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Venlafaxine and its interaction with WAY 100635: effects on serotonergic unit activity and behavior in cats

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Abstract

The therapeutic efficacy of antidepressant drugs that inhibit the reuptake of serotonin (5-hydroxytryptamine, 5-HT) may be enhanced by blocking their indirect activation of 5-HT_{1A} autoreceptors, which mediate feedback inhibition of serotonergic neuronal activity. In this study, we examined the effects of venlafaxine, a dual 5-HT/noradrenaline reuptake inhibitor, alone and in combination with the selective 5-HT_{1A} receptor antagonist *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl) cyclohexanecarboxamide (WAY 100635), on the single-unit activity of serotonergic dorsal raphe neurons and concurrent behavior in freely moving cats. Systemic administration of venlafaxine (0.05–1.0 mg/kg, i.v.) produced a dose-dependent decrease in firing rate (ED_{50} = 0.19 mg/kg), with virtually complete inhibition of neuronal discharge at the highest dose tested. The subsequent administration of WAY 100635 (0.1 mg/kg, i.v.) rapidly reversed the neuronal suppression produced by venlafaxine and significantly elevated the firing rate above baseline levels. The overshoot in neuronal activity was associated with the onset of an adverse behavioral reaction resembling the 5-HT syndrome resulting from excessive levels of brain 5-HT. The intensity of this reaction paralleled the degree of neuronal restoration induced by WAY 100635, suggesting a causal relationship. Such behavioral responses were either not observed previously, or of a low intensity, when WAY 100635 was combined with selective 5-HT reuptake inhibitors. Overall, these results suggest that the risk of inducing adverse effects, such as the 5-HT syndrome, may be higher with dual 5-HT/noradrenaline reuptake inhibitors than with selective 5-HT reuptake inhibitors, when these agents are combined with a potent 5-HT_{1A} autoreceptor antagonist. Possible mechanisms that might account for these differences in drug interaction are discussed.

Keywords: Venlafaxine; WAY 100635; 5-HT(5-hydroxytryptamine, serotonin); 5-HT_{1A} autoreceptor; Dorsal raphe nucleus; 5-HT syndrome; (Cat)

1. Introduction

A large body of evidence suggests a dysfunction of brain serotonin (5-hydroxytryptamine, 5-HT) in major depression (Meltzer, 1989; Mann, 1999). Furthermore, most antidepressant therapies are thought to act by increasing central serotonergic neurotransmission (Blier and de Montigny, 1994). Other neurotransmitters, notably noradrenaline, have also been implicated in the pathophysiology of depression and in the mechanism of action of certain antidepressant drugs (Delgado and Moreno, 2000). The first generation of antidepressants, the tricyclics, inhibit the neuronal reuptake of 5-HT and/or noradrenaline, but additionally interact with multiple neurotransmitter receptors, including cholinergic, histaminergic, and α-adrenergic receptors (Briley and Moret, 1997). These receptor interactions are primarily responsible for many of the undesirable side effects (e.g., dry mouth, constipation, blurred vision, sedation, orthostatic hypotension) of the tricyclics, and result in poor patient compliance. Recently, a new class of antidepressant drugs has been introduced, which inhibit the neuronal reuptake of both 5-HT and noradrenaline, while having negligible affinity for various neurotransmitter receptors (Muth et al., 1986; Owens et al., 1997). Venlafaxine is an example of these so-called specific 5-HT/noradrenaline reuptake inhibitors. These drugs may be more

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efficacious than other antidepressant drugs targeting only one neurotransmitter system, such as the selective 5-HT reuptake inhibitors, which are presently the most widely prescribed class of compounds (for review, see Artigas, 1995; Preskorn et al., 1995; Andrews et al., 1996; Briley and Moret, 1997). However, as with other antidepressants, several weeks of treatment are usually required to achieve a therapeutic response.

The delayed onset of action of antidepressant drugs acting via brain 5-HT may be attributed to their indirect activation of somatodendritic 5-HT_{1A} autoreceptors, which leads to an inhibition of serotonergic neuronal activity. Consequently, little or no increase in extracellular concentrations of forebrain 5-HT are seen following the acute application of these drugs (Invernizzi et al., 1992; Gartside et al., 1995; Artigas et al., 1996). However, upon chronic administration, tolerance develops to the acute inhibitory action of these drugs on neuronal activity, apparently due to a desensitization of 5-HT_{1A} autoreceptors (Blier et al., 1987). The recovery of serotonergic neuronal discharge (and hence, neurotransmitter release) in the presence of sustained reuptake inhibition is thought to lead to an overall enhancement of serotonergic neurotransmission, which may underlie the therapeutic effect.

Neurochemical studies have shown that 5-HT_{1A} receptor antagonists, in general, markedly potentiate the ability of 5-HT reuptake inhibitors to increase extracellular 5-HT levels in the brain, presumably by blocking autoreceptor-mediated feedback inhibition of serotonergic neuronal activity (Romero et al., 1996; Dawson et al., 1999). Based on these findings, it has been hypothesized that the combined administration of a 5-HT_{1A} autoreceptor antagonist and a 5-HT reuptake inhibitor might lead to a more rapid or more effective antidepressant response. Clinical trials with pindolol, a β-adrenoceptor blocker with putative 5-HT_{1A} autoreceptor antagonist properties, support this idea in general (for review, see Blier and Bergeron, 1998) and have stimulated considerable interest in the development of novel 5-HT_{1A} autoreceptor antagonists, which can be used in depressed patients to enhance the efficacy of antidepressant medications.

A possible complication associated with this therapeutic approach is the induction of the 5-HT syndrome, a potentially life-threatening condition, caused by excessive synaptic concentrations of 5-HT (Mills, 1995; Sporer, 1995). This hyperserotonergic state is usually produced by the concurrent administration of two or more 5-HT-potentiating agents acting via distinct mechanisms. Although classically associated with monoamine oxidase inhibitors, the syndrome can be produced by any of a variety of drugs that enhance central serotonergic neurotransmission, including 5-HT reuptake inhibitors, which, due to their widespread use, have been increasingly implicated in this drug interaction. Clinical manifestations of the syndrome are mediated via stimulation of postsynaptic 5-HT receptors and include alterations in mental status, autonomic function, and neuromuscular activity, with changes in the latter being the most prominent. While most cases of the 5-HT syndrome are mild and often resolve within several hours with minor supportive care alone, severe complications and fatalities have been reported.

