{1A}$ receptor antagonist BMY 7378 completely prevented the suppressant effect of WAY 100635 (Figure 7c). However, it has previously been shown that the systemic administration of BMY 7378 suppresses the activity of dorsal raphe 5-HT neurones (Chaput & de Montigny, 1988; Cox *et al*., 1993). It is therefore possible that BMY 7378 prevented the suppressant effect of WAY 100635 on the firing activity of LC NA neurones by shutting off the firing of 5-HT neurones as a result of either its α_{1D}-adrenoceptor antagonistic activity (Goetz *et al*., 1995) or of its partial 5-HT$_{1A}$ agonistic activity (Chaput & de Montigny, 1988; Cox *et al*., 1993). It has been shown that the $\alpha_{1A/1B/1D}$-adrenoceptor antagonist prazosin suppresses the spontaneous firing activity of dorsal raphe 5-HT neurones ($ED_{50} = 50$ μg kg^{-1}, i.v.) without modifying that of LC NA neurones (Marwaha & Aghajanian, 1982). In the present study, the suppressant effect of WAY 100635 on the firing activity of LC NA neurones was significantly prevented by prazosin (100 μg kg^{-1}, i.v.), providing further evidence for the crucial role of the 5-HT input in this effect of WAY 100635.

It has been shown that 5-HT$_2$ receptors are located postsynaptically (Leyson *et al*., 1982; Stockmeier & Kellar, 1986;

Fischette *et al*., 1987) and an *in situ* hybridization study has revealed the presence of 5-HT_{2C} mRNA in the LC (Pompeiano *et al*., 1994), whereas Wright *et al*. (1995) did not observe the presence of 5-$HT_{1A/2}$ mRNAs in this nucleus. In the present study, the non-selective 5-HT receptor antagonist metergoline, but not spiperone, increased by itself the firing activity of LC NA neurones and yet, both drugs prevented the suppressant effect of WAY 100635 on the firing activity of LC NA neurones. Binding studies have shown that metergoline has high affinity for 5-$HT_{1A/B/D}$, 5-$HT_{2A/C}$ and 5-HT_7 sites, whereas spiperone has high affinity for 5-HT_{1A}, 5-HT_{2A} and 5-HT_7 sites, and (−)-pindolol has only high affinity for 5-$HT_{1A/B}$ re-

