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Potentiation of the antinociceptive effect of clomipramine by a 5-ht$_{1A}$ antagonist in neuropathic pain in rats

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1 The benefit of antidepressant treatment in human neuropathic pain is now well documented, but the effect is limited and slow to appear. It has been demonstrated that the association of a 5-HT$_{1A}$ antagonist and a serotoninergic antidepressant reduced the delay of action and increases the thymoanaleptic effect of the drug.

2 The purpose of this work was to evaluate the combination of an antidepressant and a 5-HT$_{1A}$ antagonist in animal models of chronic neuropathic pain. We studied the antinociceptive effect of the co-administration of clomipramine and a 5-HT$_{1A}$ antagonist (WAY 100,635) in a pain test applied in normal rats and in two models of neurogenic sustained pain (mononeuropathic and diabetic rats).

3 The results show an increase in the antinociceptive effect of acutely injected clomipramine due to WAY 100,635 in these models, which is majored when the two drugs are repeatedly injected. The 5-HT$_{1A}$ antagonist reduced the delay of onset and increased the maximal antinociceptive effect of clomipramine.

4 These new findings argue for using the combination of an antidepressant and a 5-HT$_{1A}$ antagonist in human neuropathic pain therapy.
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Keywords: Antidepressant; clomipramine; neuropathic pain; 5-HT$_{1A}$ antagonist; antinociception; rat

Abbreviations: ANOVA, analysis of variance; AUC, area under the curve; CMI, clomipramine; 5-HT, serotonin; MPE, maximum per cent of effect

Introduction

Together with anticonvulsive drugs, antidepressants are the most frequent treatment for neurogenic pain. Several reviews (Eschalier, 1990; Magni, 1991) and two meta-analyses (Onghena & Van Houdenhove, 1992; McQuay *et al*., 1996) confirm their therapeutic usefulness but underline their shortcomings.

New findings concerning the effect of antidepressants on monoamines offer new interesting perspectives in pain relief. *In vivo*, microdialysis studies showed that acute administration of a serotonergic antidepressant induced only a slight increase in synaptic 5-HT level in the frontal cortex, which might seem surprising given their reuptake blockade property (Adell & Artigas, 1991; Bel & Artigas, 1992; Invernizzi *et al*., 1992). This effect is due to the activation of 5-HT$_{1A}$ autoreceptors, present in high densities on the cell bodies and dendrites of serotonergic neurones in the raphe nuclei (Pazos & Palacios, 1985; Verge *et al*., 1986). These receptors inhibit neuronal firing (Aghajanian, 1978) and thus axonal serotonin release (Adell & Artigas, 1991; Invernizzi *et al*., 1992). However, after chronic administration, the same antidepressant drugs induced a down-regulation of these receptors (Chaput *et al*., 1986; Blier *et al*., 1987; Le Poul *et al*., 1995; Artigas *et al*., 1996) and increased 5-HT synaptic level (Bel & Artigas, 1992). A possible implication of the 5-HT$_{1A}$ receptors in the delay of the psychotropic action of antidepressants was suggested and so a blockade of 5-HT$_{1A}$ receptors was proposed to shorten this delay (Artigas *et al*., 1994; Blier & Bergeron, 1995). This hypothesis was confirmed by experimental studies, which showed that blockade of 5-HT$_{1A}$ autoreceptors concomitant with administration of antidepressants induced a rapid rise in 5-HT in the terminal fields (Artigas *et al*., 1996; Invernizzy *et al*., 1992; Hjorth, 1993). Clinical studies performed in depressed patients have confirmed that coadministration of pindolol (a 5HT$_{1A}$ antagonist) and antidepressants reduces the delay of action of thymoanaleptic drugs and increases their efficacy (Artigas *et al*., 1994; Blier & Bergeron, 1995; Artigas *et al*., 1996; Perez *et al*., 1997; Tome *et al*., 1997).

Concerning pain relief by antidepressant drugs, it is known that 5-HT is involved in the central inhibitory control of pain. Several studies have reported that 5-HT is antinociceptive and we have shown that at the spinal level this effect depends on the nature of the stimulus (Bardin *et al*., 1997). It has been found that 5-HT antagonists inhibit the antinociceptive effect of some antidepressants such as clomipramine (CMI) (Eschalier *et al*., 1981), amitriptyline or imipramine (De Felipe *et al*., 1986). Some clinical studies point to an analgesic effect of selective reuptake inhibitors of 5-HT such as paroxetine (Sindrup *et al*., 1990).

The purpose of this study was to evaluate whether a 5-HT$_{1A}$ serotonergic receptor antagonist could increase the antinociceptive potency of the serotonergic antidepressant

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CMI. WAY 100,635 was chosen for its high affinity and specificity for the 5-HT_{1A} serotonergic receptors and its weak or nil affinity for the others receptors (Gozlan *et al*., 1995). The study was performed in normal rats and in chronic pain models of mononeuropathic pain including the model described by Bennett & Xie (1988) (mononeuropathy), in which CMI showed a high efficacy (Ardid & Guilbaud, 1992), and diabetic rats, in which the antidepressant was less active (Courteix *et al*., 1994). In order to mimic the clinical situation, the study was performed after acute but also repeated injections of CMI (i.e., performed every half-life time previously determined in rats). In these conditions Ardid & Guilbaud (1992) demonstrated a higher antinociceptive effect of CMI than after an acute injection.

Methods

Normal animals

Male Sprague-Dawley rats (CD1 Charles River, France), weighing 250–300 g, were used. They were housed in standard laboratory conditions with free access to food and water 1 week before experiments.

Induction of mononeuropathy

Male Sprague-Dawley rats (CD1 Charles River, France), initially weighing 200–250 g were used. They were housed four per cage under standard laboratory conditions and were given food and water *ad libitum*. Preliminary thresholds to paw pressure (the mean of two consecutive stable values which do not differ more than 10%) were determined before surgery. Ligatures were applied around the common sciatic nerve of the right hind paw, according to the method detailed by Bennett & Xie (1988) and Attal *et al*. (1990). Briefly, rats were anaesthetized with sodium pentobarbital (50 mg kg^{-1}, i.p.) and four chromic gut (5-0) ligatures were tied loosely (with about 1 mm spacing) around the common sciatic nerve. The nerve was constricted to a barely discernible degree, so that circulation through the epineurial vasculature was not interrupted.

