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Effects of the co-administration of mirtazapine and paroxetine on serotonergic neurotransmission in the rat brain

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Abstract

The α_2-adrenoreceptor antagonist mirtazapine, which is also a 5-HT_2, 5-HT_3 and H_1 receptors antagonist and the selective serotonin (5-HT) reuptake inhibitor paroxetine are effective antidepressant drugs which enhance 5-HT neurotransmission via different mechanisms. The present studies were undertaken to determine whether the mirtazapine–paroxetine combination could induce an earlier and/or a greater effect on the 5-HT system than either drug alone. Using in vivo electrophysiological paradigms, the firing activity of dorsal raphe 5-HT neurons was decreased by 70% in rats treated with paroxetine (10 mg/kg/day, s.c.) for 2 days and was back to normal after 21 days. In contrast, a 2-day treatment with mirtazapine (5 mg/kg/day, s.c.) did not alter the firing of 5-HT neurons whereas it was increased by 60% after 21 days of treatment. A low dose of mirtazapine (5 mg/kg/day, s.c.×2 days) failed to offset the decremental effect of paroxetine on the 5-HT neuron firing activity, but a higher dose (10 mg/kg/day, s.c.×2 days) did attenuate the decremental effect of paroxetine. In the dorsal hippocampus, neither mirtazapine (5 mg/kg/day, s.c.) nor a paroxetine (10 mg/kg/day, s.c.) treatment altered the responsiveness of 5-HT_{1A} receptors to microiontophoretically-applied 5-HT. Both in controls and in rats treated for 2 days with paroxetine alone, the administration of the 5-HT_{1A} antagonist WAY 100635 (25–100 μg/kg, i.v.) did not change the firing activity of dorsal hippocampus CA_3 pyramidal neurons. However, WAY 100635 increased significantly the firing activity of these neurons in rats treated with mirtazapine alone but to a greater extent with both mirtazapine and paroxetine for 2 days. After 21 days of treatment, WAY 100635 increased to a greater degree the firing rate of CA_3 pyramidal neurons in rats which received the combination over rats given either drug alone. It is concluded that the mirtazapine–paroxetine combination shortened the delay in enhancing the tonic activation of postsynaptic 5-HT_{1A} receptors and produced a greater activation of the postsynaptic 5-HT_{1A} receptors than either drug given alone. The present results suggested that mirtazapine may have a faster onset of action than a SSRI, and that the co-administration of mirtazapine and paroxetine may accelerate the antidepressant response and as well as being more effective than either drug alone.

Keywords: Mirtazapine; Paroxetine; Serotonin; Dorsal raphe; Dorsal hippocampus

1. Introduction

Although the physiopathology of major depression is not fully defined, there is a growing body of evidence suggesting the implication of the serotonin (5-HT) system in the therapeutic effect of antidepressant treatments (Heninger and Charney, 1987; Price et al., 1990; Van Praag et al., 1990; Cummings, 1993; Blier and de Montigny, 1994; Maes and Meltzer, 1995). It has been shown that long-term tricyclic antidepressant (TCA) and repeated electroconvulsive shock (ECS) administration lead to an enhanced 5-HT neurotransmission through a sensitization of the postsynaptic 5-HT_{1A} receptors (de Montigny and Aghajanian, 1978; de Montigny, 1984; Welner et al., 1989; Nowak and Dulinski, 1991; Stockmeier et al., 1992). Long-term treatment with either monoamine oxidase inhibitors (MAOIs) or selective 5-HT reuptake inhibitors (SSRIs) results in a desensitization of the somatodendritic 5-HT_{1A} autoreceptor of 5-HT neurons in the dorsal raphe nucleus, thereby allowing their firing rate to recover in the presence of the drugs (Blier et al., 1986; Chaput et al., 1986). In addition, long-term SSRI treatment also desensitizes terminal 5-HT_{1B} (in the rat) and 5-HT_{1D} (in the guinea-pig)

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autoreceptors, whereas long-term MAOI treatment desensitizes terminal α_2-adrenergic heteroreceptors located on 5-HT terminals (Blier and Bouchard, 1994; Mongeau et al., 1994), the role of both receptors being to inhibit 5-HT release (Göthert, 1980). Long-term treatment with the antidepressant mirtazapine, an α_2-adrenoceptor antagonist, increases 5-HT neurotransmission, as a result of a sustained increase in the firing activity of 5-HT neurons in presence of decreased function of α_2-adrenergic heteroreceptors located on 5-HT terminals (Haddjeri et al., 1997). Finally, long-term treatment with 5-HT_{1A} receptor agonists, such as gepirone, desensitizes the 5-HT_{1A} autoreceptor on 5-HT neurons, but not the postsynaptic 5-HT_{1A} receptors located on CA_3 pyramidal neurons (Blier and de Montigny, 1987; Schechter et al., 1990; Godbout et al., 1991; Fanelli and McMonagle-Strucko, 1992; Dong et al., 1998; Haddjeri et al., 1999).

Recently, novel direct evidence of an enhanced 5-HT neurotransmission by antidepressant treatments have been provided (Haddjeri et al., 1998). This study showed that long-term treatment with either the TCA imipramine, the SSRI paroxetine, the selective and reversible MAO-A befloxatone, the α_2-adrenergic antagonist mirtazapine, the 5-HT_{1A} receptor agonist gepirone or repeated ECS therapy enhanced the tonic activation of postsynaptic 5-HT_{1A} receptors in the dorsal hippocampus, as put into evidence by the enhanced disinhibition produced by the acute administration of the selective 5-HT_{1A} receptor antagonist WAY 100635 (Haddjeri et al., 1998).

The present study was undertaken to determine whether the association of mirtazapine with the SSRI paroxetine could act in synergy to enhance 5-HT neurotransmission. This was deemed of interest because these drugs have been shown to modulate the 5-HT system via different mechanisms. Thus, using in vivo electrophysiological paradigms in the rat dorsal raphe and dorsal hippocampus, the effects of short-term and long-term treatments with mirtazapine and paroxetine, given alone and in combination, on the firing activity 5-HT neurons and on the degree of activation of postsynaptic 5-HT_{1A} receptors have been assessed.