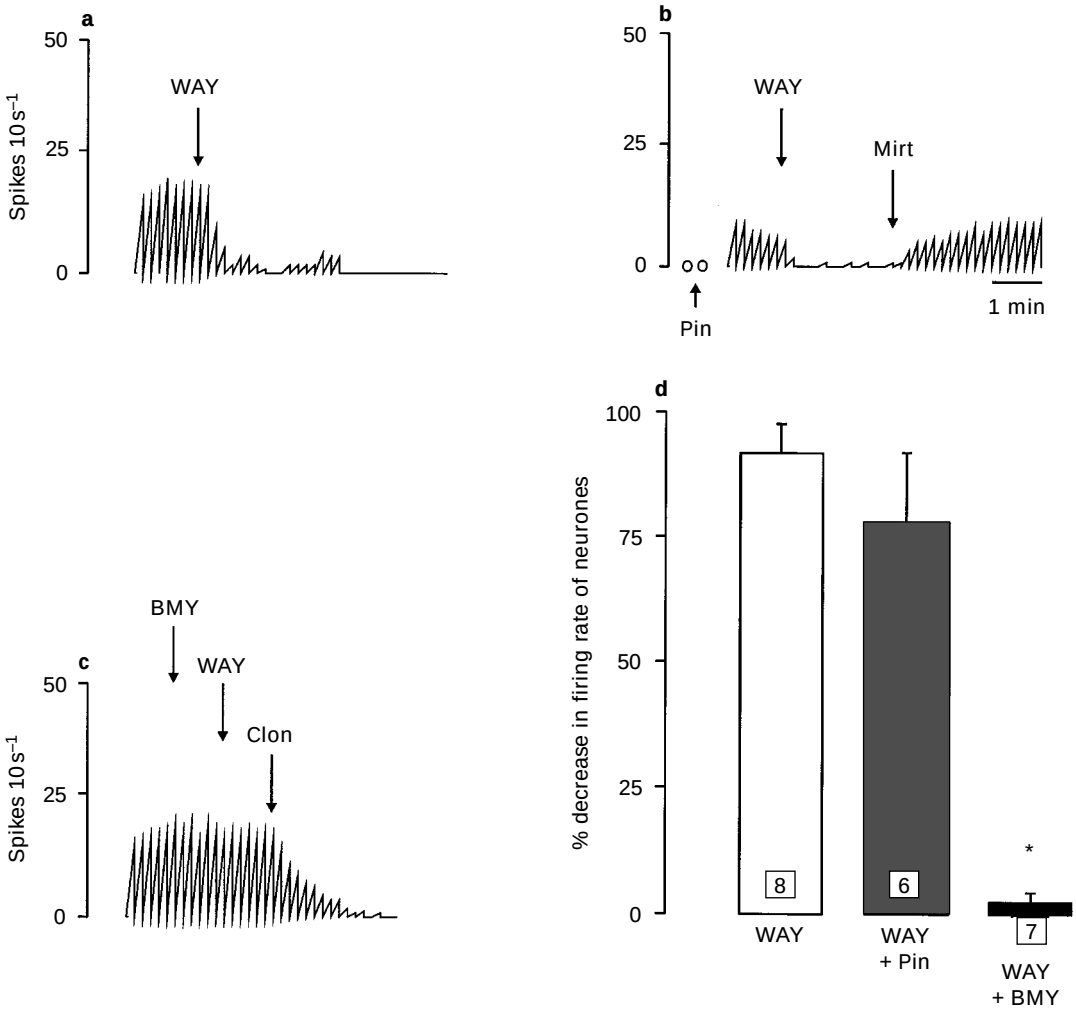

Figure 7 Integrated firing rate histogram of noradrenergic neurones recorded in the LC showing their responses to WAY 100635 (WAY, 100 μg kg^{-1}, i.v.) in control rat (a), after the administration of (−) pindolol (Pin, 15 mg kg^{-1}, i.p.) (b), and in another rat after the injection of BMY 7378 (BMY, 1 mg kg^{-1}, i.v.) (c). Responses to mirtazapine (Mirt, 250 μg kg^{-1}, i.v.) and clonidine (Clon, 5 μg kg^{-1}, i.v.) are also shown in (b) and (c), respectively, (d) Responsiveness of NA neurones to WAY 100635 (100 μg kg^{-1}, i.v.) in control, BMY 7378 and (−)-pindolol-pretreated rats (means ± s.e.mean). The numbers at the bottom of the columns indicate the number of neurones tested. *$P<0.05$ (unpaired Student's t test).

Table 1 Affinities of 5-HT receptor antagonists and their effects on the spontaneous firing rate and on the inhibitory action of WAY 100635 on locus coeruleus noradrenergic neurones

Affinities	*5-HT$_{1A}$*	*5-HT$_{2A}$*	*5-HT$_{2C}$*	*5-HT$_7$*	*Effect on firing rate*[1]	*Inhibitory action of WAY 100635*[1]
Drugs						
Ritansenin	–	+	+	+	↗	↓
Spiperone	+	+	–	+	0	↓
(–)-Pindolol	+	–	–	–	↘	0
Metergoline	+	+	+	+	↗	↓
BMY 7378	+	–	–	–	↗	↓
GR 127935	–	–	–	NA	0	0
BRL 4640A	–	–	–	NA	0	0