Induction of diabetes

Male Sprague-Dawley rats (CD1 Charles River, France), initially weighing 200–250 g were used. They were housed four per cage under standard laboratory conditions and were given food and water *ad libitum*. Preliminary thresholds to paw pressure (the mean of two consecutive stable values which do not differ more than 10%) were determined before diabetes induction. Animals were intraperitoneally injected with streptozocin (75 mg kg^{-1}) (Zanosar®, Upjohn, France) dissolved in distilled water, according to the method described by Courteix *et al*. (1993). Diabetes was confirmed 1 week later by measurement of tail vein blood glucose levels with Ames Dextrostix and a reflectance colorimeter (Ames Division, Miles Laboratories, France). Blood samples were obtained from the tail by pin prick and only animals with a final blood glucose level >14 mM were considered diabetic. This model has been shown sensitive to antidepressants, morphine, and several other pharmacological compounds, such as antagonists of cholecystokinine B receptors (Courteix *et al*., 1994; Coudore-civiale *et al*., 2000).

Nociceptive test procedures

The antinociceptive effect of the tested compounds was assessed using a mechanical noxious stimulus as previously described by Randall & Selitto (1957). Nociceptive thresholds, expressed in grams (g), were measured with a Ugo Basile analgesimeter (Bioseb) by applying an increasing pressure to the right hind paw of unrestrained rats until a squeak (vocalization threshold) and/or a struggle was obtained (a cut-off level of 750 g was applied).

The experiments were performed blind in a quiet room by a single experimenter using the method of equal blocks with randomization of treatments in order to avoid any uncontrollable environmental influence that could induce a modification in behavioural response. These experiments were monitored by a local ethical committee. Since a certain amount of suffering might result from these experiments, the guidelines of the Committee for Research and Ethical Issue of the I.A.S.P. (1983) were followed. Great care was taken, particularly with regard to housing conditions, to avoid or minimize discomfort to the animals.

Pharmacological experiments

Testing took place 3 weeks after diabetes induction and 2 weeks after sciatic nerve ligatures. Rats were randomly arranged in cages, each rat receiving either drug or saline in the same volume (0.1 ml 100 g^{-1} of body weight of i.v. and s.c. injections). Each experiment was performed with different rats.

Influence of WAY 100,635 on the effect of CMI, acutely injected
(Three series of experiments were performed)

In normal rats Eight groups of eight rats were used. Preliminary thresholds to paw pressure (the mean of two consecutive stable values which do not differ more than 10%) were determined (control predrug values). Ten minutes afterwards, two groups of rats received an i.v. injection of either saline or WAY 100,635 at one of the three doses of 0.5, 2 or 8 mg kg^{-1}. Then, 10 min after this injection they were i.v. injected with saline or CMI (6 mg kg^{-1}) ($n=8$ for each combined co-administration). Vocalisation threshold to paw pressure test was then measured every 15 min for 1 h.

In mononeuropathic rats Eight groups of eight rats were used. Preliminary thresholds to paw pressure (the mean of two consecutive stable values which do not differ more than 10%) were determined before and 14 days (control predrug values) after ligatures. Ten minutes after the control value determination, two groups of rats received an i.v. injection of either saline or WAY 100,635 at one of the three doses of 0.5, 2 or 8 mg kg^{-1}. Then, 10 min afterwards, they were i.v. injected with saline or CMI (6 mg kg^{-1}) ($n=8$ for each combined co-administration). Vocalization threshold to the paw pressure test was then measured every 15 min for 1 h.

In diabetic rats Eight groups of eight rats were used. Preliminary thresholds to paw pressure (the mean of two

consecutive stable values which do not differ more than 10%) were determined before and 3 weeks (control predrug values) after diabetes induction. Ten minutes after the control value determination, two groups of rats received an i.v. injection of either saline or WAY 100,635 at one of the three doses of 0.5, 2 or 8 mg kg^{-1} ($n=16$ for each doses). Then, 10 min after this injection they were i.v. injected with saline or CMI (6 mg kg^{-1}) ($n=8$ for each combined co-administration). Vocalization threshold to paw pressure test was then measured every 15 min for 1 h.

Influence of WAY 100,635 on the effect of CMI, repeatedly injected
(Three experiments were performed)

In normal rats Four groups of six rats were used. Preliminary thresholds to paw pressure (the mean of two consecutive stable values which do not differ more than 10%) were determined (control values). At $t=0$ (10 min after these measures) rats were treated with either saline or WAY 100,635 (6 mg kg^{-1}, s.c.) (loading dose). At $t=30$ min and every half life time of CMI, previously determined in rats (2.35 h), they received for the groups pretreated with saline, a co-administration of saline + saline or saline + CMI (1.5 mg kg^{-1}, s.c.) and for the groups pretreated with WAY 100,635, a co-administration of WAY 100,635 (2 mg kg^{-1}, s.c.) + saline or WAY 100,635 (2 mg kg^{-1}, s.c.) + CMI (1.5 mg kg^{-1}, s.c.). Vocalization thresholds were determined 30 min after each of the six co-administrations performed so as to reach the steady-state of CMI levels.

In mononeuropathic rats Four groups of six rats were used. Preliminary thresholds to paw pressure (the mean of two consecutive stable values which do not differ more than 10%) were determined 14 days after ligatures (control values) At $t=0$ (10 min after these measures) rats were treated with either saline or WAY 100,635 (6 mg kg^{-1}, s.c.) (loading dose). At $t=30$ min and every half life time of CMI, previously determined in rats (2.35 h), they received for the groups pretreated with saline, a co-administration of saline + saline or saline + CMI (1.5 mg kg^{-1}, s.c.) and for the groups pretreated with WAY 100,635, a co-administration of WAY 100,635 (2 mg kg^{-1}, s.c.) + saline or WAY 100,635 (2 mg kg^{-1}, s.c.) + CMI (1.5 mg kg^{-1}, s.c.). Vocalization thresholds were determined 30 min after each of the six co-administrations performed so as to reach the steady-state of CMI levels.

In diabetic rats Eight groups of six rats were used. Preliminary thresholds to paw pressure (the mean of two consecutive stable values which do not differ more than 10%) were determined 3 weeks after diabetes induction (control values). At $t=0$ (10 min after these measures) rats were treated with either saline or WAY 100,635 (6 mg kg^{-1}, s.c.) (loading dose). At $t=30$ min and every half life time of CMI, previously determined in rats (2.35 h), they received for the groups pretreated with saline, a co-administration of saline + saline or saline + CMI (1.5 mg kg^{-1}, s.c.) and for the groups pretreated with WAY 100,635, a co-administration of WAY 100,635 (2 mg kg^{-1}, s.c.) + saline or WAY 100,635 (2 mg kg^{-1}, s.c.) + CMI (1.5 mg kg^{-1}, s.c.). Vocalization thresholds were determined 30 min after each of the six co-administrations performed so as to reach the steady-state of CMI levels.

