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Electrophysiological Evidence for the Tonic Activation of 5-HT_{1A} Autoreceptors in the Rat Dorsal Raphe Nucleus

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Serotonin (5-hydroxytryptamine, 5-HT) and norepinephine (NE) neurons have reciprocal connections. These may thus interfere with anticipated effects of selective pharmacological agents targeting these neurons. The main goal of the present study was to assess whether the somatodendritic 5-HT_{1A} autoreceptor is tonically activated by endogenous 5-HT in anesthetised rats, using *in vivo* extracellular unitary recordings. In rats with their NE neurons lesioned using 6-hydroxydopamine (6-OHDA) and in controls administered the NE reuptake inhibitor desipramine to suppress NE neuronal firing, the α_2-adrenoceptor agonist clonidine no longer inhibited 5-HT neuron firing, therefore indicating the important modulation of the firing activity of 5-HT neurons by NE neurons. In control rats, the administration of the potent and selective 5-HT_{1A} receptor antagonist WAY 100,635 ((*N*-{2-[4(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl)cyclohexanecarboxamide trihydroxychloride) (100 μg/kg, i.v.) did not modify the spontaneous firing activity of 5-HT neurons, but in NE-lesioned rats using either 6-OHDA or DSP-4, WAY 100,635 produced a mean firing increase of 80 and 69%, respectively. When desipramine and D-amphetamine were used in control rats to prevent alterations in the availability of NE in the dorsal raphe, again WAY 100,635 produced a significant disinhibition of the firing of 5-HT neurons (83 and 53%, respectively). These data support the notion that the NE system tonically activates the firing activity of 5-HT neurons. When the fluctuations of the function of NE neurons normally produced by WAY 100,635 were prevented, a tonic activation of 5-HT_{1A} autoreceptors by endogenous 5-HT was unmasked.

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INTRODUCTION

The cell bodies of serotonin (5-hydroxytryptamine; 5-HT) neurons are concentrated in several nuclei in the brainstem, the largest number of 5-HT neurons being located in the dorsal raphe (DR). In such nuclei, the tissue concentration of 5-HT, as well as the extracellular levels of 5-HT assessed with the microdialysis technique, exceed those present in the projection areas of the 5-HT fibers (Hervas and Artigas, 1998). Consequently, one could assume that the 5-HT_{1A} autoreceptors located on the soma and dendrites of 5-HT neurons are always under a tonic activation by synaptic 5-HT, thereby being able to exert their negative feedback control on the firing rate. Indeed, at 5-HT terminals where 5-HT levels are much lower than around the cell body, local infusion of a 5-HT autoreceptor antagonist increases 5-HT release as a result of lifting the dampening action of 5-HT on this 5-HT_{1B} autoreceptor (de Groote *et al*, 2003). Unexpectedly, however, the potent and selective 5-HT_{1A} antagonist WAY 100,635 ((*N*-{2-[4(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl)cyclohexanecarboxamide trihydroxychloride) when injected on its own in anesthetized rats has not consistently been reported to enhance the firing rate of 5-HT neurons (Fletcher *et al*, 1996; Forster *et al*, 1994; Mundey *et al*, 1994; Gartside *et al*, 1995; Lejeune and Millan, 1998; Haddjeri *et al*, 1998; Martin *et al*, 1999; Hajós *et al*, 2001). Only in very active freely moving cats, when 5-HT neurons are firing at their highest rate, WAY 100,635 produces a clear disinhibition of 5-HT neuronal activity (Fornal *et al*, 1996). In contrast, the α_2-adrenergic antagonist idazoxan produces a marked disinhibition of the firing of locus coeruleus (LC) norepinephrine (NE) neurons in anesthetized rats, thereby indicating an endogenous activation of the noradrenergic cell body autoreceptor (Dong and Blier, 2001). Similarly, the dopamine D2 antagonist haloperidol produces a disinhibitory action on the firing rate of A9 and A10 dopamine neurons, again demonstrating the presence of a tonic activation of dopaminergic autoreceptors by endogenous dopamine on

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the cell body of dopamine neurons (Pucak and Grace, 1994). Given the high concentration of the three neurotransmitters in the immediate vicinity of their respective cell bodies and similar parameters controlling their release, there may be some factor preventing a consistent disinhibitory action of systemic administration of WAY 100,635 on 5-HT neuronal firing.