2. Materials and methods

2.1. Animals and treatments

Male Sprague–Dawley rats (Charles-River, Quebec, Canada) weighing 300±20 g on the day of the experiment were used. The animals were maintained on a 12:12 h light–dark cycle with free access to food and water. Rats were treated for 2 or 21 days with either mirtazapine (5 or 10 mg/kg/day), paroxetine (10 mg/kg/day), or the combination (mirtazapine–paroxetine), using osmotic minipumps (Alza, Palo Alto, CA, USA) implanted subcutaneously on the dorsal region of the rat under halothane anesthesia. Mirtazapine and paroxetine were dissolved in a water–ethanol solution (80:20, v/v and 50:50, v/v, for the 2 day- and 21 day treatments, respectively). For each series of experiments, controls were implanted with a minipump filled with the same vehicle as the corresponding treated groups. Rats receiving the mirtazapine–paroxetine combination were implanted with two minipumps. All the experiments were carried out with the minipumps on board. None of these treatments altered the normal behavior of the animals in their cages or upon handling, and the weight gain was similar in the treated groups than in controls. The regimens above have been chosen on the basis of previous experiments indicating a marked blockade of 5-HT reuptake with 10 mg/kg/day of paroxetine (Piñeyro et al., 1994), and a blockade of α_2-adrenoceptors by 5 mg/kg/day of mirtazapine after a long-term administration (Haddjeri et al., 1997).

2.2. Electrophysiological procedures

Rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and were mounted in a stereotaxic apparatus. Additional doses (100 mg/kg, i.p.) were given to maintain the anesthesia along the experiment.

2.2.1. Extracellular unitary recordings from dorsal raphe 5-HT neurons

A hole was drilled 1 mm anterior to lambda on the midline. 5-HT neurons were recorded with single-barreled glass micropipettes filled with a 2 M NaCl solution. Their impedance range was between 2 and 4 MΩ. The dorsal raphe nucleus was encountered at a depth of 5–6 mm below the surface of the brain. The 5-HT neurons were identified according to the criteria of Aghajanian (1978), by their slow (0.5–2.5 Hz) and regular firing rate and long-duration (0.8–1.2 ms) positive action potentials. Once 5-HT neurons were identified, their spontaneous firing activity was recorded for 50–100 s to determine their mean baseline firing rate.

2.2.2. Extracellular recording and microiontophoresis from dorsal hippocampus CA_3 pyramidal neurons

A hole was drilled 4.2 mm lateral and 4.2 mm anterior to lambda. Extracellular recordings of dorsal hippocampus CA_3 pyramidal neurons and microiontophoretic applications of 5-HT were performed with five-barreled glass micropipettes pulled in the conventional manner in order to achieve a tip diameter of 10–15 μm. The central recording barrel was filled with a 2 M NaCl solution saturated with fast green dye. One of the side-barrels was filled with quisqualic acid (1.5 mM in 400 mM NaCl, pH 8) since a leak or a small ejection current (+2 to −4 nA) of quisqualate was needed to activate the CA_3 hippocampus pyramidal neurons within their physiological range in anesthetized animals. These neurons were identified according to the criteria of Kandel and Spencer (1961): large amplitude and long duration complex spike discharges. Two side-barrels were filled with 5-HT (10 mM in 200

mM NaCl, pH 4). Two ejection currents of 5-HT were used (5 and 10 nA), each ejection period lasting for 50 s. The fourth barrel was filled with 2 M NaCl and served as an automatic current balance.

In order to assess the blockade of the 5-HT transporter produced by paroxetine, the RT_{50} values after microiontophoretic applications of 5-HT (5 and 10 nA) were determined. The RT_{50} value corresponds to the time (in seconds) required to achieve a 50% recovery of the firing activity of the neuron once the microiontophoretic application of 5-HT has been stopped (de Montigny et al., 1980). This parameter is a reliable index of the in vivo activity of monoamine reuptake process in the rat hippocampus and previous studies have shown an increase of the RT_{50} values for 5-HT after acute intravenous paroxetine injections or after the lesion of the 5-HT pathways by the neurotoxin 5,7-dihydroxytryptamine (Piñeyro et al., 1994).

We mainly focused our interest on overall 5-HT transmission by assessing the tonic activation of the postsynaptic 5-HT_{1A} receptors of the dorsal hippocampus CA_3 pyramidal neurons. To this end, we compared the firing rate of dorsal hippocampus CA_3 pyramidal neurons before and after the systemic injection of the selective 5-HT_{1A} receptor antagonist, WAY 100635, via a lateral tail vein cannula in control and treated groups. It is well established that the suppression of the firing activity of CA_3 pyramidal neurons by microiontophoretic applied 5-HT is mediated through the activation of 5-HT_{1A} receptors (de Montigny et al., 1984). If a tonic activation of postsynaptic 5-HT_{1A} receptors exists, the firing activity of hippocampus CA_3 pyramidal neurons will be lowered even though the responsiveness of postsynaptic 5-HT_{1A} receptors to 5-HT is unchanged. Then, the blockade of these 5-HT_{1A} receptors by WAY 100635 will disinhibit the CA_3 hippocampus pyramidal neurons resulting in an increase of their firing activity (Haddjeri et al., 1998). In this series of experiments the current of quisqualate was chosen in order to obtain a firing rate around 3 Hz in order to more readily allow the detection of enhancements in firing following administration of WAY 100635. The baseline firing was recorded for at least 2 min before the intravenous injection of WAY 100635. An intravenous injection of saline preceded the first injection of WAY 100635 to eliminate any effect due to the injection by itself. The saline injection corresponds to the 0 mg/kg dose. WAY 100635 was administered in incremental doses of 25 μg/kg at time intervals of 1–2 min. To avoid residual drug effects, only one cell was studied in each rat. Since WAY 100635, administered intravenously, did not modify the firing rate of 5-HT neurons in the dorsal raphe nucleus of anesthetized rats (Forster et al., 1995; Lejeune and Millan, 1998; Haddjeri et al. unpublished observations), it can be assumed that any changes in the firing activity of hippocampus pyramidal neurons would reflect an increased level in the tonic activation of the postsynaptic 5-HT_{1A} receptors. Therefore, in treated animals, where there would be increased extracellular levels of 5-HT in the raphe region, WAY 100635 would restore 5-HT neuronal firing activity. However, because WAY 100635 was given systemically, it would be simultaneously blocking the effects of 5-HT on postsynaptic neurons, thereby cancelling out the effect of WAY 100635 on the somatodendritic autoreceptors. Indeed, if the action of WAY 100635 at the somatodendritic 5-HT_{1A} autoreceptors was influencing the activity of the hippocampal neurons, it would serve to further inhibit their firing due to an increased release of 5-HT into the target area. Thus, it can be assumed that any increment in the firing activity of hippocampus pyramidal neurons would reflect an increased level in the tonic activation of the postsynaptic 5-HT_{1A} receptors and the degree to which WAY 100635 disinhibits this firing would be a direct measure of the tonic level of activation of these receptors by extracellular 5-HT.