Expression of results and statistical analysis

Results were expressed as variation (g) between vocalization thresholds (each measure before and after drugs injection) and preliminary threshold to paw pressure (the mean of two consecutive stable pre-drug values). To measure a global effect of the drugs, areas under the antinociceptive effect-time curves (AUC) were calculated from 0 to 60 min by the trapezoidal rule. The maximum per cent of effect (MPE) was calculated, at the peak of the time course curves, as follows: $100\times$(maximum postdrug value $-$ predrug value)/(cut-off value $-$ predrug value). The cut-off value corresponds to the maximum pressure that the apparatus allows (750 g). Data were analysed by a two-way analysis of variance (ANOVA) to compare time-course scores and by a one-way analysis of variance to compare the effect of different treatments as estimated by the AUC. These analyses were followed, when the F-value was significant, by a Tukey test. The significance level was $P<0.05$.

Drugs

CMI (clomipramine HCl, research Biochemicals international, France) and WAY 100,635 (synthesized as a sample for pharmacological studies) were dissolved in physiological saline (NaCl 0.9%). Solutions were prepared immediately before testing.

Results

Predrug control values of vocalization thresholds for normal rats (262 ± 4 g) or for neuropathic models (189 ± 5 and 191 ± 4 g for diabetic and mononeuropathic rats, respectively) correspond to the mechanical hyperalgesia previously described (Attal *et al*., 1990; Courteix *et al*., 1994).

Influence of WAY 100,635 on the effect of CMI acutely injected

In normal rats (Figure 1A) There was a significant difference when AUCs were compared (ANOVA, $F2.25=3.442$: $P=0.003$). Co-administered with saline, WAY 100,635 induced, at the high dose of 8 mg kg^{-1}, i.v., a significant antinociceptive effect, characterized by an increase in the AUC of variations ($P=0.02$ vs saline + saline treated group, Tukey test). CMI (6 mg kg^{-1}, i.v.) co-administered with saline slightly modified the vocalization threshold to paw pressure; this effect was not statistically significant. Co-administered with WAY 100,635 at the highest dose of 8 mg kg^{-1}, s.c., the same dose of CMI induced an antinociceptive effect characterized by a significant increase in the vocalization thresholds ($P=0.027$ vs saline + saline treated group, Tukey test). The result obtained with these co-administrations of CMI + WAY 100,635 was not significantly different from the result obtained with the CMI + saline treated group ($P=0.722$, Tukey test).

In mononeuropathic rats (Figure 1B) There was a significant difference when AUCs were compared (ANOVA,

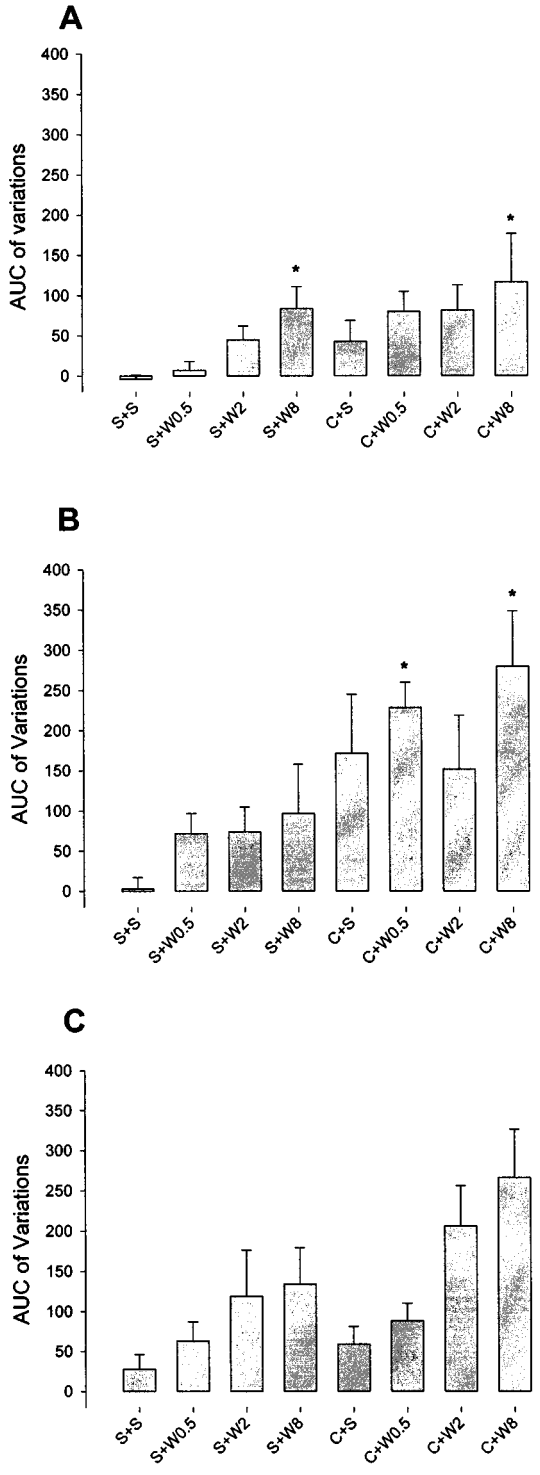

Figure 1 Effect of acute co-administration of WAY 100,635 and CMI on the vocalization to paw pressure in (A) normal rats; (B) mononeuropathic rats and (C) diabetic rats. Results are expressed as mean ± s.e.mean area under the antinociceptive effect-time curves (AUC) calculated by the trapezoidal rule. Eight groups of eight rats were used: S + S = saline + saline; S + WAY0.5 = saline + WAY 100,635 (0.5 mg kg^{-1}, i.v.); S + WAY2 = saline + WAY 100,635 (2 mg kg^{-1}, i.v.); S + WAY8 = saline + WAY 100,635 (8 mg kg^{-1}, i.v.); C + S = CMI (6 mg kg^{-1}, i.v.) + saline; C + WAY0.5 = CMI (6 mg kg^{-1}, i.v.) + WAY 100,635 (0.5 mg kg^{-1}, i.v.); C + WAY2 = CMI (6 mg kg^{-1}, i.v.) + WAY 100,635 (2 mg kg^{-1}, i.v.); C + WAY8 = CMI (6 mg kg^{-1}, i.v.) + WAY 100,635 (8 mg kg^{-1}, i.v.). *$P<0.05$ vs saline + saline treated group, Tukey test; °$P<0.05$ vs CMI (6 mg kg^{-1}, i.v.) + saline treated group, Tukey test.