In response to the growing interest in using 5-HT_{1A} autoreceptor antagonists to augment the therapeutic action of antidepressant drugs, we explored the neuronal and behavioral consequences of such an interaction, in this case, using the dual 5-HT/noradrenaline reuptake inhibitor venlafaxine. The aims of this study were: (i) to characterize the effects of venlafaxine on the single-unit activity of serotonergic neurons recorded in freely moving cats, and (ii) to assess its interaction with a selective 5-HT_{1A} receptor antagonist, while simultaneously monitoring the behavior of animals. Changes in neuronal activity were subsequently correlated with drug-induced alterations in behavior. To our knowledge, this is the first electrophysiological study on the effects of venlafaxine in conscious, freely moving animals.

2. Materials and methods

2.1. Animals

Adult male cats weighing 2.7–5.2 kg were used. They were housed individually in a temperature-controlled (22 ± 1°C) and light-controlled (lights on from 0700 to 2100 h) room and had free access to food and water. All cats were cared for and used in strict accordance to the Public Health Service Guide for the Care and Use of Laboratory Animals. All procedures were reviewed and approved by the Institutional Animal Care and Use Committee of Princeton University.

2.2. Surgical procedures and electrophysiological recordings

Under pentobarbital anesthesia, cats were stereotaxically implanted with bundles of moveable, insulated nichrome microwires in the dorsal raphe nucleus for recording single-unit activity and with gross electrodes for monitoring behavioral state, as described previously (Bjorvatn et al., 1998). In addition, a chronic indwelling jugular catheter was implanted for remote drug injections. Cats were allowed a minimum of 2 weeks to recover from surgery before experiments were initiated.

Electrical potentials were recorded from each cat using a counterweighted low-noise cable system and 24-channel slip ring assembly. Single-unit activity was monitored continuously on an oscilloscope and separated from background noise by means of a window discriminator. The output of the discriminator was used to obtain an on-line record of cell discharge through a speaker, an electronic

counter, and a polygraph. The cortical electroencephalogram (EEG), nuchal electromyogram, and electrooculogram were recorded simultaneously on the polygraph. All experimental trials were conducted between 0900 and 1800 h in an electrically shielded chamber ($65 \times 65 \times 95$ cm in height) with a transparent Plexiglas front door.

2.3. Neuronal identification

Serotonergic dorsal raphe neurons were identified according to previously established criteria (Fornal and Jacobs, 1988): (1) slow and regular discharge activity ($\sim$ 1–4 spikes/s); (2) long-duration action potentials ($\geq$ ms); (3) cessation of spontaneous activity during rapid-eye-movement (REM) sleep; (4) suppression of activity in response to systemic administration of a low dose (10 μg/kg, i.v.) of the 5-HT_{1A} autoreceptor agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT); and (5) histological localization of recording sites to the dorsal raphe nucleus.

2.4. Drug administration

Intravenous drug injections were made remotely from an adjacent room via an infusion line connected to the venous catheter. Drugs were loaded into the infusion line (0.06–0.10 ml/kg) and flushed into the animal with 2 ml of sterile saline over a 5-s period. All injections were made while cats were in a quiet but alert behavioral state, based on direct visual observations and polygraphic monitoring. Pulse injections of an equivalent volume of sterile saline served as a control.

To assess the dose–response relationship for venlafaxine on neuronal activity, venlafaxine was administered, at 5-min intervals, in increasing doses, starting at 0.05 mg/kg, until a total cumulative dose of 1 mg/kg was given. Following the last injection, the selective 5-HT_{1A} receptor antagonist *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]-ethyl]-*N*-(2-pyridinyl) cyclohexanecarboxamide (WAY 100635) (0.1 mg/kg) was administered as a single bolus and neuronal activity was monitored continuously for an additional 4 h, to assess the interaction between venlafaxine and WAY 100635. In one trial, the selective 5-HT_{1A} receptor antagonist 4-iodo-*N*-[2-[4-(methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinyl-benzamide (*p*-MPPI) (0.1 mg/kg) was administered after venlafaxine, in order to provide further evidence that the effects of WAY 100635 were attributed to its 5-HT_{1A} receptor antagonist properties. The doses of WAY 100635 and *p*-MPPI employed were previously shown to effectively block 5-HT_{1A} autoreceptors in awake cats (Fornal et al., 1996, 1999a; Bjorvatn et al., 1998).

Additionally, the effects of i.p. injection of venlafaxine (5 mg/kg) and subsequent administration of WAY 100635 (0.2 mg/kg, s.c.) were examined in a small group of neurons. In these experiments, WAY 100635 was given 60 min after venlafaxine.

In most cases, we were able to record the same cell for several days after venlafaxine administration (both i.v. and i.p.). This allowed us to follow the time course of venlafaxine's effect on neuronal activity.

2.5. Behavioral observations

The behavior of each cat was monitored continuously throughout the experiment with a video camera or by direct visual observation. Cats were observed for possible behavioral changes (e.g., signs of the 5-HT syndrome) following the sequential administration of venlafaxine and WAY 100635. The following behaviors were observed: splayed hindlimbs, arched back, protruded claws, immobility, abnormal posture, tachypnea, salivation, vocalization, piloerection, emesis, mydriasis, and impaired pupillary response to light. Most behaviors were scored according to the following four-point ranked intensity scale: 0 = not present, 1 = equivocal, 2 = definite, 3 = intense. Alternatively, abnormal posture, immobility, mydriasis, and impaired pupillary reflex were scored using a different four-point ranked intensity scale, where 0 = not present, 1 to 3 = increasing levels of intensity. Effects on behavior were subsequently correlated with drug-induced changes in neuronal activity.

2.6. Data collection

Firing rate data for the drug conditions were obtained during comparable behavioral states, based on polygraphic and behavioral criteria, because the activity of serotonergic neurons varies directly with the level of behavioral arousal (Fornal and Jacobs, 1988). For the venlafaxine cumulative dose–response trials, firing rates were calculated from consecutive 10-s samples beginning 1 min prior to the first drug injection (baseline) and continuing for 5 min after each successive drug injection. The percentage of change in firing rate was determined by comparing the baseline discharge rate to the rate taken over a 1-min period (i.e., six consecutive 10-s samples) during the time of maximum drug effect. The dose of venlafaxine required to produce a 50% suppression in neuronal activity (ED_{50}) was calculated for each cell using least-squares linear regression analysis of a semilogarithmic plot of percent suppression vs. dose. For the drug interaction trials, firing rates were calculated from six consecutive 10-s samples obtained at predefined time intervals and were compared to baseline.