2.3. Drugs

Mirtazapine (ORG 3770) was provided by Organon (Oss, The Netherlands), paroxetine by SmithKline Beecham (Harlow, UK) and WAY 100635 by Wyeth–Ayerst (Princeton, NJ, USA). 5-HT creatinine sulfate and quisqualic acid were purchased from Sigma (St. Louis, MO, USA).

2.4. Statistical analyses

Results were expressed as mean±S.E.M. ANOVA followed by the Student Newman–Keuls test with a 0.05 statistic level of significance for the pairwise comparisons were used for all the statistical analyses except for the analysis of the results obtained with the short-term treatments in the dorsal raphe. In this latter series of experiments two doses of mirtazapine (5 and 10 mg/kg/day) were used. However, we were a priori interested in comparing the effects of the mirtazapine–paroxetine combination with either drug given alone and not in comparing the effect of one dose of mirtazapine to the other. For this reason, it was more appropriate to use Student's *t*-tests followed by the Bonferroni correction for this set of data.

One- and two-way repeated measure ANOVA were used to determine the effects of the different treatments on the firing rate of dorsal hippocampus CA_3 pyramidal neurons when repeated doses of WAY 100635 were given.

3. Results

3.1. Effects of mirtazapine and paroxetine on the spontaneous firing activity of 5-HT neurons of the dorsal raphe nucleus

Following a 2-day treatment with paroxetine (10 mg/kg/day, s.c.), there was a 75% reduction of the firing activity of dorsal raphe 5-HT neurons (Fig. 1). In rats

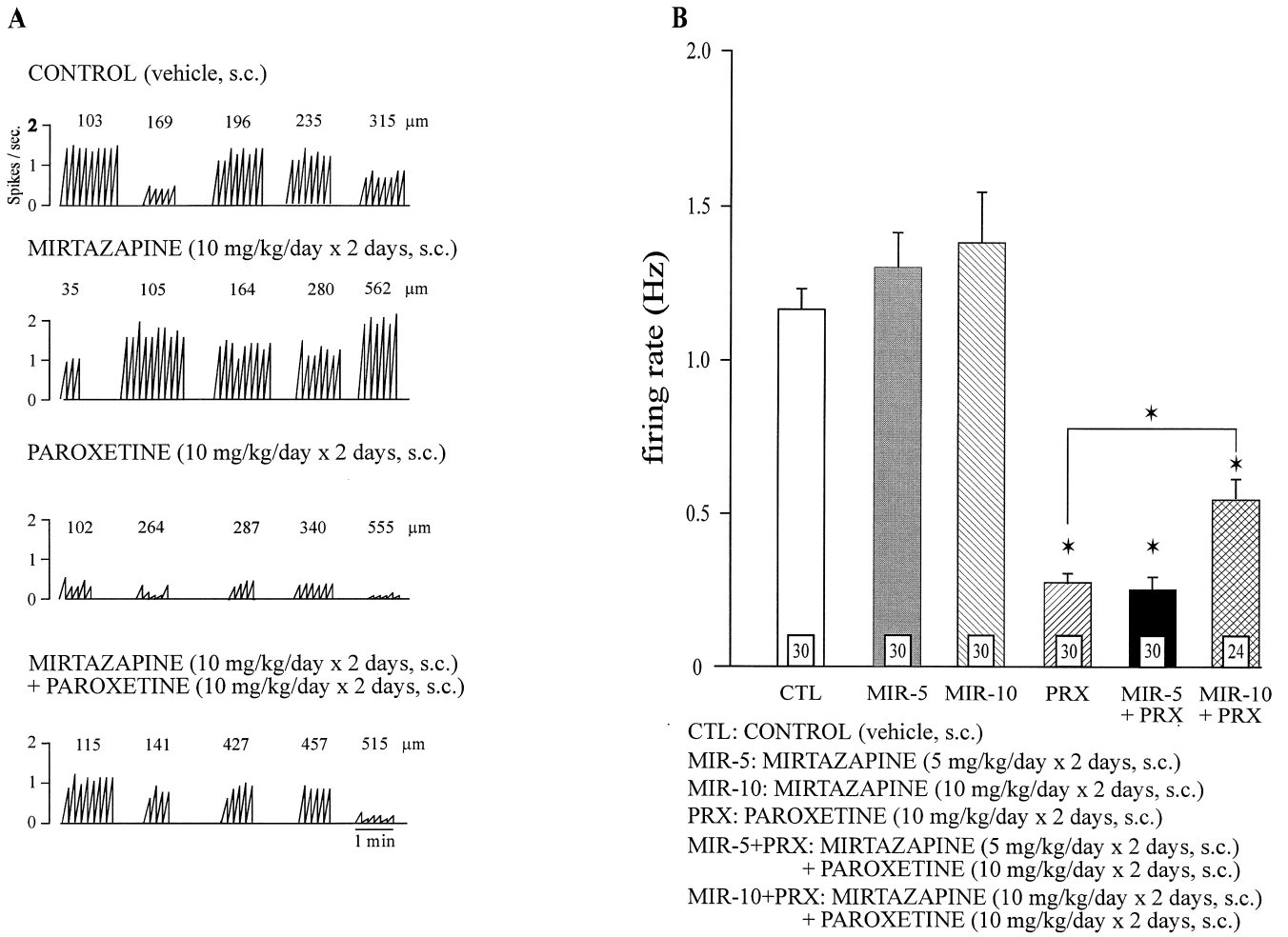

Fig. 1. Integrated firing rate histograms of 5-HT neurons in the dorsal raphe nucleus showing their spontaneous firing activity in control rats and in rats treated with MIR, PRX or both for 2 days (A). The number above each neuron indicates the depth from the ventral border of the sylvius aqueduct at which the neuron was recorded. The time base applies to all traces. (B) Effects of short-term treatments with MIR, PRX and their combination on the spontaneous firing activity of dorsal raphe 5-HT neurons (means±S.E.M.). Numbers in the bottom of the columns indicate the number of neurons tested. *$P<0.05$ between the treated group and the corresponding control group. *$P<0.05$ between the MIR-PRX treated group and the group treated with PRX alone. The Student's t test with the Bonferroni correction was used for the statistical analyses.

treated with mirtazapine (5 or 10 mg/kg/day, s.c.) for 2 days, the firing activity of 5-HT neurons appeared to be increased in a dose-dependent manner (12% and 19% with 5 and 10 mg/kg/day of mirtazapine, respectively; Fig. 1), although this did not reach statistical significance. When the low dose of mirtazapine (5 mg/kg/day, s.c.) was co-administered with paroxetine for 2 days, the firing rate of 5-HT neurons was similar to that observed with of paroxetine alone (Fig. 1). However, the co-administration of the high dose of mirtazapine (10 mg/kg/day, s.c.) significantly attenuated the reduction of the serotonergic firing activity produced by paroxetine (Fig. 1).