F2.25 = 3.605: $P=0.004$). Co-administered with saline, WAY 100,635 did not modify the vocalization thresholds to paw pressure. CMI (6 mg kg^{-1}, i.v.) co-administered with saline increased these thresholds but this increase was not significant ($P=0.220$ vs saline + saline treated group, Tukey test). Co-administered with WAY 100,635 at the two doses of 0.5 and 8 mg kg^{-1}, s.c., the same dose of CMI induced an antinociceptive effect characterized by a significant increase in the vocalisation thresholds ($P=0.026$ and $P=0.005$ for the two doses of WAY 100,635 compared with the saline + saline treated group respectively, Tukey test). None of the results obtained with these co-administration of CMI + WAY 100,635 was significantly different from the results obtained with the CMI + saline treated group.

In diabetic rats (Figure 1C) There was a significant difference when AUCs were compared (ANOVA, *F2.25* = 3.747: $P=0.002$). Co-administered with saline, WAY 100,635 did not modify the vocalization thresholds to paw pressure. CMI (6 mg kg^{-1}, i.v.) co-administered with saline slightly modified the vocalization threshold to paw pressure; this effect was not statistically different from the saline + saline treated group. Co-administered with WAY 100,635 at the highest dose of 8 mg kg^{-1}, s.c., the same dose of CMI induced a significant antinociceptive effect ($P=0.005$ vs saline + saline treated group, Tukey test). The effect of this co-administration of CMI + WAY 100,635 (8 mg kg^{-1}) was significantly different from the result obtained with the CMI + saline group ($P=0.016$, Tukey test) but not saline + WAY 100,635 (8 mg kg^{-1}) treated group ($P=0.328$, Tukey test).

Influence of WAY 100,635 on the effect of CMI repeatedly injected

In normal rats (Figure 2) Six repeated injections of saline + saline and saline + WAY 100,635 (6 mg kg^{-1}, s.c.) did not modify the vocalization threshold to paw pressure (ANOVA, *F2.25* = 1.154 : $P=0.344$ and *F2.25* = 0.279 : $P=0.279$, respectively). Repeated injections of CMI (1.5 mg kg^{-1}, s.c.) + saline induced an increase in the vocalization threshold (ANOVA, *F2.25* = 5.837 : $P<0.001$). This increase became significant and peaked after the fifth co-injection (+65%, $P=0.009$, Tukey test). Repeated injections of CMI (1.5 mg kg^{-1}, s.c.) + WAY 100,635 (6 mg kg^{-1}, s.c.) induced an increase in the vocalization threshold (ANOVA, *F2.21* = 18.132 : $P<0.001$). This increase became significant after the second co-injection ($P=0.041$, Tukey test) and was greatest after the fourth one (+113%, $P<0.001$, Tukey test), before reaching a maximum.

The study of the AUC of variation showed a significant increase (ANOVA, *F2.95* = 22.896: $P<0.001$) in the CMI (1.5 mg kg^{-1}, s.c.) + saline and CMI (1.5 mg kg^{-1}, s.c.) + WAY 100,635 (6 mg kg^{-1}, s.c.) groups ($P=0.006$ and $P<0.0001$ vs saline + saline treated group, Tukey test). There was a statistical difference between these two groups ($P=0.003$, Tukey test) and between CMI (1.5 mg kg^{-1}, s.c.) + WAY 100,635 (6 mg kg^{-1}, s.c.) and saline + WAY 100,635 (6 mg kg^{-1}, s.c.) groups ($P<0.001$, Tukey test).

In mononeuropathic rats (Figure 3) Six repeated injections of saline + saline did not modify the vocalization threshold to

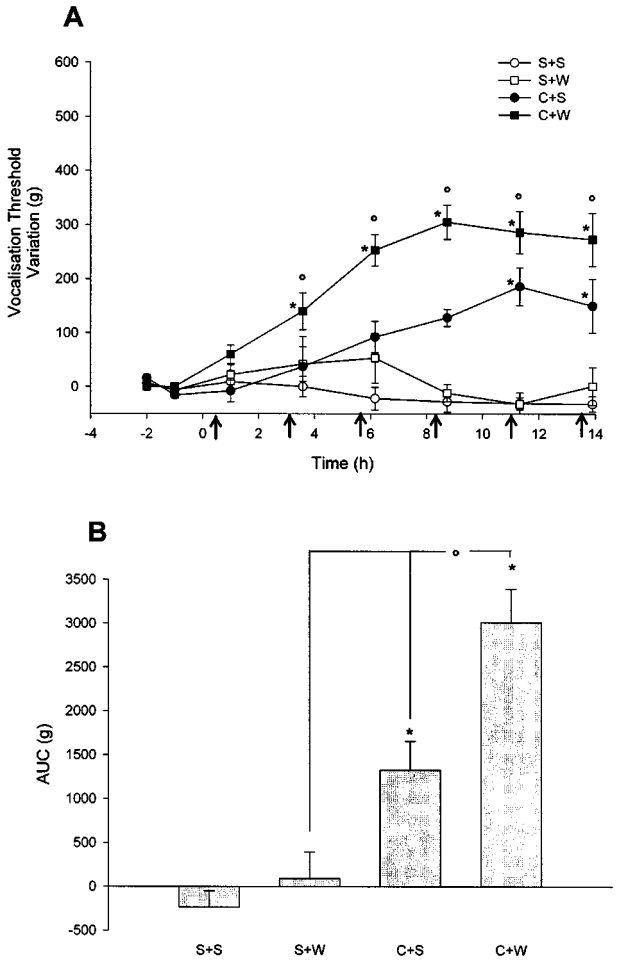

Figure 2 Effect of repeated co-administration of WAY 100,635 and CMI on the vocalization to paw pressure in normal rats. Results are expressed (A) by the time-course curve of the mean ± s.e.mean variation in grams of the vocalization threshold calculated by the difference between the mean of the two consecutive stable pre-drug values and all the thresholds obtained before and after drug treatment and (B) by the mean ± s.e.mean area under the antinociceptive effect-time curves (AUC) calculated by the trapezoidal rule. Four groups of six rats were used. They were treated with either saline or WAY 100,635 (6 mg kg^{-1}, s.c.) (loading dose). At 30 min after and every half life time of CMI (2.35 h), they received for the groups previously treated with saline, a co-administration of saline + saline (S + S) or saline + CMI (1.5 mg kg^{-1}, s.c.) (C + S) and for the groups previously treated with WAY 100,635 a co-administration of WAY 100,635 (2 mg kg^{-1}, s.c.) + saline (S + W) or WAY 100,635 (2 mg kg^{-1}, s.c.) + CMI (1.5 mg kg^{-1}, s.c.) (C + W). Vocalisation thresholds were determined 30 min after each of the six co-administrations (black arrows). (A) $^{*}P<0.05$ vs pre-drug value, Tukey test and $^{\circ}P<0.05$ vs Saline + CMI (1.5 mg kg^{-1}, s.c.) treated group, Tukey test. (B) $^{*}P<0.05$ vs S + S treated group, Tukey test and $^{\circ}P<0.05$ vs corresponding group, Tukey test.