It is well established that DR 5-HT neurons receive NE projections from the LC (Loizou, 1969; Anderson *et al*, 1977; Baraban and Aghajanian, 1981; Jones and Yang, 1985; Luppi *et al*, 1995, Peyron *et al*, 1996), and pharmacological studies have demonstrated that the firing activity of 5-HT neurons in the DR nucleus is under a tonic activation by an NE input. For instance, intravenous (i.v.) injection of the α_2-adrenergic agonist clonidine suppresses the firing activity of DR 5-HT neurons. This inhibitory action of clonidine is believed to result from the activation of α_2-adrenergic autoreceptors on the cell body and terminals of NE neurons, thereby decreasing the endogenous NE input to excitatory α_1-adrenoceptors on 5-HT neurons in the DR nucleus (Svensson *et al*, 1975; Baraban and Aghajanian, 1980; Clement *et al*, 1992). Accordingly, acute administration of the α_2-adrenergic antagonists idazoxan and mirtazapine increase the spontaneous firing activity of DR 5-HT neurons and antagonize the suppressant effect of clonidine on these neurons (Freedman and Aghajanian, 1984; Garrat *et al*, 1991; Clement *et al*, 1992; Haddjeri *et al*, 1996). Furthermore, the enhancing effect of mirtazapine on the firing activity of 5-HT neurons is abolished by lesioning NE neurons, indicating that this effect of mirtazapine is mediated via NE neurons (Haddjeri *et al*, 1996). In contrast, it was previously reported that the i.v. injection of WAY 100,635 produces a complete suppression of the firing activity of LC NE neurons, an effect that is prevented by lesioning 5-HT neurons and reversed by the selective 5-HT_{2A} antagonist MDL 100,907 in naïve rats (Haddjeri *et al*, 1997; Szabo and Blier, 2001a, b). Taken together, these observations reveal important reciprocal interactions between 5-HT and NE neurons.

The present study was thus aimed at providing further evidence for the crucial role of the firing rate of NE neurons in the modulation of 5-HT neuronal firing, and then determine whether NE neurons could interfere with the detection of a tonic activation of cell body 5-HT_{1A} autoreceptors by synaptic 5-HT using WAY 100,635 in anesthetized animals. To this end, the action of clonidine and WAY 100,635 on the firing activity of 5-HT neurons was studied in NE-lesioned rats and in naïve rats that had the firing activity of their NE neurons interrupted with an NE reuptake inhibitor. It was hypothesized that clonidine would not suppress 5-HT neuronal firing because it could no longer alter NE neuronal function, and that WAY 100,635 would produce a disinhibition of the firing of 5-HT neurons because it would no longer alter the NE neuronal function, thus unveiling 5-HT_{1A} autoreceptor activation.

MATERIALS AND METHODS

Animals and Treatments

The experiments were carried out in male Sprague–Dawley rats (Charles River, St Constant, Quebec, Canada) weighing 250–300 g, which were kept under standard laboratory conditions (12 : 12 light–dark cycle with free access to food and water). The animals were anesthetized with chloral hydrate (400 mg/kg, intraperitoneal (i.p.). Supplemental doses were given to maintain constant anesthesia and to prevent any nociceptive reaction to a tail pinch. Body temperature was monitored using a rectal probe and maintained at $37.0 \pm 0.5^{\circ}$C using a thermostatic water heated pad.

Lesions of NE neurons were performed under chloral hydrate anesthesia by injecting 6-hydroxydopamine (6-OHDA) intracerebroventricularly (i.c.v.) (120 μg free base in 20 μl of 0.9% NaCl and 0.1% ascorbic acid) 1 h after the injection of the 5-HT reuptake blocker fluoxetine (10 mg/kg, i.p.) to protect 5-HT neurons. The rats were tested 10 days later. Sham-operated rats were used as controls and received the same volume of vehicle injected i.c.v. NE neurons were also lesioned using the selective neurotoxin DSP-4 in a different group of rats, since 6-OHDA destroys both NE and dopamine neurons. As described previously (Cheetham *et al*, 1996; Hughes and Stanford, 1998; Bortolozzi and Artigas, 2003), a dose of 40 mg/kg of DSP-4 was injected i.p. and rats were tested 5 days later. Although differential effects of DSP-4 administration on regional brain NE level and turnover has been described, this compound has been shown to produce a robust decrease (90%) of NE level in the hippocampus and its action is restricted to LC axons (Logue *et al*, 1985; Grzanna *et al*, 1989). All animals were handled according to the guidelines approved by the Society of Neuroscience and all animal use procedures were approved by the Faculty ethical committee.