In rats treated with paroxetine (10 mg/kg/day, s.c.) for 21 days, the firing activity of dorsal raphe 5-HT neurons was not significantly different from the control group (Fig. 2). In rats treated with mirtazapine (5 mg/kg/day, s.c.) for 21 days, there was a significant increase (60%) of the firing rate of dorsal raphe 5-HT neurons. In the mirtazapine–paroxetine group, the firing activity of 5-HT neurons was similar to that of controls (Fig. 2).

3.2. Recovery time of dorsal hippocampus pyramidal neurons after microiontophoretic applications of 5-HT

Both the 2- and 21-day treatments with paroxetine (10 mg/kg/day, s.c.) significantly prolonged the RT_{50} following the microiontophoretic application of 5-HT with both ejections currents used (5 and 10 nA), whereas mirtazapine (5 mg/kg/day, s.c.) was without effect on this parameter. The paroxetine-induced prolongation of the RT_{50} was not modified by the co-administration of mirtazapine (Fig. 3).

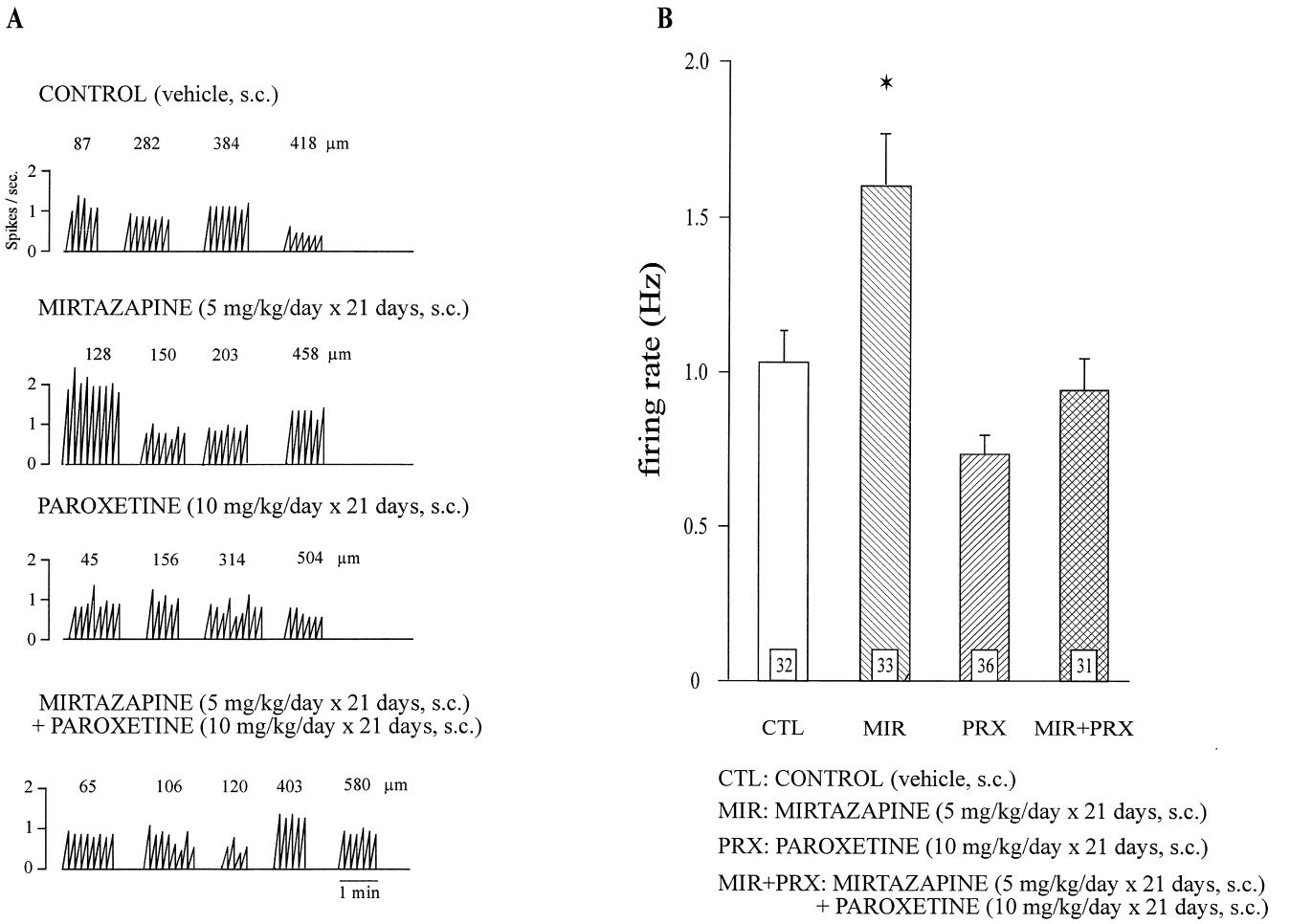

Fig. 2. Integrated firing rate histograms of 5-HT neurons in the dorsal raphe nucleus showing their spontaneous firing activity in control rats and in rats treated with MIR, PRX or both for 21 days (A). The number above each neuron indicates the depth from the ventral border of the sylvius aqueduct at which the neuron was recorded. The time base applies to all traces. (B) Effects of long-term treatments with MIR, PRX and their combination on the spontaneous firing activity of dorsal raphe 5-HT neurons (means±S.E.M.). Numbers in the bottom of the columns indicate the number of neurons tested. *$P<0.05$ between the treated group and the corresponding control group. The one-way ANOVA followed by Student's t test for the 2×2 comparisons was used for the statistical analyses.

3.3. Tonic activation of the postsynaptic 5-HT$_{1A}$ receptors on the dorsal CA$_3$ hippocampus pyramidal neurons by mirtazapine and paroxetine

As illustrated in Fig. 4, the injection of saline did not alter the firing activity of the dorsal hippocampus CA_3 pyramidal neurons in any of the groups. In control rats, the intravenous injection of the 5-HT_{1A} antagonist WAY 100635 (Haddjeri et al., 1998) did not modify the firing activity of CA_3 pyramidal neurons within a dose range of 25–100 µg/kg (Rueter et al., 1998; Figs. 4A and 6A). Similarly, in rats treated with paroxetine for 2 days, the intravenous injection of WAY 100635 failed to modify the firing activity of CA_3 pyramidal neurons (Figs. 4C and 6A). In rats treated with mirtazapine for 2 days, an increase in the mean firing rate of CA_3 pyramidal neurons was observed in response to intravenous injection of WAY 100635 (38%, 68%, 62% from baseline with 50, 75, 100 µg/kg of WAY 100635, respectively; Fig. 6A). In fact, these increases did reach statistical significance ($P=0.008$, using the Kruskal–Wallis one-way ANOVA on ranks). Moreover, following the 2-day co-administration of mirtazapine and paroxetine for 2 days, WAY 100635 induced a clear dose-dependent increase of the firing activity of hippocampus CA_3 pyramidal neurons (Fig. 6A).