paw pressure (ANOVA, $F2.25 = 1.431$: $P = 0.227$). Repeated injections of saline + WAY 100,635 (6 mg kg^{-1}, s.c.) and CMI (1.5 mg kg^{-1}, s.c.) + saline induced an increase in the vocalization threshold (ANOVA, $F2.25 = 4.885$: $P < 0.001$ and $F2.25 = 16.223$: $P < 0.001$, respectively). This increase became significant for saline + WAY 100,635 (6 mg kg^{-1}, s.c.) for the second and third co-injection (maximal effect: +90%, $P = 0.011$, Tukey test) and for CMI (1.5 mg kg^{-1}, s.c.) + saline after the third co-injection ($P < 0.001$, Tukey test; maximal after the fifth injection +121%, $P < 0.001$, Tukey test). Repeated injections of CMI (1.5 mg kg^{-1}, s.c.) + WAY 100,635 (6 mg kg^{-1}, s.c.) induced an increase in the vocalization threshold (ANOVA, $F2.25 = 25.396$: $P < 0.001$). This increase became significant after the second co-injection ($P = 0.001$, Tukey test) and was greatest after the fourth one (+267%, $P < 0.001$, Tukey test).

The determination of the AUCs of variation showed a significant increase in these AUCs (ANOVA, $F3.13 = 20.216$: $P < 0.001$) in the saline + WAY 100,635 (6 mg kg^{-1}, s.c.), CMI (1.5 mg kg^{-1}, s.c.) + saline and CMI (1.5 mg kg^{-1}, s.c.) + WAY 100,635 (6 mg kg^{-1}, s.c.) groups ($P = 0.039$, $P = 0.001$ and $P < 0.001$ vs saline + saline treated group respectively, Tukey test). There was a statistical difference between CMI (1.5 mg kg^{-1}, s.c.) + WAY 100,635 (6 mg kg^{-1}, s.c.) group vs the CMI (1.5 mg kg^{-1}, s.c.) + saline and saline + WAY 100,635 (6 mg kg^{-1}, s.c.) groups ($P < 0.001$ for the two comparisons, Tukey test).

In diabetic rats (Figure 4) Six repeated injections of saline + saline and saline + WAY 100,635 (6 mg kg^{-1}, s.c.) did not modify the vocalization threshold to paw pressure (ANOVA, $F2.20 = 0.799$: $P = 0.592$ and $F2.20 = 2.054$: $P = 0.064$). Repeated injections of CMI (1.5 mg kg^{-1}, s.c.) + saline induced an increase in the vocalization threshold (ANOVA, $F2.20 = 12.495$: $P < 0.001$). This increase became significant after the third co-injection (maximal effect after the fourth co-injection : +77%, $P < 0.001$, Tukey test). Repeated injections of CMI (1.5 mg kg^{-1}, s.c.) + WAY 100,635 (6 mg kg^{-1}, s.c.) induced an increase in the vocalization threshold (ANOVA, $F2.20 = 9.184$: $P < 0.001$). This increase became significant after the first co-injection ($P = 0.005$, Tukey test) and was greatest after the fourth one (+131%, $P < 0.001$, Tukey test).

The study of the AUCs of variation showed a significant increase in these AUCs (ANOVA, $F2.95 = 23.805$: $P < 0.001$) in the CMI (1.5 mg kg^{-1}, s.c.) + saline and CMI (1.5 mg kg^{-1}, s.c.) + WAY 100,635 (6 mg kg^{-1}, s.c.) groups ($P = 0.001$ and $P < 0.001$ vs saline + saline treated group, Tukey test). There was a statistical difference between the two groups ($P = 0.004$, Tukey test) and between CMI (1.5 mg kg^{-1}, s.c.) + WAY 100,635 (6 mg kg^{-1}, s.c.) and saline + WAY 100,635 (6 mg kg^{-1}, s.c.) groups ($P < 0.001$, Tukey test).

Discussion

This study shows for the first time to our knowledge that the combination of a 5-HT$_{1A}$ antagonist with CMI can increase the antinociceptive effect of the antidepressant, especially after repeated injections.

Whereas after acute administration the effect of CMI was not statistically significant in any of the pain model used, the combination of CMI + WAY 100,635 always induced a significant increase in vocalization thresholds, mainly when the high dose of the 5-HT$_{1A}$ receptor antagonist (8 mg kg^{-1}, i.v.) was used. After acute injection, CMI (6 mg kg^{-1}, i.v.) slightly raised the vocalization threshold and its effect was not significant. This is not surprising because in normal animals (without sustained pain) the doses that induce antinociception are higher (e.g. 40 mg kg^{-1}, i.p. for Gail-