Recordings of DR 5-HT Neurons

Extracellular recordings were performed with single-barreled glass micropipettes preloaded with fiberglass filaments in order to facilitate filling. The tip was broken back to 2–4 μm and filled with a 2 M NaCl solution. Typically, these electrodes had an impedance between 3 and 8 MΩ. The rats were placed in a stereotaxic frame and a burr hole was drilled on the midline 1 mm anterior to lambda. DR 5-HT neurons were encountered over a distance of 1 mm starting immediately below the ventral border of the Sylvius aqueduct. These neurons were identified using the criteria of Aghajanian (1978): a slow (0.5–2.5 Hz) and regular firing rate and long-duration (0.8–1.2 ms) positive action potentials. All drugs used for the i.v. injections were dissolved in saline and each dose was given in a volume of approximately 0.1 ml. Using a gauge 26 catheter inserted in a lateral vein of the tail, the responsiveness of DR 5-HT neurons to the i.v. administration of WAY 100,635 (100 μg/kg) and the prototypical α_2-adrenoceptor agonist clonidine (5–20 μg/kg) was assessed in controls prior to and after the i.v. administration of the NE reuptake blocker desipramine (500 μg/kg, i.v.) as well as in rats pretreated with the neurotoxin 6-OHDA. In the case of clonidine, only the suppressant effect of the first injection corresponding to the dose 5 μg/kg will be taken into account for the analysis. The change of the firing activity was assessed by calculating the mean of firing rate of cells from about 1 min (until a

'plateau') prior to and after drug administration. Only one neuron was tested in each rat.

Drugs

WAY 100,635, clonidine, 6-OHDA HCl, desipramine HCl, DSP-4 HCl and D-amphetamine were purchased to Research Biochemicals, (Natick, MA, USA); LSD is obtained from the Ministry of Health and Welfare (Ottawa, Canada); fluoxetine was a gift from Eli Lilly (Indianapolis, IN). 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.

Statistics

All values are expressed as means ± their standard error. Comparisons between groups were made using the two-tailed unpaired Student *t*-test using a correction factor for multiple comparisons of experimental to a single control group. A probability level smaller than 0.05 was considered significant.

RESULTS

The mean firing frequency of 5-HT neurons in 6-OHDA and DSP-4 rats (1.5 ± 0.2 Hz, $n = 15$ and 1.31 ± 0.25 Hz, $n = 6$, respectively) was not significantly different from that observed in the controls (1.22 ± 0.11, $n = 29$). As exemplified in Figure 1a and summarized in Figure 1d, an i.v. dose of 5 μg/kg of clonidine produced a robust inhibition of 5-HT neurons firing and a complete one using 10 μg/kg ($n = 6$, data not shown). In rats with NE neurons lesioned with 6-OHDA, the same dose of clonidine did not produce a significant inhibition of the firing activity, but the 5-HT autoreceptor agonist LSD was still effective to inhibit the firing activity (Figure 1b). As reported previously, the selective NE reuptake inhibitor desipramine did not alter 5-HT neuronal firing rate (Scuvée-Moreau and Dresse, 1979; $-1 \pm 4\%$, $n = 12$, Figure 1c). This dose of desipramine produces a complete suppression of the firing rate of NE neurons, but likely leaves synaptic availability of NE in the DR unaltered because 5-HT neuronal is unaffected (Scuvée-Moreau and Dresse, 1979; Béïque *et al*, 1999), thereby maintaining NE levels in the DR (Bortolozzi and Artigas, 2003). Under this pharmacological condition, clonidine no longer inhibited the firing rate of 5-HT neurons, whereas the 5-HT autoreceptor agonist LSD still exerted its typical inhibitory action (Figure 1b and c). Finally, WAY 100,635, presumably because of its capacity to suppress the firing activity of NE neurons at this i.v. dose of 100 μg/kg (Haddjeri *et al*, 1997; Szabo and Blier, 2001a, b), also prevented the inhibitory action of clonidine on the firing activity of DR 5-HT neurons (Figure 1d and e). However, neither desipramine (Figure 1a) nor WAY 100,635 (data not