As for the 21-day treatments with either mirtazapine or paroxetine alone, WAY 100635 induced a dose-dependent increase of the mean firing activity of CA_3 pyramidal neurons (Fig. 5). The degrees of increased in firing activity of these neurons induced by WAY 100635 in the mirtazapine- and in the paroxetine-treated groups were similar (Fig. 6B). When mirtazapine and paroxetine were co-administered, the increase of the firing activity of CA_3 pyramidal neurons in response to WAY 100635 was much

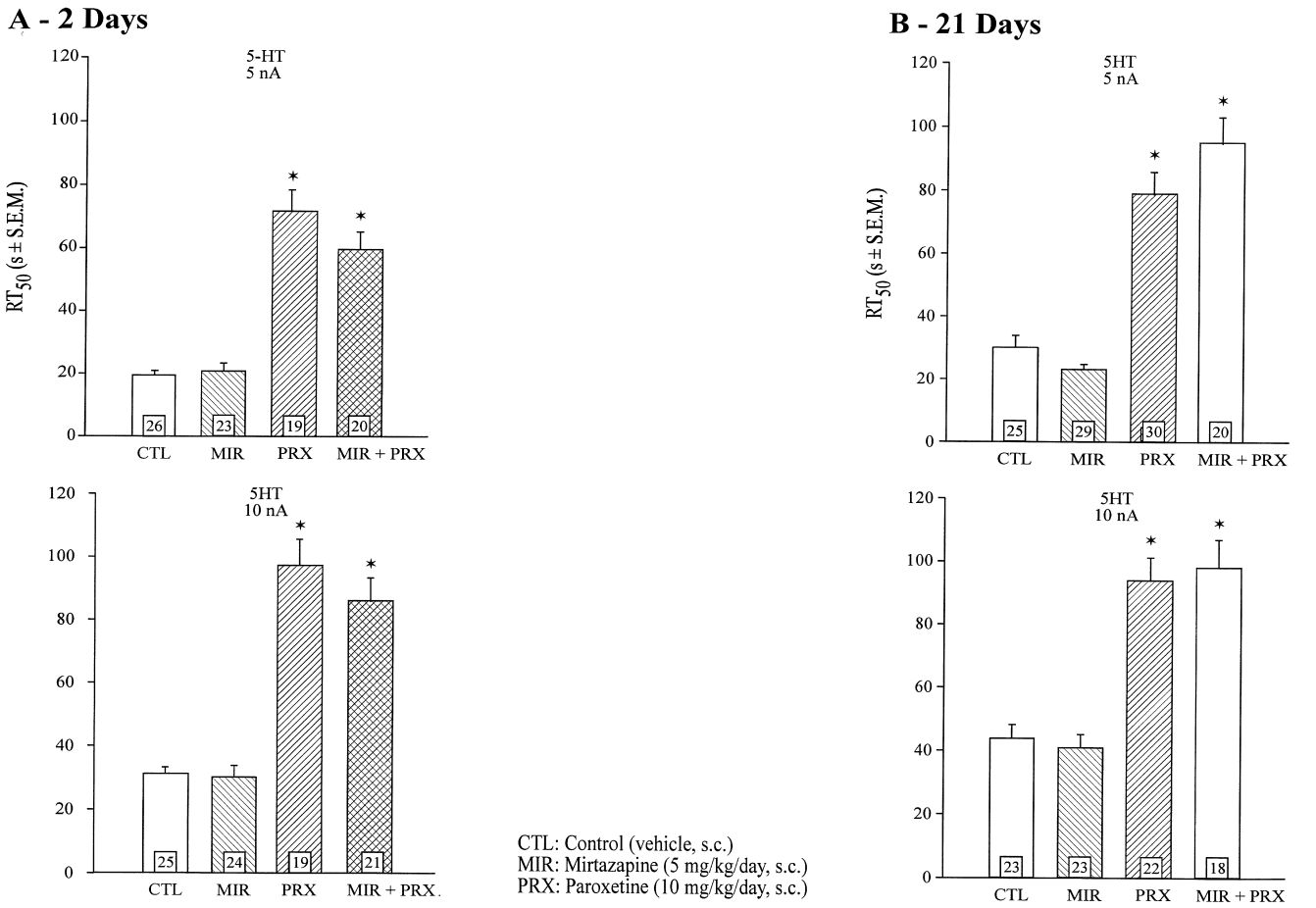

Fig. 3. Recovery time of dorsal hippocampus CA_3 pyramidal neurons, expressed as RT50 values (means±S.E.M.), from microiontophoretic applied 5-HT in control rats and in rats treated with MIR, PRX or both for 2 days (A) or 21 days (B). The numbers in the bottom of the columns indicate the number of neurons tested. *$P<0.05$ between the treated group and the control group, using the one-way ANOVA followed by Student's t test for the 2×2 comparisons.

greater than that observed with paroxetine or mirtazapine alone (Figs. 5 and 6B).

4. Discussion

Two main points of interest emerge from the present electrophysiological studies concerning the concomitant administration of the α_2-adrenoreceptor antagonist mirtazapine and the SSRI paroxetine. First, the delay for obtaining an increased tonic activation of postsynaptic 5-HT_{1A} receptors is shortened by the combination of mirtazapine and paroxetine. Second, the combination induced a greater enhancement of the tonic activation of postsynaptic 5-HT_{1A} receptors than either drug given alone.

The marked decrease of the firing activity of dorsal raphe 5-HT neurons and followed by recovery, after the short-term and the long-term administration of paroxetine respectively (Figs. 1 and 2), is in agreement with previous studies (see Blier and de Montigny, 1994 for review; Romero et al., 1996). The decreased firing activity of 5-HT neurons induced by short-term treatments with various SSRIs has been suggested to result from a greater activation of somatodentritic 5-HT_{1A} autoreceptors due to the increased levels of extracellular 5-HT in the dorsal raphe produced by SSRIs (Bel and Artigas, 1992; Hjorth et al., 1995; Romero et al., 1996). The recovery of firing activity upon pursuing the SSRI treatment results from a desensitization of these somatodentritic 5-HT_{1A} autoreceptors (see Blier and de Montigny, 1994 for review).