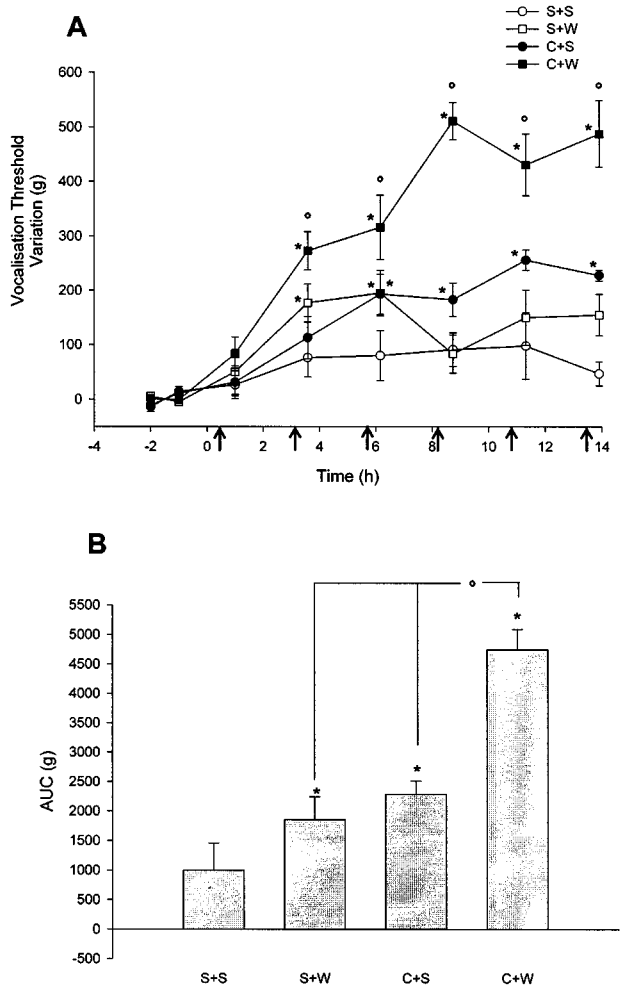

Figure 3 Effect of repeated co-administration of WAY 100,635 and CMI on the vocalization to paw pressure in mononeuropathic rats. Results are expressed (A) by the time-course curve of the mean ± s.e.mean variation in grams of the vocalization threshold calculated by the difference between the mean of the two consecutive stable pre-drug values and all the thresholds obtained before and after drug treatment and (B) by the mean ± s.e.mean area under the antinociceptive effect-time curves (AUC) calculated by the trapezoidal rule. Four groups of six rats were used. They were treated with either saline or WAY 100,635 (6 mg kg^{-1}, s.c.) (loading dose). At 30 min after and every half life time of CMI (02.35 h), they received for the groups previously treated with saline, a co-administration of saline + saline (S + S) or saline + CMI (1.5 mg kg^{-1}, s.c.) (C + S) and for the groups previously treated with WAY 100,635 a co-administration of WAY 100,635 (2 mg kg^{-1}, s.c.) + saline (S + W) or WAY 100,635 (2 mg kg^{-1}, s.c.) + CMI (1.5 mg kg^{-1}, s.c.) (C + W). Vocalization thresholds were determined 30 min after each of the six co-administrations (black arrows). (A) $^{*}P<0.05$ vs pre-drug value, Tukey test and $^{\circ}P<0.05$ vs saline + CMI (1.5 mg kg^{-1}, s.c.) treated group, Tukey test. (B) $^{*}P<0.05$ vs S + S treated group, Tukey test and $^{\circ}P<0.05$ vs corresponding group, Tukey test.

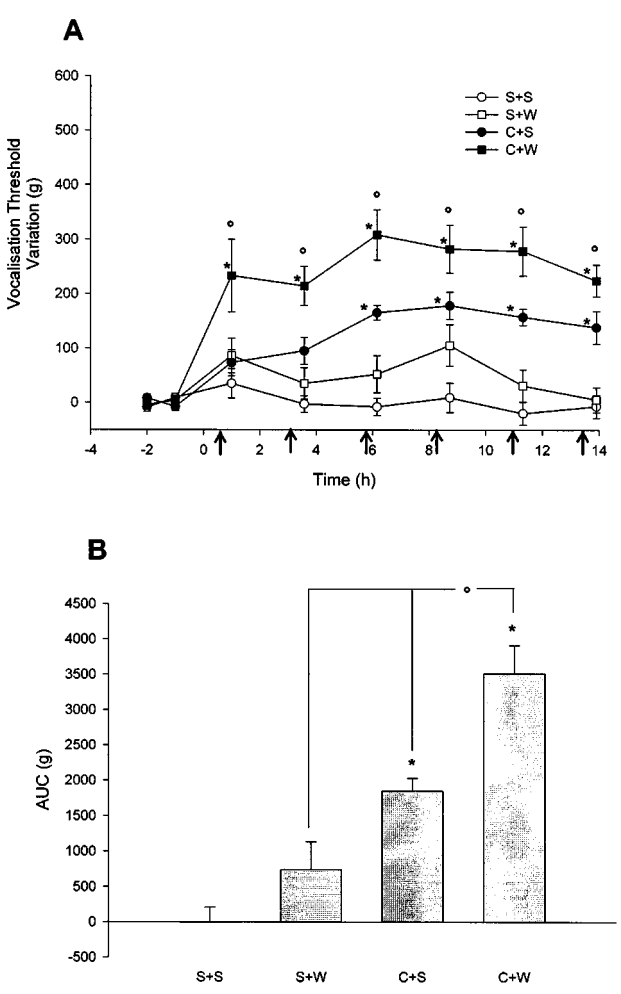

Figure 4 Effect of repeated co-administration of WAY 100,635 and CMI on the vocalization to paw pressure in diabetic rats. Results are expressed (A) by the time-course curve of the mean ± s.e.mean variation in grams of the vocalization threshold calculated by the difference between the mean of the two consecutive stable pre-drug values and all the thresholds obtained before and after drug treatment and (B) by the mean ± s.e.mean area under the antinociceptive effect-time curves (AUC) calculated by the trapezoidal rule. Four groups of six rats were used. They were treated with either saline or WAY 100,635 (6 mg kg^{-1}, s.c.) (loading dose). At 30 min after and every half life time of CMI (2.35 h), they received for the groups previously treated with saline, a co-administration of saline + saline (S + S) or saline + CMI (1.5 mg kg^{-1}, s.c.) (C + S) and for the groups previously treated with WAY 100,635 a co-administration of WAY 100,635 (2 mg kg^{-1}, s.c.) + saline (S + W) or WAY 100,635 (2 mg kg^{-1}, s.c.) + CMI (1.5 mg kg^{-1}, s.c.) (C + W). Vocalization thresholds were determined 30 min after each of the six co-administrations (black arrows). (A) $^{*}P<0.05$ vs pre-drug value, Tukey test and $^{\circ}P<0.05$ vs saline + CMI (1.5 mg kg^{-1}, s.c.) treated group, Tukey test. (B) $^{*}P<0.05$ vs S + S treated group, Tukey test and $^{\circ}P<0.05$ vs corresponding group, Tukey test.