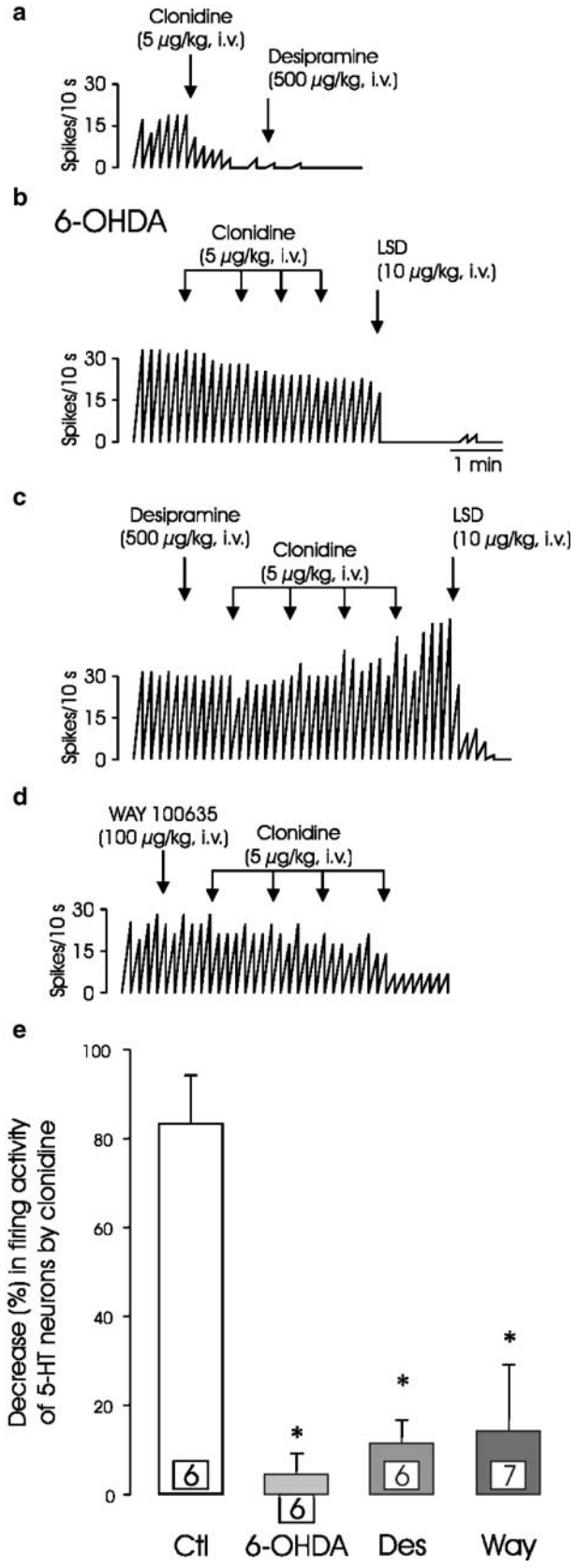

Figure 1 Integrated firing rate histogram of 5-HT neurons recorded in the DR nucleus showing their responses to clonidine (5–20 μg/kg, i.v.) in a control rat (a), a 6-OHDA-lesioned rat (b), after the administration of desipramine (500 μg/kg, i.v.) (c), and in another rat after the injection of WAY 100,635 (100 μg/kg, i.v.) (d). (e) Represents the responsiveness of 5-HT neurons to the first dose of clonidine (5 μg/kg, i.v.) in control (Ctl), 6-OHDA-, desipramine- (Des), and WAY 100,635- (Way) pretreated rats (means ± SEM). The numbers at the bottom of the columns indicate the number of neurons tested. *$P < 0.05$ using unpaired Student's *t*-test. LSD was used to confirm the 5-HT nature of the neuron recorded.

shown) reversed the suppressant effect of clonidine (5 or 10 μg/kg) on the firing activity of DR 5-HT neurons.