[1]All drugs were injected i.v. (except pindolol, i.p.) before WAY 100635 (0.1 mg kg^{-1} i.v.), 0 = no change, ↗ = increase, ↓ = decrease. [2](+) = pK_i > 7 and (–) = pK_i < 6; NA = not avaliable. Data from Baxter *et al*. (1995); Fiorella *et al*. (1995); Hoyer *et al*. (1994); Ruat *et al*. (1993); Shen *et al*. (1993); Zgombick *et al*. (1995).

ceptor subtypes (see Table 1). Ritanserin, which has high affinity for both 5-$HT_{2A/B/C}$ and 5-HT_7 receptors prevented the effect of WAY 100635 on the firing activity of LC NA neurones. However, in the LC, it has been suggested that the effects 5-HT_2 receptor ligands are indirect (Chiang & Aston-Jones, 1993).

High levels of 5-HT_7 receptor mRNA were observed in rat thalamus, hypothalamus, brainstem (including dorsal raphe) and hippocampus (Lovenberg *et al*., 1993; Ruat 1993). Using [^{3}H]-lysergic acid diethylamide and transfected cell lines, these groups have shown that metergoline, ritanserin and spiperone have high affinities for the recombinant 5-HT_7 receptors. Using whole-cell voltage-clamp recordings from rat suprachiasmatic neurones Kawahara *et al*. (1994) have shown that 5-HT inhibits a GABA-activated current, presumably via 5-HT_7 receptors. This latter effect of 5-HT was antagonized by ritanserin, but not by pindolol nor by the 5-$HT_{2A/2C}$ receptors antagonist ketanserin. In the present study, metergoline, ritanserin and spiperone, which also have a high affinity for the 5-HT_7 receptors (Zgombick *et al*., 1995), prevented the suppressant effect of WAY 100635 on the firing activity of LC NA neurones. On the other hand, BMY 7378 has very low affinity (≈ 2 μM) for the 5-HT_7 binding sites (F.D. Yocca, personal communication) and since WAY 100635 has no significant affinity for these receptors (Fletcher *et al*., 1996), their involvement can be ruled out. Taking into account the affinities for the different 5-HT receptor subtypes of the drugs used in the present study (see Table 1) and the fact that prazosin prevented the effect of WAY 100635, the present results suggest that, the suppressant effect of WAY 100635 on the firing activity of LC NA neurones is mediated via 5-HT_{1A} receptors located presynaptically on the NA neurones. Given that spiperone, ritanserin and metergoline are 5-HT_{2A} receptor antagonists which, unlike BMY 7378, do not alter the firing activity of dorsal raphe 5-HT neurones in anaesthetized rats, it is possible that postsynaptic 5-HT_{2A} receptors might also be involved in the suppressant effect of WAY 100635 on the firing activity of LC NA neurones. Using microdialysis in freely moving rats Bosker *et al*. (1996) have recently shown that WAY 100635 (>50 μg kg^{-1}, s.c.) tended to increase extracellular 5-HT in the median raphe and the dorsal hippocampus and we have shown that WAY 100635 (100 μg kg^{-1}, i.v.) increased the duration of suppression of firing (corresponding to the endogenous 5-HT release) of dorsal hippocampus CA_3 pyramidal neurones induced by the electrical stimulation of the ascending 5-HT pathway (Author's unpublished observations). It is conceivable that the effect of WAY 100635 on the firing activity of LC NA neurones could be due to a direct blockade of presynaptic 5-HT_{1A} receptors, leading to an increase of 5-HT in the LC which results ultimately in the activation of postsynaptic 5-HT_{2A} receptors. It is also possible, on the other hand, that this suppressant effect of WAY 100635 on the LC NA neurones firing could be mediated through the 5-HT-mediated suppression of the excitatory glutamatergic input from the paragigantocellularis nucleus to LC neurones, as has been previously described (Aston-Jones *et al*., 1991). Other experiments are needed to confirm the nature of the pre- and postsynaptic 5-HT receptors involved in the effect of WAY 100635 on LC NA neurones firing activity and to elucidate further this complex 5-HT neuromodulation of the LC noradrenergic system.

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