2.7. Drugs

The following drugs were used: (±)-venlafaxine hydrochloride (courtesy of Wyeth-Ayerst Research, Princeton,

USA), WAY 100635 trihydrochloride (courtesy of Wyeth Research, Taplow, UK), *p*-MPPI hydrochloride and (±)-8-OH-DPAT hydrobromide (Research Biochemicals International, Natick, USA). Venlafaxine, WAY 100635 and 8-OH-DPAT were dissolved in sterile bacteriostatic saline, whereas *p*-MPPI was dissolved in sterile water. Drugs were prepared immediately before each trial. Venlafaxine, *p*-MPPI and 8-OH-DPAT required mild sonication to dissolve. Dosages of all drugs refer to the salt.

2.8. Statistical analysis

Data are expressed as means ± S.E.M. Unit activity and behavior were analyzed by a one-way repeated-measures analysis of variance (ANOVA) and post hoc Student–Newman–Keuls test. A probability value ≤ 0.05 was taken as statistically significant.

3. Results

3.1. General characteristics of serotonergic neuronal activity

Data were obtained from 10 serotonergic neurons recorded in seven cats. All neurons exhibited a relatively slow (range = 1.3–4.7 spikes/s), regular, discharge pattern during quiet waking. The activity of these cells was strongly suppressed both during REM sleep (mean decrease, 94 ± 4%) and in response to systemic administration of the 5-HT_{1A} autoreceptor agonist 8-OH-DPAT (mean decrease, 99 ± 1%). Upon histological examination, all cells were found to be localized on, or near, the midline within the dorsal raphe nucleus.

3.2. Effects of saline control

The i.v. administration of saline (0.1 ml/kg) had no apparent effect on either the spontaneous activity of serotonergic dorsal raphe neurons or behavior.

3.3. Effects of venlafaxine

Administration of cumulative doses of venlafaxine (0.05–1 mg/kg, i.v.) depressed the spontaneous activity of serotonergic dorsal raphe neurons in a dose-dependent manner (Figs. 1 and 2). The decrease in firing rate produced by venlafaxine ranged from approximately 20% at 0.05 mg/kg to 95% at 1 mg/kg. The mean ED_{50} value, derived from individual dose–response curves, for venlafaxine-induced neuronal inhibition was 0.19 ± 0.05 mg/kg, i.v. ($n = 7$). The decrease in neuronal activity produced by each dose of venlafaxine was significantly different from control saline injections, which only decreased firing rate by 5 ± 2% ($n = 7$). The inhibitory action of venlafaxine was long lasting. Neuronal activity remained significantly reduced from baseline levels by

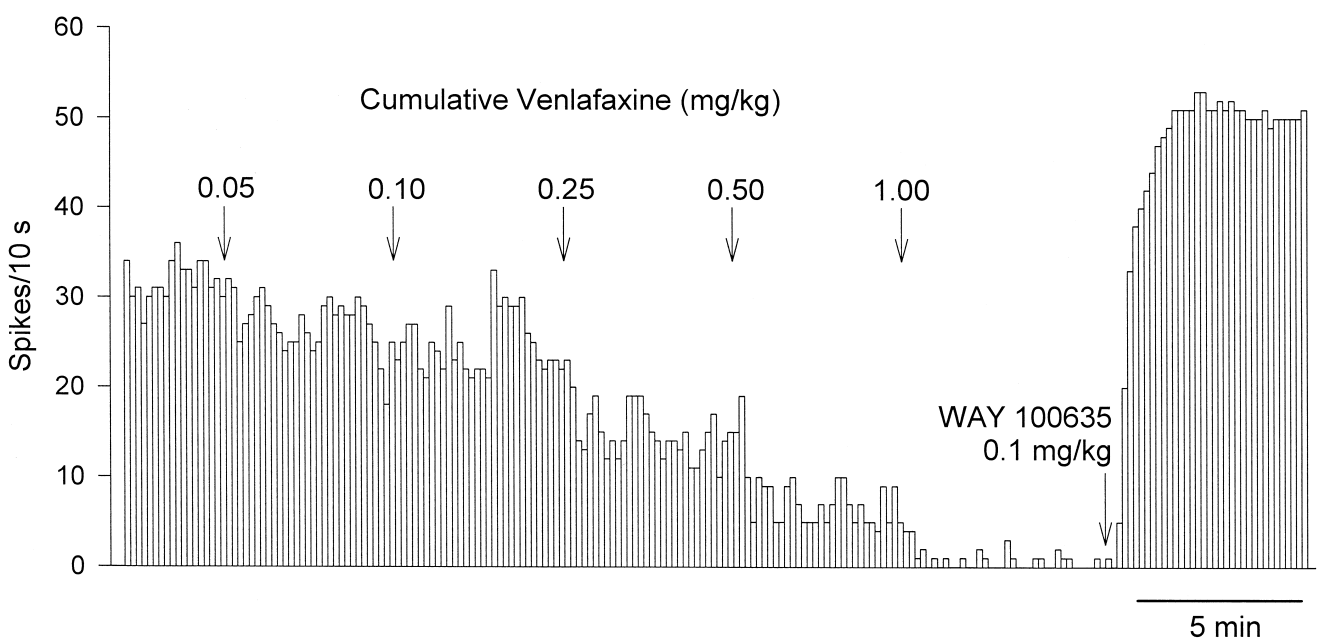

Fig. 1. Integrated firing rate histogram showing the response of a single serotonergic dorsal raphe neuron to i.v. administration of cumulative doses of venlafaxine (0.05–1 mg/kg). Arrows indicate time of injections. The activity of this cell was markedly suppressed by venlafaxine. This effect was rapidly reversed by the subsequent administration of WAY 100635 (0.1 mg/kg, i.v.).