Previous studies have established that the firing activity of 5-HT neurons in the dorsal raphe nucleus is dependent on the tonic activation of α_1-adrenoreceptors located on the soma and dendrites of these neurons (Svensson et al., 1975; Baraban and Aghajanian, 1980a,b). The transient increase of the firing activity of dorsal raphe 5-HT neurons induced by the acute administration of mirtazapine (250 μg/kg, i.v.) has been shown to be due to the enhancement of noradrenaline (NA) release, leading to a greater activation of their α_1-adrenoreceptors (Haddjeri et al., 1996). In the present study, the 2-day treatment with mirtazapine did

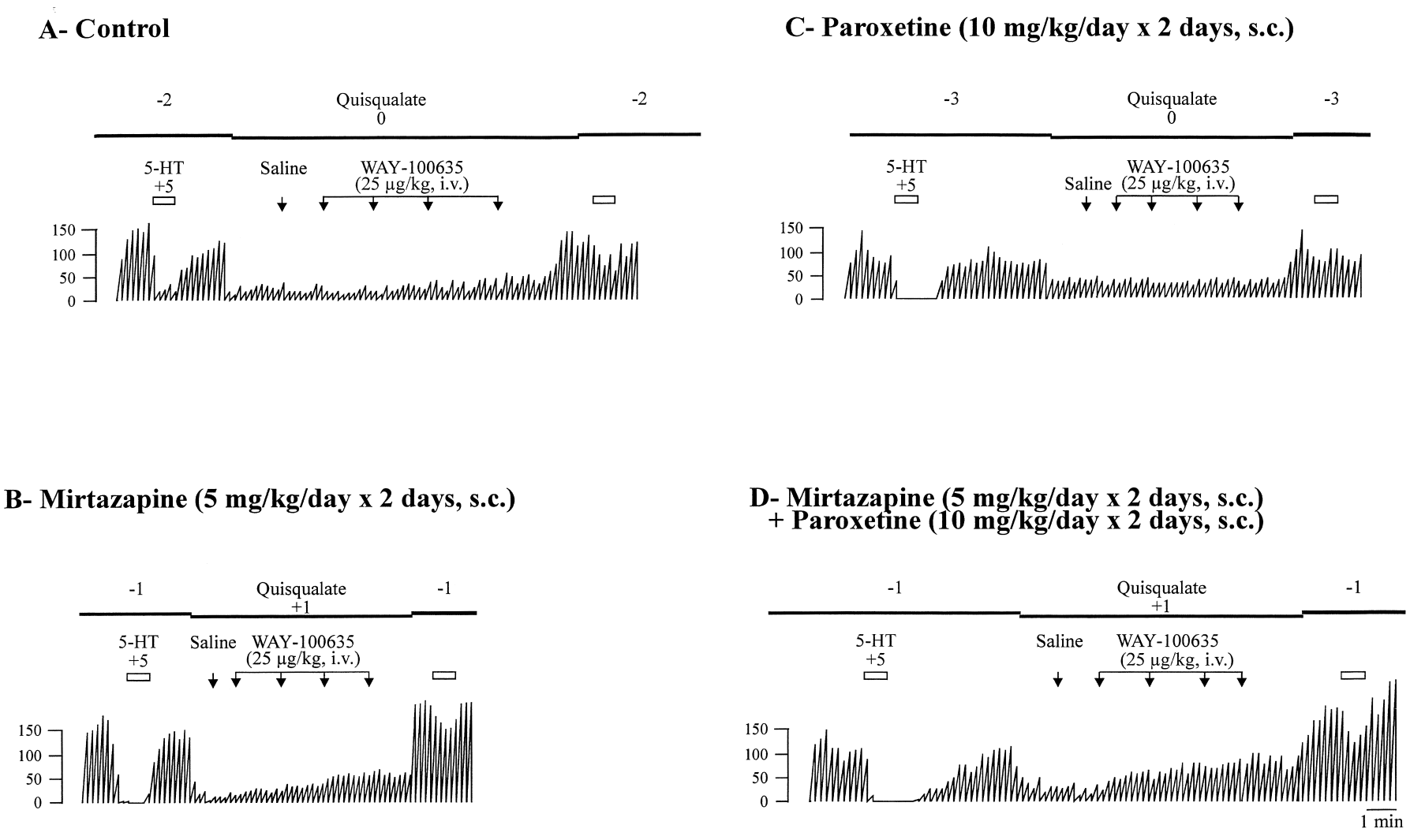

Fig. 4. Integrated firing rate histograms of dorsal hippocampus CA_3 pyramidal neurons showing their responsiveness to microiontophoretic applications of 5-HT and to acute injections of cumulative doses of WAY 100635 (25–100 µg/kg, i.v.) in control rats and in rats treated with MIR, PRX and their combination for 2 days. The neurons were activated by microiontophoretic applications of quisqualate (+1 to −3 nA). White horizontal bars indicate the duration of 5-HT applications. Arrows indicate the moment of the injection of WAY 100635 (25 µg/kg, i.v.).The time base applies to all traces.

not increase significantly the firing activity of 5-HT neurons. This contrasts with the 80% increase of the firing activity of dorsal raphe 5-HT neurons produced by an acute injection of mirtazapine (250 μg/kg, i.v.; Haddjeri et al., 1996). This apparent discrepancy might be due to lower cerebral concentrations of mirtazapine attained after a 2-day subcutaneous administration than after an intravenous bolus. However, after 21 days of administration, mirtazapine (5 mg/kg/day, s.c.) significantly increased (60%) the spontaneous firing activity of dorsal raphe 5-HT neurons, possibly due to a desensitization of α_2-adrenergic heteroreceptors located on 5-HT terminals (Haddjeri et al., 1997), as previously reported.

When the two drugs were co-administered for 2 days, the low dose of mirtazapine (5 mg/kg/day, s.c.) failed to reverse the paroxetine-induced reduction of the firing activity of dorsal raphe 5-HT neurons. However, the high dose of mirtazapine (10 mg/kg/day, s.c.) did attenuate the suppressant effect of paroxetine (Fig. 1). When the two antidepressant drugs were co-administered during 21 days, the mirtazapine-induced increase of the firing rate of 5-HT neurons was no longer present. Altogether these results suggest that a physiological opposition between the 5-HT_{1A} autoreceptor and the postsynaptic α_1-adrenoceptor may exist, that is the opposing actions of paroxetine and mirtazapine on 5-HT neuronal firing activity would tend to cancel each other.