lard-Plaza *et al*., 1982; 30 and 40 mg kg^{-1}, i.p. for Reichenberg *et al*., 1985). In the context of sustained pain, Ardid & Guilbaud (1992) found that a dose of 2 mg kg^{-1}, i.v. was only slightly effective and less so than a lower dose of 0.5 mg kg^{-1} in mononeuropathic rats, i.v. and Courteix *et al.* (1994) demonstrated that a high dose of 8 mg kg^{-1} was ineffective in diabetic rats. The 5-HT_{1A} antagonist, WAY 100,635 induced an increase in the vocalization threshold at the highest dose (8 mg kg^{-1}, s.c.) that was significantly different from controls in normal rats. This is surprising because some studies have shown that 5-HT_{1A} agonists induced antinociception. For instance, Robles *et al.* (1996) demonstrated the antinociceptive effect of several 5-HT_{1A} agonists in the hot plate test in mice; Galeotti *et al.* (1997) demonstrated the antinociceptive effect of buspirone, gepirone and 8-OHDPAT in the hot plate and writhing tests in

mice. However, results are conflicting. Murphy & Zemlan (1990) demonstrated that 5-HT_{1A} agonists increased sensitivity to noxious stimulation (effect blocked by a 5-HT_{1A} antagonist). Millan (1994) showed that alprenolol and WAY 100135 induced antinociception in the writhing test in mice, and Millan *et al*. (1996) demonstrated that partial and specific 5-HT_{1A} antagonists alprenolol, tertatolol, WAY 100135 and S 15931 induced antinociception in the formalin and writhing tests. In our study, the antinociceptive effect of WAY 100,635 was only found after acute injection in the normal rats, which limits our conclusion concerning a potent antinociceptive effect of this drug. The combined acute administration of these two drugs induced an increase in the vocalization thresholds in all the pain contexts. The benefit was, however, limited because the vocalization threshold obtained was most often not different from either the CMI + saline or the saline + WAY 100,635 group.

Interestingly, the influence of WAY 100,635 was markedly higher when it was repeatedly administered simultaneously with CMI. In these conditions, the effect of the antidepressant alone was significant, reaching a maximum when the steady-state of the plasma level was obtained, as previously observed in mice (Eschalier *et al*., 1988) and in mononeuropathic rats (Ardid & Guilbaud, 1992). The addition of the 5-HT_{1A} receptor antagonist induced a significantly prompter and greater antinociceptive effect than after administrations of CMI alone. The antinociceptive effect became significant after the first or second injection, whereas the antidepressant alone induced a significant effect only after the third or fifth injection. The delay of onset was thus shortened by 2.35 h. Moreover, the intensity of the effect was markedly increased as shown either by both time-course effects and AUCs. Thus CMI in mononeuropathic and diabetic rats, repeatedly injected alone, induced a 121 and 77% increase in the vocalization threshold, which reached 267 and 131% when administered with WAY 100,635. Comparatively, morphine induced a 93 and a 88% increase of vocalization thresholds for 1 mg kg^{-1} in mononeuropathic rats (Attal *et al*., 1991) and for 4 mg kg^{-1} in diabetic rats (Courteix *et al*., 1994), respectively.

It is now well documented that the effect of antidepressants on 5-HT release is decreased at the beginning of treatment by a presynaptic involvement of 5-HT_{1A} autoreceptor, which explains the delayed action. Thus Esteban *et al*. (1999) report that chronic fluoxetine and zimelidine (two SSRIs) acutely administered activated inhibitory 5-HT_{1A} autoreceptor in the rat brain resulting in a decrease in 5-HT synthesis, whereas chronic treatment with these drugs was followed by a desensitization of these presynaptic receptors. Dawson *et al*. (1999) found that a combination treatment with WAY 100,635 and venlafaxine produced a dose-dependent increase in extracellular 5-HT concentrations, an effect not found with the antidepressant alone. Likewise WAY 100,635 potentiates the increase in levels of 5-HT in rat brain elicited by fluoxetine (Gobert & Millan, 1999).

The potentiation of thymoanaleptic activity of antidepressants by 5-HT_{1A} antagonist was demonstrated in clinical trials (e.g. Artigas *et al*., 1994; Blier & Bergeron, 1995) but results are conflicting in animal models. Some studies show that antidepressant activity can be potentiated by 5-HT_{1A} antagonist. Mitchell & Redfern (1997) showed that the reduction of aggressive behaviour by venlafaxine could be potentiated by WAY 100,635; Trillat *et al*. (1998) found a clear potentiation of fluoxetine-induced decrease of food intake in food-deprived rats by WAY 100,635; Millan *et al*. (1998) showed that duloxetine (a mixed antidepressant) was inactive alone and dose-dependently reduced immobility in the forced swimming test in the presence of WAY 100,635. Some other studies failed to find this potentiation. Thus Cryan *et al*. (1999), using the olfactory bulbectomy-induced behavioural syndrome, showed no potentially faster onset of antidepressant action by 5-HT_{1A} receptor antagonist. These discrepancies can be explained by the site of the 5-HT_{1A} autoreceptor action, Hervas & Artigas (1998) having demonstrated that WAY 100,635 potentiates the effect of fluoxetine more in the frontal cortex than in the dorsal hippocampus or by the hypothesis of Davidson & Stamford (1998), which postulates that there are two populations of 5-HT_{1A} receptors one of which controls the 5-HT release and the other the 5-HT firing.

Concerning pain, we can hypothesize that the site of 5-HT_{1A} antagonist potentiation might be the dorsal raphe nucleus, well known for its implication in pain control (see Willis & Westlund, 1997). This region contained a high density of 5-HT_{1A} autoreceptors (Pazos & Palacios, 1985). Romero & Artigas (1997) demonstrated the potentiation of the elevation of extracellular concentration of 5-HT induced by fluoxetine at the level of the dorsal raphe neurones projecting to the frontal cortex using WAY 100,635. A dorsal raphe level, several inhibitory bulbospinal 5-HT neurones projected to the spinal cord, using the dorsolateral funiculus, and Ardid *et al*. (1995) have demonstrated that the effect of intravenously administered CMI is significantly decreased after lesions of this funiculus.

In conclusion, this study clearly demonstrates the beneficial effect of antidepressant and 5-HT_{1A} combination in the treatment of neurogenic sustained pain in rats. This combination reduces the delay of the antinociceptive effect of the antidepressant and increases its potency. These new findings argue for reducing the delay of action of antidepressant therapy by co-administering a 5-HT_{1A} antagonist in human chronic pain.

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References

ADELL, A. & ARTIGAS, G. (1991). Differential effects of clomipramine given locally or systemically on extracellular 5-hydroxytryptamine in raphe nuclei and frontal cortex. An in vivo brain microdialysis study. *Naunyn Schmiedebergs Arch. Pharmacol.*, **343,** 237–244.

AGHAJANIAN, G.K. (1978). Feedback regulation of central monoaminergic neurons: evidence for single cell recording studies. In *Essays in neurochemistry and neuropharmacology*. ed. Youdin, M.B.H., Lovenberg, W., Sharman, D.F. & Lagnado, J.R. pp. 1–33. New York: John Wiley & Sons.

ARDID, D. & GUILBAUD, G. (1992). Antinociceptive effects of acute and "chronic" injections of tricyclic antidepressant drugs in a new model of mononeuropathy in rats. *Pain,* **49,** 253 – 256.