In intact animals, WAY 100,635 (100 μg/kg) did not significantly alter the firing rate of DR 5-HT neurons (Figures 1d, 2a and 3a). In fact, in two of the rats tested,

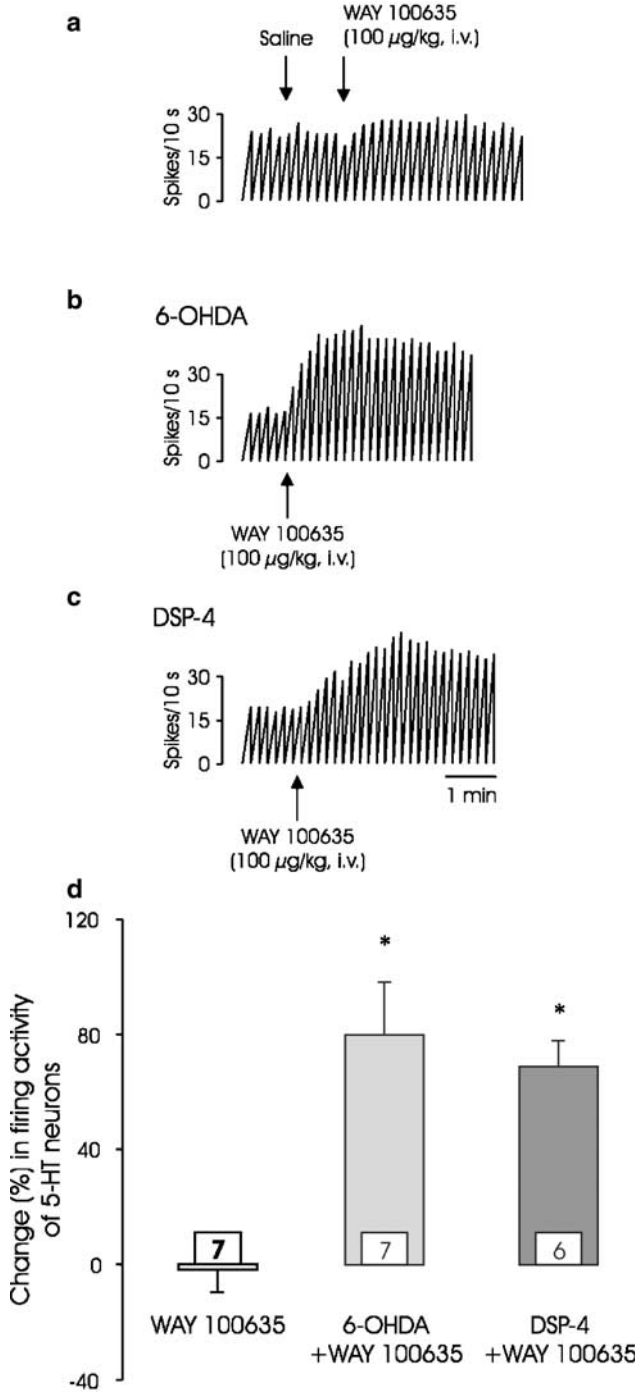

Figure 2 Integrated firing rate histogram of 5-HT neurons recorded in the DR nucleus showing their responses to WAY 100,635 (100 μg/kg, i.v.) in a control rat (a), in a 6-OHDA (b), and a DSP-4 pretreated (c). (d) Represents the responsiveness of 5-HT neurons to WAY 100,635 (100 μg/kg, i.v.) in control (WAY 100635), in 6-OHDA- (6-OHDA + Way), and DSP-4- (DSP-4 + Way)-pretreated rats (means ± SEM). The numbers at the bottom of the columns indicate the number of neurons tested. *$P < 0.05$, using the unpaired Student's *t*-test.