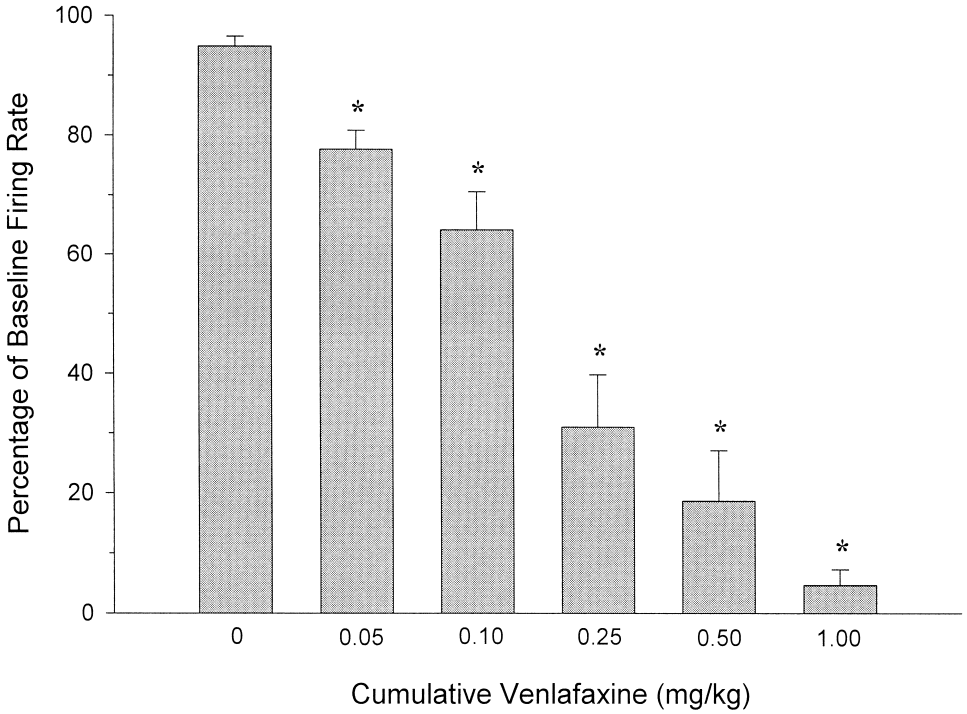

Fig. 2. Effect of i.v. administration of venlafaxine on the spontaneous activity of serotonergic dorsal raphe neurons in awake cats. Values are means ± S.E.M.; $n = 7$ cells. Venlafaxine produced a dose-dependent inhibition of unit activity. $^{*}P < 0.05$ vs. 0 mg/kg (saline control) by one-way ANOVA and Student–Newman–Keuls test.

32 ± 10% ($n = 6$) 24 h after injection, but recovered to within baseline levels by 48 h ($n = 5$).

The i.p. administration of a single dose of venlafaxine (5 mg/kg) also produced a virtually complete inhibition of serotonergic neuronal activity ($n = 3$). This suppressant action was apparent within 15 min of injection and lasted for 2–3 days (data not shown).

Cats treated with i.v. venlafaxine displayed mydriasis (and diminished pupillary responses to light), but no distinct changes in behavior (Table 1) or polygraphic indices.

Table 1
Effects of sequential i.v. adminstration of saline, venlafaxine (1 mg/kg, total cumulative dose), and WAY 100635 (0.1 mg/kg) on various behaviors in cats

Behaviors	Post-saline	Post-venlafaxine	Post-WAY 100635			
			5 min	30 min	60 min	120 min
Hindlimb abduction[a]	0	0	2.4 ± 0.2[b,c]	1.8 ± 0.4[b,c]	1.0 ± 0.3[b,c]	0
Piloerection[a]	0	0	1.6 ± 0.2[b,c]	0.8 ± 0.2[b,c]	0.4 ± 0.2	0
Abnormal breathing[a]	0	0	0	0.4 ± 0.4	0.4 ± 0.4	0
Salivation[a]	0	0	0.8 ± 0.6	0.2 ± 0.2	0	0
Vocalization[a]	0	0	2.4 ± 0.2[b,c]	0.8 ± 0.5	0.2 ± 0.2	0
Protrusion of claws[a]	0	0	2.0 ± 0.3[b,c]	0.8 ± 0.6	0.4 ± 0.2	0.4 ± 0.2
Arched back[a]	0	0	1.8 ± 0.6[b,c]	1.8 ± 0.4[b,c]	1.0 ± 0.3	0
Emesis[a]	0	0	0.6 ± 0.6	0	0	0
Abnormal posture[d]	0	0	2.4 ± 0.2[b,c]	1.8 ± 0.5[b,c]	0.8 ± 0.4	0.2 ± 0.2
Immobility[d]	0	0	2.4 ± 0.2[b,c]	2.0 ± 0.4[b,c]	1.0 ± 0.4[b,c]	0.4 ± 0.2
Mydriasis[d]	0	1.6 ± 0.2[b]	3.0 ± 0.0[b,c]	3.0 ± 0.0[b,c]	2.4 ± 0.2[b,c]	2.0 ± 0.3[b]
Diminished pupillary reflex[d]	0	0.6 ± 0.4	1.8 ± 0.2[b,c]	1.8 ± 0.2[b,c]	1.4 ± 0.2[b,c]	0.8 ± 0.4[b]

Values are means ± S.E.M. $n = 6$.
[a] Behavior was scored using a four-point ranked intensity scale, where 0 = not present; 1 = equivocal; 2 = definite; 3 = intense.
[b] $P < 0.05$ vs. saline control.
[c] $P < 0.05$ vs. venlafaxine, by one-way ANOVA and Student–Newman–Keuls test.
[d] Behavior was scored using a four-point ranked intensity scale, where 0 = not present; 1–3 = increasing levels of intensity.