A pharmacokinetic interaction between mirtazapine and paroxetine is unlikely to account for the above results since mirtazapine does not alter the activity of the cytochrome P450 isoenzymes (Delbressine and Vos, 1997), and the inhibitory effect of paroxetine on the activity of CYP2D6 (Bauman, 1996; Lane, 1996) would rather lead to increased plasma levels of mirtazapine. A competitive interaction at the receptor level can also be ruled out since both mirtazapine and paroxetine have negligible affinity for 5-HT_{1A} receptors (Tulloch and Johnson, 1992; De Boer, 1996; Goodwin, 1996), and paroxetine has no affinity for adrenoreceptors (Goodwin, 1996; Frazer, 1997).

An interaction at the level of the locus coeruleus can, however, be involved in the effects of the combination of mirtazapine and paroxetine (Fig. 2). NA neurons of the locus coeruleus receive a dense 5-HT input (Steinbusch, 1984) and send projections to the dorsal raphe nucleus (Loizou, 1969; Anderson et al., 1977). Previous studies from our laboratory have provided evidence for a decreased firing activity of locus coeruleus NA neurons following a long-term treatment with paroxetine (10 mg/kg/day, s.c.; Szabo et al., 1999). Preliminary results suggest that when mirtazapine (5 mg/kg/day, s.c.) and paroxetine (10 mg/kg/day, s.c.) are co-administered, the increased firing activity of locus coeruleus NA neurons induced by mirtazapine alone is prevented by concomitant paroxetine administration. Thus, the decremental effect of paroxetine on the firing activity of locus coeruleus NA neurons might dampen the incremental one of mirtazapine which acts as antagonist at somatodentritic α_2-adrenergic autoreceptors on these neurons. This would prevent the enhancement of NA release in the dorsal raphe nucleus and the consequent activation of the α_1-adrenoceptors located on 5-HT which would prevent the mirtazapine–paroxetine combination from enhancing 5-HT neuronal firing activity (Fig. 2).

The results obtained in the dorsal hippocampus indicated that the inhibition of 5-HT transporter by paroxetine was not modified either by short- or long-term mirtazapine administration. The assertion that the inhibitory effect of 5-HT on the firing activity of CA_3 pyramidal neurons is mediated by the 5-HT_{1A} receptor subtype is supported by the observation that the selective 5-HT_{1A} receptor antagonist WAY 100635 produced a marked reduction of the effect of microiontophoretically-applied (Figs. 4 and 5). Should there be a greater tonic activation of these postsynaptic 5-HT_{1A} receptors, one would expect their blockade by WAY 100635 to result in a greater enhancement of the baseline firing activity of CA_3 pyramidal neurons. WAY 100635 (25–100 μg/kg, i.v.) did not produce such disinhibition in control rats (Figs. 4–6). As previously reported (Haddjeri et al., 1998), this result suggests a lack of a tonic activation of postsynaptic 5-HT_{1A} receptors in the anesthetized untreated rats. Interestingly, it was recently reported that WAY 100635 disinhibit CA_1 pyramidal neurons firing activity, but not in 5-HT depleted, freely moving rats (Suzuki et al., 1999). In the present study, in rats receiving paroxetine alone for 2 days, WAY 100635 did not induce any significant disinhibition of CA_3 pyramidal neurons. However, it did induce a significant disinhibition of the firing activity of these neurons in rats treated with mirtazapine or paroxetine for 21 days. This is in keeping with recent studies from our laboratory showing that WAY 100635 produces a disinhibition of CA_3 pyramidal neurons following long-term administration of various antidepressant treatments (Haddjeri et al., 1998; Rueter et al., 1998). The enhanced tonic activation of postsynaptic 5-HT_{1A} receptors observed after the long-term treatment with paroxetine can be accounted for by higher levels of extracellular 5-HT in the hippocampus, as a result of the recovery of the firing rate of 5-HT neurons associated with the blockade of 5-HT reuptake. This assumption is consistent with previous studies showing increased extracellular concentrations of 5-HT in terminal brain areas (frontal cortex, hypothalamus) after long-term SSRI treatments (Bel and Artigas, 1993; Rutter et al., 1994). The enhanced tonic activation of postsynaptic 5-HT_{1A} receptors observed after the long-term treatment with mirtazapine can also be explained by increased levels of 5-HT in the hippocampus but, in contrast to that produced by paroxetine, it would result from the increase of 5-HT firing activity and the desensitization of α_2-adrenergic heteroreceptors located on terminal 5-HT neurons (Haddjeri et al., 1997).