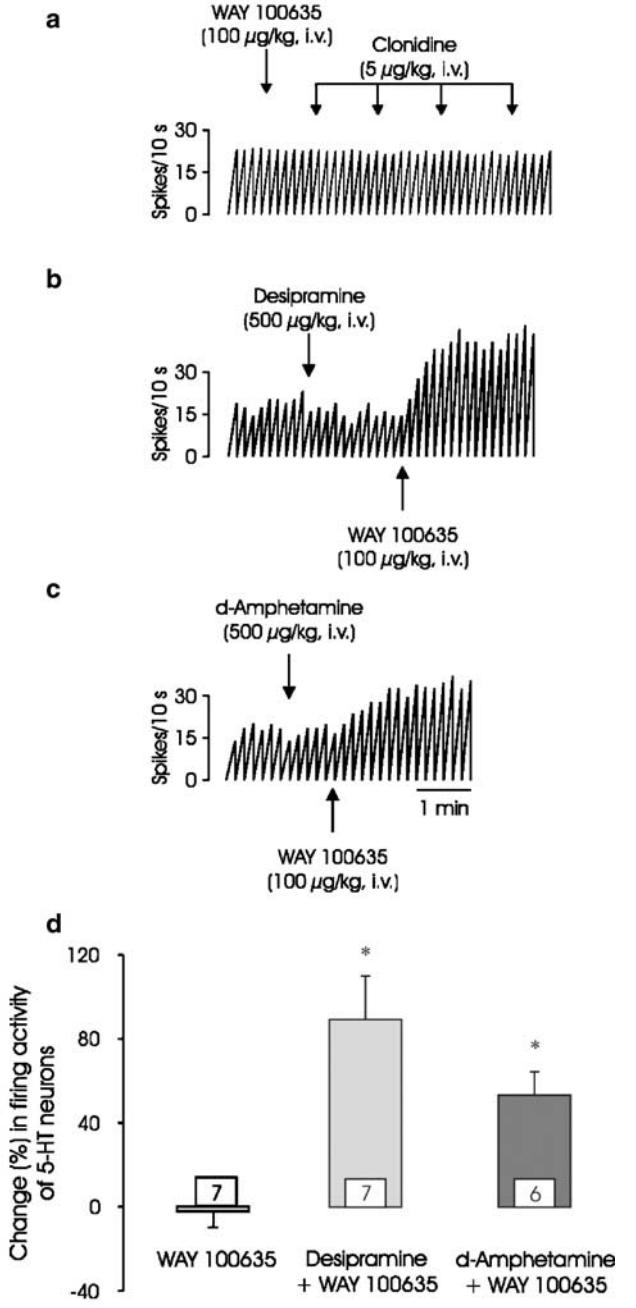

Figure 3 Integrated firing rate histogram of 5-HT neurons recorded in the DR nucleus showing their responses to WAY 100,635 (100 μg/kg, i.v.) in a control rat (a), in a desipramine- (500 μg/kg, i.v.) (b), and in a D-amphetamine-pretreated (500 μg/kg, i.v.) rat (c). (d) Represents the responsiveness of 5-HT neurons to WAY 100,635 in control, desipramine-, and amphetamine-pretreated rats (means ± SEM). The numbers at the bottom of the columns indicate the number of neurons tested. *$P < 0.05$, using the unpaired Student's *t*-test.

WAY 100,635 increased the firing rate by about 25%, whereas three neurons showed a small decrease (~20%) of their firing rate, and in two further rats the firing activity was not modified at all by WAY 100,635. In order to determine a possible involvement of NE neurons in the lack of effect of WAY 100,635 on the firing activity of 5-HT neurons, NE neurons were lesioned using the neurotoxins

6-OHDA or DSP-4. As illustrated in Figure 2b and c, WAY 100,635 (100 μg/kg, i.v.) markedly increased the firing activity of DR 5-HT neurons in rats that had a lesion of NE neurons, either with 6-OHDA or DSP-4.

In order to provide further evidence for the disinhibitory action of WAY 100,635 on 5-HT neurons manifesting itself when NE firing is not allowed to fluctuate, the NE reuptake blocker desipramine and the NE releaser/reuptake blocker D-amphetamine were used (both at 500 μg/kg, i.v.). WAY 100,635 (100 μg/kg, i.v.) increased the firing activity of DR 5-HT neurons by 89% after the injection of desipramine and by 67% after the injection of D-amphetamine (Figure 3).

DISCUSSION

The results of the present experiments first confirmed that the inhibitory action of the α_2-adrenergic agonist clonidine on the firing of 5-HT neurons is due to its action on NE neurons. Second, they showed that the 5-HT_{1A} autoreceptor is under tonic activation by 5-HT, even in anesthetized animals, but the reason why the disinhibiting action of the 5-HT_{1A} antagonist WAY 100,635 generally does not manifest itself in intact rats is because of its suppressant action on LC NE neuronal firing.