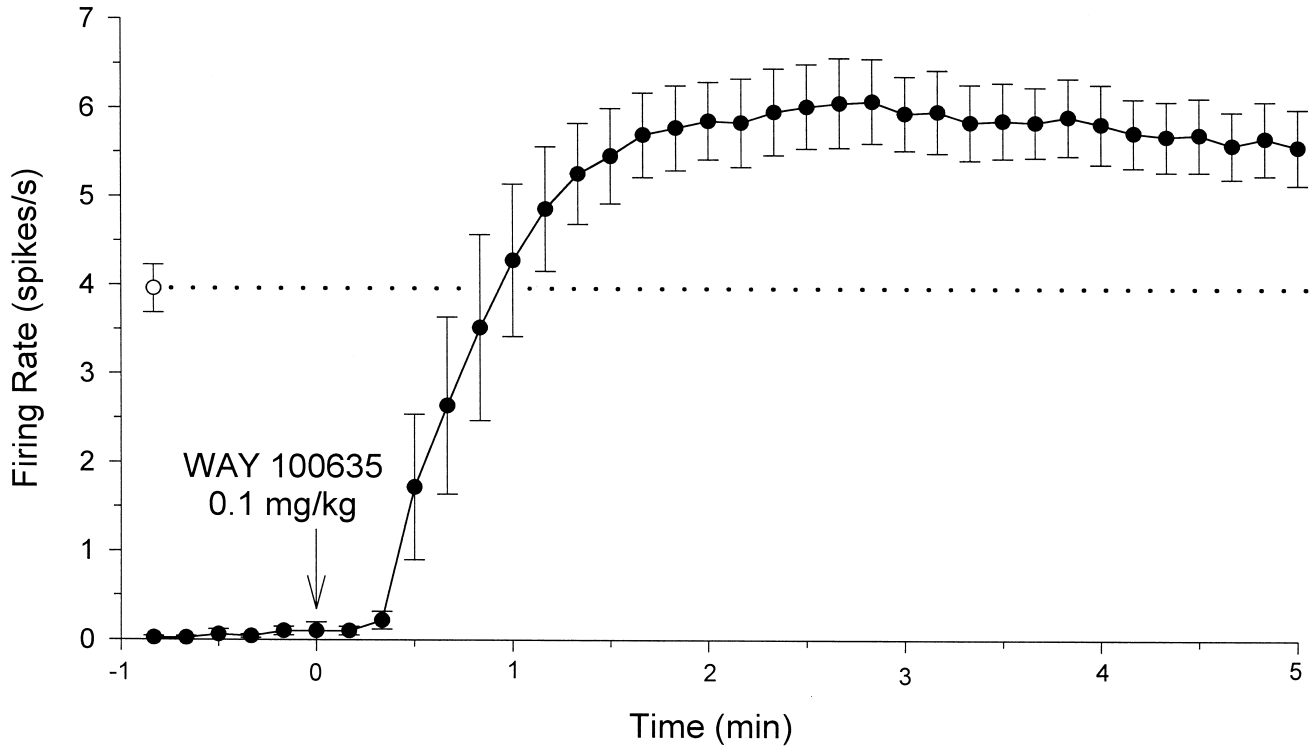

Fig. 3. Effect of i.v. administration of WAY 100635 (0.1 mg/kg) on the suppression of serotonergic dorsal raphe neuronal activity produced by venlafaxine (1 mg/kg, total cumulative dose). Values are means ± S.E.M.; $n = 5$ cells. Arrow indicates time of injection. WAY 100635 reversed the effect of venlafaxine and significantly elevated the firing rate above pre-venlafaxine baseline levels. The restoration of neuronal activity by WAY 100635 in venlafaxine-pretreated animals was associated with the onset of an adverse behavioral reaction resembling the 5-HT syndrome. The horizontal dashed line represents the baseline firing rate of these neurons prior to venlafaxine administration. One cell was excluded due to missing data points.

All cats appeared normal and were responsive to the experimenter. Following i.p. venlafaxine, however, slight salivation was observed in one cat and increased respiratory rate in another.

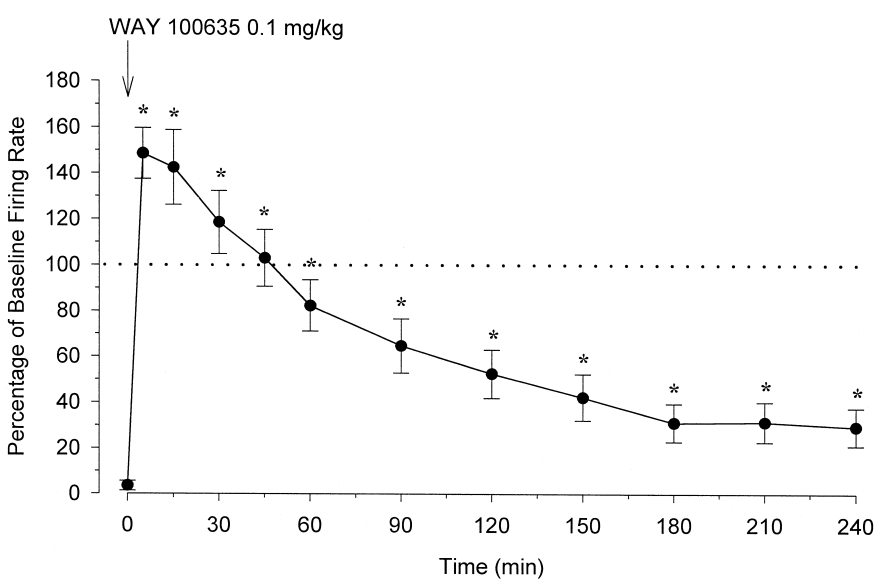

Fig. 4. Time course of the effect of WAY 100635 (0.1 mg/kg, i.v.) on the suppression of serotonergic dorsal raphe neuronal activity produced by venlafaxine (1 mg/kg, total cumulative dose). Values are means ± S.E.M.; $n = 6$ cells, except for the last two time points where $n = 5$ cells. Arrow indicates time of injection. WAY 100635 reversed the effect of venlafaxine in a time-dependent manner. The degree of neuronal restoration was temporally correlated with the intensity of the adverse behavioral reaction induced by WAY 100635 in venlafaxine-pretreated animals. The horizontal dashed line represents the baseline firing rate of these neurons prior to venlafaxine administration. $^{*}P < 0.05$ vs. venlafaxine baseline level (time 0) by one-way ANOVA and Student–Newman–Keuls test.

3.4. Interaction of venlafaxine with WAY 100635

To examine the role of 5-HT_{1A} autoreceptors in the action of venlafaxine, the selective 5-HT_{1A} receptor antagonist WAY 100635 (0.1 mg/kg) was administered after venlafaxine (1 mg/kg, total dose), at a time when neuronal activity was almost completely suppressed. As shown in Fig. 3, WAY 100635 rapidly (within 1 min) reversed the neuronal suppression produced by venlafaxine in all six cells tested. In fact, WAY 100635 significantly elevated the firing rate of these neurons to approximately 150% of predrug baseline levels, presumably by blocking the tonic activation of 5-HT_{1A} autoreceptors by endogenous 5-HT. This overshoot persisted for about 30 min and was accompanied by pronounced behavioral changes in the animals, as described below. After the peak drug effect was reached, neuronal activity gradually declined towards the suppressed venlafaxine baseline level, as the antagonist activity of WAY 100635 dissipated over time (Figs. 4 and 5). By 180 min, neuronal activity stabilized at a level somewhat higher than the original venlafaxine baseline, presumably due to a loss of venlafaxine's inhibitory activity.

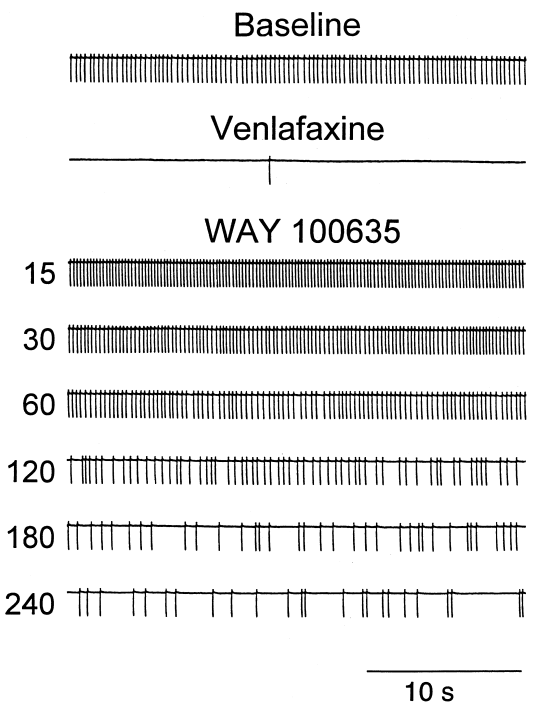

Fig. 5. Polygraph event mark traces of unit activity for a single serotonergic dorsal raphe neuron showing WAY 100635 (0.1 mg/kg, i.v.) antagonism of the inhibitory action of venlafaxine (1 mg/kg, i.v.; total cumulative dose). Each pen deflection = 1 action potential. The venlafaxine trace was obtained immediately prior to WAY 100635 administration. The WAY 100635 traces were obtained after drug administration at the times (min) indicated in the left margin. Note the marked overshoot in neuronal activity 15 min after WAY 100635 and the subsequent decline in activity towards the suppressed venlafaxine baseline level, as the effect of WAY 100635 dissipated over time. Five out of six cells tested with WAY 100635 displayed increases in unit activity above pre-venlafaxine baseline levels.