A striking finding was the marked enhanced tonic

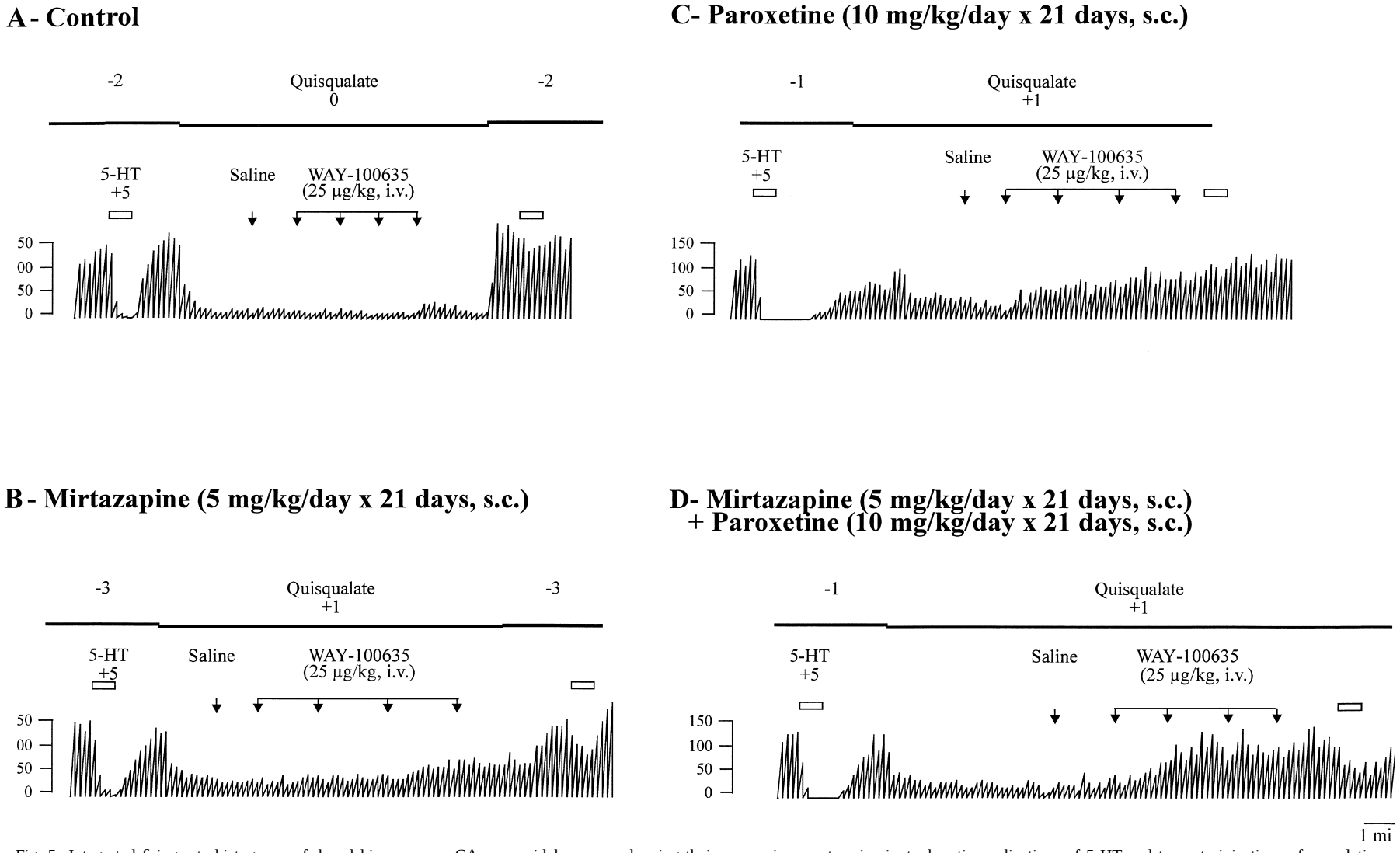

Fig. 5. Integrated firing rate histograms of dorsal hippocampus CA_3 pyramidal neurons showing their responsiveness to microiontophoretic applications of 5-HT and to acute injections of cumulative doses of WAY 100635 (25–100 µg/kg, i.v.) in control rats and in rats treated with MIR, PRX and their combination during 21 days. The neurons were activated by microiontophoretic applications of quisqualate (+1 to −3 nA). White horizontal bars indicate the duration of 5-HT applications. Arrows indicate the moment of the injection of WAY 100635 (25 µg/kg, i.v.). The time base applies to all traces.

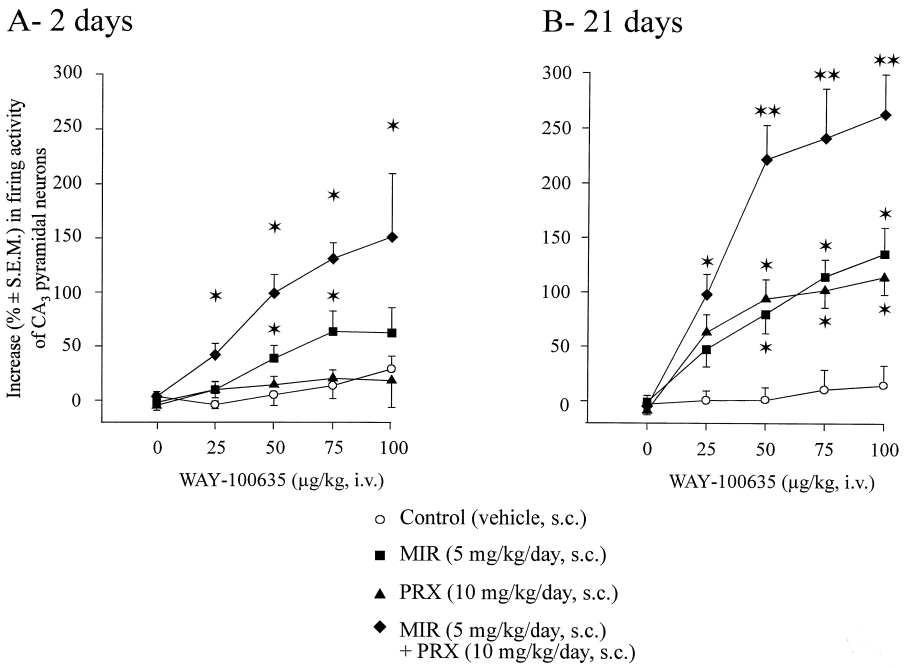

Fig. 6. Dose–response curves of the effects of intravenous injections of WAY-100635 in control rats and in rats treated with MIR, PRX and their combination for 2 days (A) or 21 days (B). *$P<0.05$ between the treated groups and the control group. **$P<0.05$ between the group treated with the MIR–PRX combination and the group treated with PRX or treated with MIR alone. Number of neurons tested: 9–12 per group.

activation of postsynaptic 5-HT_{1A} receptors observed after the short-term treatment with the mirtazapine–paroxetine combination while the firing activity of dorsal raphe 5-HT neurons was still significantly reduced (Fig. 1). In addition, the 2-day mirtazapine treatment, which did not modify 5-HT neuronal firing, also increased the disinhibitory action of WAY 100635, albeit to a lesser extent than in the combined treatment group. Taken together, these results suggest an enhanced 5-HT release in the dorsal hippocampus which is independent of the 5-HT neuronal firing rate in the dorsal raphe nucleus (Haddjeri et al., 1998; Rueter et al., 1998). Another interesting finding was the greater tonic activation of postsynaptic 5-HT_{1A} receptors observed following the long-term treatment with mirtazapine–paroxetine compared to each drug given alone (Fig. 6). A higher degree of 5-HT neuronal firing activity cannot account for this observation as the mean firing rate of dorsal raphe 5-HT neurons in mirtazapine–paroxetine treated rats was not increased (Figs. 1 and 2). Altogether, these results suggest that the co-administration of mirtazapine with paroxetine enhances the tonic activation of postsynaptic 5-HT_{1A} receptors due to the differential effects of the two drugs on 5-HT neurotransmission in the CA_3 hippocampus: the blockade of terminal 5-HT transporters and desensitization of 5-HT_{1B} autoreceptors by paroxetine (Chaput et al., 1986, 1991; Piñeyro et al., 1994) and with the increased 5-HT release induced by mirtazapine through the blockade of the terminal α_2-adrenergic heteroreceptors located on 5-HT terminals (Haddjeri et al., 1997).

In conclusion, the present study provides evidence for an earlier increase in 5-HT neurotransmission with mirtazapine than with a SSRI. At least one SSRI-controlled trial supports this possibility of a more rapid onset of action of mirtazapine (Thompson, 1999). The combination of mirtazapine and paroxetine induced a more rapid and greater enhancement of 5-HT neurotransmission than either drug given alone. In the light of previous evidence of the key role of postsynaptic 5-HT_{1A} receptors site in the therapeutic effect of antidepressant drugs, the co-administration of mirtazapine and paroxetine to patients presenting major depression might accelerate the antidepressant response and/or might prove more effective than either drug alone. A double-blind controlled trial is presently underway at our center to assess these possibilities.

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