The activation of α_2-adrenoceptors by clonidine decreases the firing activity of DR 5-HT neurons, as well as 5-HT release in the DR. This effect has been postulated to result from a reduction of the endogenous NE excitatory input to α_1-adrenergic receptors (Svensson *et al*, 1975; Baraban and Aghajanian, 1980; Pudovkina *et al*, 2003; Bortolozzi and Artigas, 2003). Indeed, Svensson *et al* (1975) had previously shown that the suppressant effect of clonidine on the firing activity of DR 5-HT neurons was prevented by lesioning NE neurons produced by the neurotoxin 6-OHDA. The electrophysiological experiments presented herein were nevertheless essential because an unaltered effect of clonidine on 5-HT neuronal firing in rats with neonatal NE lesions had also been reported (Lanfumey and Adrien, 1988).

The dose of desipramine used in the present study produces a complete inhibition of the firing of NE neurons (Scuvée-Moreau and Dresse, 1979; Béïque *et al*, 1999). Therefore, the observation that clonidine no longer inhibited 5-HT neuronal firing following desipramine administration in intact rats provides additional evidence that clonidine mediates its action on raphe firing through its interference with synaptic NE levels. In further support of the latter assertion, neurokinin 1 receptor antagonism, which promptly attenuates the inhibitory action of clonidine on LC neuronal firing, also decreases the suppressant effect of clonidine on DR neurons (Haddjeri and Blier, 2000).

Thus far, WAY 100,635 has proven to be a potent and selective antagonist shown to be active at pre- and most postsynaptic 5-HT_{1A} receptors, and lacks the affinity for other 5-HT receptors (Fletcher *et al*, 1996). Nevertheless, despite the availability of such a 5-HT_{1A} receptor antagonist, the existence of a tonic activation of somatodendritic 5-HT_{1A} autoreceptors by endogenous 5-HT in the DR nucleus using this drug had not yet been clearly established. Testing WAY 100,635 on DR slices using bath application does not necessarily circumvent the issue of the NE innervation of 5-HT neurons. This is because 5-HT neurons have to be artificially driven by a large concentration of the α_1-adrenoceptor agonist phenylephrine, since they do not discharge spontaneously upon acutely depriving them from their endogenous NE input. Indeed, WAY 100,635 does not consistently alter the basal firing rate of rat and guinea-pig DR 5-HT neurons in such a preparation (Craven *et al*, 1994; Johnson *et al*, 2002). Only a small increase had been reported in one report (Corradetti *et al*, 1998). Similar to results obtained *in vitro* by Fletcher *et al* (1996), WAY 100,635 (at doses $\leqslant$100 μg/kg, i.v.) in the rat did not alter the firing activity of DR 5-HT neurons, although occasionally, WAY 100,635 produces some increases of the firing activity of DR 5-HT neurons without, however, achieving statistical significance (Forster *et al*, 1994; Gartside *et al*, 1995; Fletcher *et al*, 1996; Lejeune and Millan, 1998). Mundey *et al* (1996) reported consistent increases in 5-HT neuronal firing in anesthetised guinea-pig, but apparently in 5-HT neurons firing at a very slow rate, on average 0.6 Hz. Fornal *et al* (1996) reported in freely moving cats that the administration of WAY 100,635 increased the firing activity of DR 5-HT neurons. The same phenomenon was observed using the much less selective 5-HT_{1A} antagonist spiperone (Fornal *et al*, 1994). The latter increases were evident during wakefulness, when DR 5-HT neurons have a relatively high level of firing activity, but not during quiet waking or sleep, when DR 5-HT neurons display little spontaneous activity. Finally, a suppressant action of WAY 100,635 on the firing activity of rat DR 5-HT neurons was even observed at high doses due to its α_1-adrenergic antagonism at such nonselective regimens (Haddjeri *et al*, 1998; Martin *et al*, 1999).