The i.v. administration of WAY 100635 to venlafaxine-pretreated animals elicited a number of behaviors, including hindlimb abduction, arching of the back, abnormal posture, immobility, protrusion of the claws, piloerection and vocalization (Table 1), which are consistent with the induction of the 5-HT syndrome in cats (Bogdanski et al., 1958; Rodriguez et al., 1973). The intensity of these behavioral effects varied across individual animals and was most pronounced during the first 5 min after the administration of WAY 100635. During this period, cats appeared to be in distress and vocalized continually (up to as many times as 15–20/min). Each cat typically adopted a crouched or haunched, immobile posture with hindlimbs splayed, head down and with a fixed gaze. The cortical EEG was characterized by a low-voltage fast (desynchronized) pattern. The pupils were fully dilated and less reactive to light, compared to venlafaxine treatment alone. Micturition and defecation were not observed, although one cat vomited shortly (1–2 min) after WAY 100635. This cat also exhibited marked salivation a few minutes following emesis. Other behavioral effects that were observed (but not scored) in individual animals included backward treading, splaying of the forelimbs, hindlimb ataxia, and sham rage. None of the cats exhibited hyperlocomotion, tremor, rigidity, head and/or body shakes. Respiratory rate and depth appeared unaltered. The behavioral changes induced by WAY 100635 largely subsided within 30–60 min. The cortical EEG became less desynchronized over time and soon thereafter large amplitude slow waves began to appear as the animals became drowsy, with most cats falling asleep during the second hour after WAY 100635 administration. The general appearance and activity of the cats returned to normal by this time.

The effects of WAY 100635 do not appear to be specific to the i.v. route of administration (or the rapidity of drug action), since similar behavioral responses were observed after s.c. administration of WAY 100635 (0.2 mg/kg) in the two cats pretreated with i.p. venlafaxine (data not shown). One of these cats also exhibited continuous panting (respiratory rate $\sim$ 180 breaths/min) for up to 5 h after the injection of WAY 100635. The close temporal relationship between the increase in neuronal activity and the appearance of adverse side effects after both i.v. and s.c. administration of WAY 100635 suggests a causal effect.

3.5. Interaction of venlafaxine with p-MPPI

To determine whether the effects of WAY 100635 generalize to another selective 5-HT_{1A} receptor antagonist, *p*-MPPI (0.1 mg/kg, i.v.) was administered after i.v. venlafaxine (1 mg/kg, total cumulative dose) in one cat. Like WAY 100635, *p*-MPPI rapidly restored neuronal

firing to an elevated level (115% of baseline) and precipitated a similar behavioral reaction, characterized by both motor (hindlimb abduction, abnormal posture, immobility) and autonomic (emesis, piloerection, pupil dilation) disturbances. These responses almost completely subsided within 30–60 min (data not shown). Thus, the effects of WAY 100635 on both unit activity and behavior appear to be related to its 5-HT_{1A} receptor antagonist properties and not to a nonspecific effect of the drug.

4. Discussion

The present findings indicate that venlafaxine, a combined 5-HT/noradrenaline reuptake inhibitor, strongly inhibits the activity of serotonergic dorsal raphe neurons in freely moving cats, at relatively low doses. Moreover, this action is long lasting (>24 h) and completely reversible with time. The potency of venlafaxine in suppressing serotonergic dorsal raphe neurons in freely moving cats ($ED_{50} = 0.19$ mg/kg, i.v.) was similar to that observed in anesthetized rats (ED_{50}s $= 0.16$ and 0.23 mg/kg, i.v.) (Gartside et al., 1997; Béïque et al., 1999). Although venlafaxine is a relatively weak inhibitor of 5-HT reuptake in vitro in comparison to selective 5-HT reuptake blockers (Bolden-Watson and Richelson, 1993), its potency in suppressing serotonergic neurons in vivo is nearly identical to that of paroxetine, one of the most potent 5-HT reuptake inhibitors. The high in vivo potency of venlafaxine observed in electrophysiological studies may be related, at least in part, to its comparatively low plasma protein and tissue binding (Morton et al., 1995), which results in higher concentrations of active drug for entry into the brain. Overall, these data indicate that venlafaxine is a potent inhibitor of serotonergic neuronal activity and are consistent with the hypothesis that venlafaxine potentiates the action of neuronally released 5-HT at central synapses via reuptake inhibition.

The suppression of serotonergic neuronal activity produced by venlafaxine appears to be mediated via activation of 5-HT_{1A} autoreceptors, since this effect was readily reversed by WAY 100635, a highly selective 5-HT_{1A} receptor antagonist (Forster et al., 1995). Because venlafaxine lacks appreciable affinity for 5-HT_{1A} receptors (Owens et al., 1997), a direct stimulatory action on 5-HT_{1A} autoreceptors cannot account for its effects on neuronal activity. Moreover, venlafaxine neither inhibits monoamine oxidase (Muth et al., 1986) nor releases 5-HT from presynaptic nerve terminals (Béïque et al., 1999). Therefore, the most plausible mechanism by which venlafaxine inhibits the activity of serotonergic neurons is by increasing the availability of synaptic 5-HT at somatodendritic 5-HT_{1A} autoreceptors, as a consequence of blocking the neuronal reuptake of 5-HT in the raphe region. Venlafaxine has been shown to increase extracellular 5-HT levels in the rat brain after systemic administration (Gur et al., 1999), although another study found no such effect (Dawson et al., 1999).

Similar to antidepressants, which block noradrenaline reuptake, venlafaxine also inhibits the electrical activity of noradrenergic locus coeruleus neurons in the anesthetized rat, by indirectly activating inhibitory somatodendritic α_2 autoreceptors (Haskins et al., 1985; Béïque et al., 1999). However, this effect occurs at somewhat higher doses than those required to inhibit the activity of serotonergic dorsal raphe neurons. Thus, venlafaxine is approximately three to four times less potent in inhibiting noradrenergic locus coeruleus neurons than serotonergic dorsal raphe neurons (Haskins et al., 1985; Gartside et al., 1997; Béïque et al., 1999), suggesting a preferential action of venlafaxine on the central serotonergic system. This is in accordance with various in vitro studies, which indicate that venlafaxine is a more potent 5-HT than noradrenaline reuptake inhibitor (Muth et al., 1986; Bolden-Watson and Richelson, 1993; Owens et al., 1997). Nonetheless, at the doses used in the present study, venlafaxine would be expected to inhibit the neuronal reuptake of noradrenaline (as well as 5-HT) in the brain, since the reported ED_{50} values for venlafaxine-induced suppression of noradrenergic neurons (0.68 mg/kg and 0.73 mg/kg, i.v.) fall within our dose range.

Theoretically, venlafaxine and other dual 5-HT/noradrenaline reuptake inhibitors may suppress the activity of serotonergic neurons to a lesser degree than selective 5-HT reuptake inhibitors, by indirectly activating excitatory α_1-adrenoceptors in the dorsal raphe nucleus (Auerbach et al., 1995; Gartside et al., 1997; Mongeau et al., 1997). These receptors are thought to play a critical role in maintaining the spontaneous discharge rate of serotonergic dorsal raphe neurons, at least in the anesthetized animal. Since blockade of α_1-adrenoceptors abolishes neuronal activity (Baraban and Aghajanian, 1980), an increased activation of these receptors by endogenous noradrenaline, secondary to reuptake inhibition, may offset the inhibitory action of 5-HT at somatodendritic 5-HT_{1A} autoreceptors. Such an effect could conceivably explain the reported rapid onset of antidepressant activity and greater therapeutic effectiveness of dual 5-HT/noradrenaline reuptake inhibitors in the treatment of major depression. However, dual 5-HT/noradrenaline reuptake inhibitors, such as venlafaxine and duloxetine, appear to be as potent, if not more potent, than most selective 5-HT reuptake inhibitors in suppressing serotonergic neuronal activity (cf. Gartside et al., 1997; Smith and Lakoski, 1997; Béïque et al., 1999). Moreover, pretreatment with a selective noradrenaline reuptake inhibitor does not attenuate the inhibitory response of serotonergic neurons to a selective 5-HT reuptake inhibitor (Gartside et al., 1997), as expected if noradrenaline was able to counteract the effects of 5-HT on neuronal activity. Overall, these results suggest that the excitatory influence of noradrenaline on serotonergic neuronal activity is essentially maximal under basal conditions (in both awake and anesthetized preparations).

One of the most significant findings of the present study was that the restoration of serotonergic neuronal activity produced by WAY 100635 in venlafaxine-pretreated animals was associated with an adverse behavioral reaction. Thus, cats displayed hindlimb abduction, arched back, immobility, protruded claws, piloerection, vocalization and several other abnormal behavioral responses (Table 1). The intensity of this reaction was temporally related to the degree of neuronal restoration. Thus, the behavioral changes were most pronounced immediately after the i.v. injection of WAY 100635 when neuronal activity was maximally elevated by the antagonist. As neuronal activity declined, the behavioral responses diminished as well, until they could no longer be discriminated. Since the abnormal behaviors closely paralleled the overshoot in neuronal activity, they are likely to be mediated by an increase in central serotonergic function. We believe that this drug combination induced the 5-HT syndrome in these cats. The 5-HT syndrome is linked to excessive serotonergic stimulation (Jacobs, 1976) and several of the behavioral changes seen in the cats are consistent with such a syndrome (Bogdanski et al., 1958; Rodriguez et al., 1973). Importantly, these effects were observed at low clinically relevant doses of venlafaxine and they were reproduced using another highly selective 5-HT_{1A} autoreceptor antagonist, *p*-MPPI, in combination with venlafaxine.

Previous electrophysiological studies have also reported that WAY 100635 can reverse the acute inhibitory effect of venlafaxine on the activity of serotonergic dorsal raphe neurons recorded in anesthetized rats (Gartside et al., 1997; Béïque et al., 1999). However, the use of anesthesia in those studies obviously precluded the assessment of behavior, let alone the possible correlation between the changes in neuronal activity and behavior that might have resulted from this drug combination. Moreover, WAY 100635 appears to be somewhat less effective in the anesthetized animal, since it does not completely restore neuronal activity after venlafaxine administration, despite using comparable doses (Gartside et al., 1997). This may be related to the fact that WAY 100635 alone can inhibit the spontaneous activity of serotonergic neurons under anesthesia, apparently by blocking the tonic excitatory action of noradrenaline on these cells (Martin et al., 1999), whereas, in the freely moving animal, such an effect is not observed.

In the awake animal, the vast majority of serotonergic neurons appear to be under tonic feedback inhibition, mediated by 5-HT_{1A} autoreceptors. Thus, systemic administration of both selective and nonselective 5-HT_{1A} autoreceptor antagonists (WAY 100635, *p*-MPPI and spiperone) has been shown to increase the spontaneous discharge rate of most serotonergic dorsal raphe neurons in freely moving cats (Fornal et al., 1994, 1996; Bjorvatn et al., 1998). Moreover, these antagonists not only reverse the effects of 5-HT_{1A} agonists, but actually elevate neuronal activity above baseline levels, as observed in the present study when WAY 100635 (or *p*-MPPI) was administered after venlafaxine. This overshoot in neuronal activity may have been responsible for the adverse effects observed with these antagonists in combination with venlafaxine, since the abnormal behaviors largely corresponded with the overshoot, when 5-HT release should be maximally elevated. Although WAY 100635 and *p*-MPPI increase neuronal activity by themselves, these antagonists produce no overt behavioral changes, even when administered at doses fivefold, or greater, than those used in the present study (Fornal et al., 1996; Bjorvatn et al., 1998). However, unlike the combination of a 5-HT_{1A} autoreceptor antagonist and a 5-HT reuptake inhibitor, the administration of 5-HT_{1A} autoreceptor antagonists alone produces little or no increase in basal extracellular 5-HT levels (Romero et al., 1996; Dawson and Nguyen, 1998), which may explain their general lack of effect on gross behavior.

The adverse behavioral reaction produced by venlafaxine and WAY 100635 was unexpected because previously we had not observed any marked behavioral changes in cats when WAY 100635 was combined with either the selective 5-HT reuptake inhibitor fluoxetine or sertraline, even though the effects on neuronal activity were strikingly similar (Fornal et al., 1999a; unpublished results). These results suggest that the venlafaxine/WAY 100635 combination is associated with a greater potentiation of serotonergic neurotransmission, when compared to combinations of selective 5-HT reuptake inhibitors and WAY 100635.