Consistent with the majority of the above-mentioned reports, the i.v. administration of WAY 100,635 at a dose of 100 μg/kg did not modify the firing activity of DR 5-HT neurons in the present study. However, after lesioning NE neurons, with either 6-OHDA or DSP-4, WAY 100635 significantly increased the firing activity of DR 5-HT neurons. Also, after an injection of desipramine that suppresses LC NE firing activity, thereby maintaining a tonic activation of excitatory α_1-adrenoceptors on 5-HT neurons due to NE reuptake blockade in the DR nucleus, WAY 100,635 also produced a disinhibition of the firing rate of 5-HT neurons. In this pharmacological condition, WAY 100,635 could no longer modify NE neuron firing because of the overactivation of the somatodendritic α_2-adrenergic autoreceptors resulting from NE reuptake inhibition by desipramine. The use of D-amphetamine produced similar results as it is well known to be both an NE releaser as well as an NE reuptake inhibitor. Indeed, D-amphetamine reverses the suppressant effect of α_1-adrenergic antagonists on 5-HT neuronal firing (Baraban and Aghajanian, 1981). The present results therefore suggest that the suppression of the firing activity of LC NE neurons by WAY 100,635 observed with a dose of 100 μg/kg in intact animals (Haddjeri *et al*, 1997; Szabo and Blier, 2001a, b) would thus prevent the detection of the tonic activation of somatodendritic 5-HT_{1A} autoreceptors. The effective antagonism of the 5-HT_{1A} autoreceptors by WAY 100,635 would have the tendency to drive up 5-HT neuronal firing, but the attenuated activation of excitatory α_1-adrenoceptors

resulting from the concomitant inhibition of LC NE neuron firing has the opposite effect on DRN firing, the net result being an unaltered firing rate for most 5-HT neurons. Microdialysis studies using WAY 100,635 presumably also failed to demonstrate that somatodendritic 5-HT_{1A} autoreceptors are tonically activated by endogenous 5-HT likely due to its indirect NE action mentioned above (Fletcher *et al*, 1996; Gurling *et al*, 1994; Invernizzi *et al*, 1997; Dawson and Nguyen, 1998).

It may appear paradoxical that WAY 100,635 suppresses the firing rate of NE neurons while not altering that of 5-HT neurons. However, this suppressant action of WAY 100,635 has been shown to be dependent on the presence of 5-HT neurons, and possibly results from its antagonism of 5-HT_{1A} receptors on glutamate neurons within the neurocircuitry controlling 5-HT transmission to the LC (Szabo and Blier, 2001a, b). These results emphasize the notion that even very selective agents for a single neuronal element may produce profound actions on other neurochemical systems. With respect to WAY 100,635, the anxiolytic-like effect of this compound may thus be due in part to its dampening effect on the function of the NE system (Cao and Rodgers, 1997; Griebel *et al*, 2000). Similarly, since WAY 100,635 was administered systemically in the present and prior studies, it is possible that other brain structures also contribute to its lack of disinhibitory action on 5-HT neurons.

Apart from WAY 100,635, there are few selective 5-HT_{1A} receptor antagonists, the most extensively studied being robalzotan (NAD-299). As for WAY 100,635, this drug does not consistently produce a disinhibition of 5-HT neuronal firing in anesthetized rats (Arborelius *et al*, 1999; Martin *et al*, 1999). As robalzotan has good oral bioavailability, unlike WAY 100,635, it was recently given to depressed patients to determine whether it could act as an antidepressant. Negative results were obtained in a placebo- and paroxetine-controlled trial (Ybema *et al*, 2003). These results can be understood on the basis that this antagonist likely did not enhance 5-HT neuronal firing in most phases of the sleep–wake cycle, thereby not likely leading to increased 5-HT release. In addition, robalzotan is an effective antagonist of postsynaptic 5-HT_{1A} receptors in laboratory animals and in humans (Johansson *et al*, 1997; Andree *et al*, 2003). Such receptors are believed to play an important role in the antidepressant response of serotonergic agents (Blier and Ward, 2003; Santarelli *et al*, 2003).

In summary, the present studies support the notion that NE system tonically modulates 5-HT neurotransmission. Interference with NE neuronal impulse flow unmasked the tonic activation of somatodendritic 5-HT_{1A} autoreceptors by endogenous 5-HT, as revealed by the increasing effect of WAY 100,635 on the DR 5-HT neurons firing activity following a lesion of NE neurons. Consequently, the term silent antagonist attributed to WAY 100,635 still applies to its lack of effect on 5-HT neuronal firing *per se*, but is misleading because it implies that the 5-HT_{1A} autoreceptor is not tonically activated. To the contrary, the present results obtained in anesthetized animals, together with the observations by Fornal *et al* (1994, 1996) in very active freely moving cats indicate that the 5-HT_{1A} autoreceptor receives a tonic activation by endogenous 5-HT in most stages of the sleep–wake cycle, with the possible exception of paradoxical sleep.

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