Neurochemical studies have shown that WAY 100635 can potentiate the effect of venlafaxine on extracellular levels of 5-HT but not those of noradrenaline (Dawson et al., 1999). Moreover, WAY 100635 was reported to produce a much larger increase in extracellular 5-HT when combined with venlafaxine than when combined with fluoxetine. Such results cannot be explained on the basis of single-unit activity, since WAY 100635 is equally effective in restoring neuronal activity after both venlafaxine and fluoxetine, but may instead be attributed to the distinct actions of these antidepressant drugs at nerve terminals.

Recent evidence suggests an interaction between noradrenergic and serotonergic neurons with respect to monoamine reuptake. For example, by measuring the disappearance of locally applied 5-HT, Daws et al. (1998) demonstrated that exogenous 5-HT can be taken up by both serotonergic and noradrenergic nerve terminals in brain areas receiving dual monoaminergic input. In a related study, Bel and Artigas (1996) showed that the increases in extracellular 5-HT levels produced by systemic administration of fluoxetine were potentiated by a selective noradrenaline reuptake inhibitor, which alone had no effect on extracellular 5-HT. These results suggest that under certain conditions blockade of the noradrenaline transporter may increase extracellular concentrations of 5-HT, presumably by preventing the reuptake of 5-HT into noradrenergic neurons. Thus, following the combined adminis-

tration of a 5-HT_{1A} autoreceptor antagonist and a selective 5-HT reuptake inhibitor, such as fluoxetine, a significant amount of the enhanced extracellular 5-HT may escape from the synaptic cleft and come into contact with neighboring noradrenergic nerve terminals, and subsequently be taken up by the noradrenaline transporter. However, by simultaneously blocking the noradrenaline transporter, dual 5-HT/noradrenaline reuptake inhibitors, such as venlafaxine, further prevent the clearance of 5-HT by noradrenergic neurons, thereby resulting in greater accumulations of extracellular 5-HT, which may then affect more distal 5-HT receptors. This may result in excessive stimulation of postsynaptic 5-HT receptors and, consequently, to the emergence of the 5-HT syndrome.

Another mechanism by which dual 5-HT/noradrenaline reuptake inhibitors may produce the 5-HT syndrome when combined with a 5-HT_{1A} autoreceptor antagonist involves a synergistic interaction between 5-HT and noradrenaline at postsynaptic target sites. Thus, in the mammalian central nervous system, 5-HT and noradrenaline often have similar, or complementary, actions on many physiological systems, including those likely to mediate various aspects of the 5-HT syndrome. For example, both 5-HT and noradrenaline (acting via distinct receptor mechanisms) have been shown to facilitate the excitability of brainstem and spinal motoneurons (McCall and Aghajanian, 1979; White and Neuman, 1980), as well as sympathetic preganglionic neurons located in the spinal cord (Lewis and Coote, 1996). 5-HT and noradrenaline may act as gain setters, modulating the responsiveness of these cells to excitatory inputs. Therefore, in the presence of increased noradrenergic tone induced by venlafaxine, the potentiation of serotonergic neurotransmission by WAY 100635 may now lead to overstimulation of motor and autonomic centers, culminating in the 5-HT syndrome. The finding that virtually all components of the 5-HT motor syndrome in rats can be attenuated, or abolished, by disrupting noradrenergic neurotransmission is consistent with this hypothesis (Tricklebank et al., 1985).

Previous studies in rodents have indicated that the 5-HT syndrome is largely mediated by activation of postsynaptic 5-HT_{1A} receptors (Smith and Peroutka, 1986; Yamada et al., 1988). Since WAY 100635 blocks these receptors (in addition to presynaptic 5-HT_{1A} autoreceptors), it would be expected to prevent the 5-HT syndrome that may result from its ability to potentiate venlafaxine's effects on extracellular 5-HT levels. However, since WAY 100635 is a competitive antagonist, it is possible that the increases in extracellular 5-HT produced by this drug combination overwhelmed the postsynaptic receptor blockade induced by WAY 100635. Thus, at the doses used in the present study, WAY 100635 may have only partially blocked the action of 5-HT at postsynaptic 5-HT_{1A} receptors; hence, the appearance of behavioral signs of the 5-HT syndrome. Alternatively, the behavioral responses observed in cats after venlafaxine and WAY 100635 may be mediated via non-5-HT_{1A} receptors. In this regard, 5-HT_{2A} and 5-HT_{2C} receptors have also been implicated in the 5-HT syndrome (Pranzatelli, 1990) and 5-$HT_{2A/2C}$ receptor agonists, such as 2,5-dimethoxy-4-methylamphetamine (DOM), have been shown to elicit behavioral effects in cats similar to those observed with venlafaxine and a 5-HT_{1A} autoreceptor antagonist (Wallach et al., 1972).

Recently, the putative 5-HT_{1A} autoreceptor antagonist pindolol has been used to augment the therapeutic action of selective 5-HT reuptake inhibitors in depressed patients (Blier and Bergeron, 1998). Although the addition of pindolol to these antidepressant medications has not been associated with serious side effects, such as the 5-HT syndrome, pindolol may not be an effective 5-HT_{1A} autoreceptor antagonist, as initially claimed. Thus, we have shown that pindolol lacks appreciable antagonist activity at 5-HT_{1A} autoreceptors, as indicated by its inability to reverse the inhibitory action of both fluoxetine and 8-OH-DPAT on serotonergic neuronal activity in freely moving cats (Fornal et al., 1999a,b). In fact, pindolol appears to act as an agonist at 5-HT_{1A} autoreceptors, since it strongly suppressed the spontaneous activity of serotonergic neurons by a WAY 100635-sensitive mechanism. Therefore, it remains to be determined clinically whether augmentation therapy with a highly potent and selective 5-HT_{1A} autoreceptor antagonist is associated with an increased risk of developing the 5-HT syndrome.

In summary, due to the inherent limitations of currently available antidepressant drugs, these medications are often combined in clinical practice with various other drugs for the purpose of enhancing their therapeutic efficacy. One current strategy consists of combining a 5-HT_{1A} autoreceptor antagonist with a selective 5-HT reuptake inhibitor. Although this drug combination may improve efficacy, by further enhancing 5-HT neurotransmission, it may also increase the potential risk of developing the 5-HT syndrome. Our results suggest that this risk may be higher when 5-HT_{1A} autoreceptor antagonists are combined with dual 5-HT/noradrenaline reuptake inhibitors than with selective 5-HT reuptake inhibitors.

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