ARDID, D., JOURDAN, D., MESTRE, C., VILLANUEVA, L., LE BARS, D. & ESCHALIER, A. (1995). Involvement of bulbospinal pathways in the antinociceptive effect of clomipramine in the rat. *Brain Res.,* **695,** 253 – 256.

ARTIGAS, F., PEREZ, V. & ALVAREZ, E. (1994). Pindolol induces a rapid improvement of depressed patients treated with serotonin reuptake inhibitors. *Arch. Gen. Psychiatry,* **51,** 248 – 251.

ARTIGAS, F., ROMERO, L., DE MONTIGNY, C. & BLIER, P. (1996). Acceleration of the effect of selected antidepressant drugs in major depression by 5-HT_{1A} antagonists. *Trends Neurosci.,* **19,** 378 – 383.

ATTAL, N., CHEN, Y.L., KAYZER, V. & GUILBAUD, G. (1991). Behavioural evidence that systemic morphine may modulate a phasic pain-related behaviour in a rat model of peripheral mononeuropathy. *Pain,* **47,** 65 – 70.

ATTAL, N., JAZAT, F., KAYZER, V. & GUILBAUD, G. (1990). Further evidence for 'pain-related' behaviours in a model of unilateral peripheral mononeuropathy. *Pain,* **41,** 235 – 251.

BARDIN, L., BARDIN, M., LAVARENNE, J. & ESCHALIER, A. (1997). Effect of intrathecal serotonin on nociception in rats: influence of the pain test used. *Exp. Brain Res.,* **113,** 81 – 87.

BEL, N. & ARTIGAS, F. (1992). Fluvoxamine preferentially increases extracellular 5-hydroxytryptamine in the raphe nuclei: an in vivo microdialysis study. *Eur. J. Pharmacol.,* **229,** 101 – 103.

BENNETT, G.J. & XIE, Y.K.A. (1988). peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. *Pain,* **33,** 87 – 107.

BLIER, P., DE MONTIGNY, C. & CHAPUT, Y. (1987). Modifications of the serotonin system by antidepressant treatments: implication for the therapeutic response in major depression. *J. Clin. Psychopharmacol.,* **7**(Suppl. 6): 24S – 35S.

BLIER, P. & BERGERON, R. (1995). Effectiveness of pindolol with selected antidepressant drugs in the treatment of major depression. *J. Clin. Psychopharmacol.,* **15,** 217 – 222.

CHAPUT, Y., DE MONTIGNY, C. & BLIER, P. (1986). Presynaptic and postsynaptic modifications of the serotonin system by long-term administration of antidepressant treatments. An in vivo electrophysiologic study in the rat. *Neuropsychopharmacology,* **5,** 219 – 229.

Committee for Research and Ethical Issues of the I.A.S.P. (1983). Ethical standarts for investigations of experimental pain in animals. *Pain,* **16,** 109 – 110.

COUDORE-CIVIALE, M., COURTEIX, C., BOUCHER, M., FIALIP, J. & ESCHALIER, A. (2000). Evidence for an involvement of tachykinins in allodynia in streptozocin-induced diabetic rats. *Eur. J. Pharmacol.,* **401,** 47 – 53.

COURTEIX, C., BARDIN, M., CHANTELAUZE, C., LAVARENNE, J. & ESCHALIER, A. (1994). A study of the sensitivity of the diabetes-induced pain model in rats to a range of analgesics. *Pain,* **57,** 153 – 160.

COURTEIX, C., ESCHALIER, A. & LAVARENNE, J. (1993). Streptozocin-induced diabetic rats: behavioural evidence for a model of chronic pain. *Pain,* **53,** 81 – 88.

CRYAN, J.F., MCGRATH, C., LEONARD, B.E. & NORMAN, T.R. (1999). Onset of the effects of the 5-HT1A antagonist, WAY-100,635, alone, and in combination with paroxetine, on olfactory bulbectomy and 8-OH-DPAT-induced changes in the rat. *Pharmacol. Biochem. Behav.,* **63,** 333 – 338.

DAVIDSON, C. & STAMFORD, J.A. (1998). Contrasting effects of chronic paroxetine on 5-HT1A control of dorsal raphe cell firing and 5-HT release. *Neuroreport,* **9,** 2535 – 2538.

DAWSON, L.A., NGUYEN, H.Q. & GEIGER, A. (1999). Effects of venlafaxine on extracellular concentrations of 5-HT and noradrenaline in the rat frontal cortex: augmentation via 5-HT1A receptor antagonism. *Neuropharmacology,* **38,** 1153 – 1163.

DE FELIPE, M.D.C., DE CEBALLOS, M.L. & FUENTES, J.A. (1986). Hypoalgesia induced by antidepressants in mice : a case for opioids and serotonin. *Eur. J. Pharmacol.,* **125,** 193 – 199.

ESCHALIER, A. (1990). Antidepressants and pain management. In *Serotonin and Pain. Proceedings of the International Symposium on Serotonin and Pain.* ed. Besson, J.M., pp. 305 – 325. La Roque-Gageac: Excerpta Medica, International Congress Series 879.

ESCHALIER, A., FIALIP, J., VAROQUAUX, O., MAKAMBILA, M.C., MARTY, H. & BASTIDE, P. (1988). Pharmacokinetic patterns of repeated administrations of antidepressants in animals. I. Implications for the antinociceptive action of clomipramine in mice. *J. Pharmacol. Exp. Ther.,* **245,** 963 – 968.

ESCHALIER, A., MONTASTRUC, J.L., DEVOIZE, J.L., RIGAL, F., GAILLARD-PLAZA, G. & PECHADRE, J.C. (1981). Influence of naloxone and methysergide on the analgesic effect of clomipramine in rats. *Eur. J. Pharmacol.,* **74,** 1 – 7.

ESTEBAN, S., LLADO, J., SASTRE-COLL, A. & GARCIA-SEVILLA, J.A. (1999). Activation and desensitization by cyclic antidepressant drugs of alpha2-autoreceptors, alpha2-heteroreceptors and 5-HT1A-autoreceptors regulating monamine synthesis in the rat brain in vivo. *Naunyn Schmiedebergs Arch. Pharmacol.,* **360,** 135 – 143.

GAILLARD PLAZA, G., TRAN, M.A., CHARLET, J.P., COTONAT, J. & MONTASTRUC, J.L. (1982). Plasma levels and analgesic effects of clomipramine during acute treatment in rats. *J. Pharmacol.,* **13,** 509 – 513.

GALEOTTI, N., GHELARDINI, C. & BARTOLINI, A. (1997). 5-HT1A agonists induce central cholinergic antinociception. *Pharmacol. Biochem. Behav.,* **57,** 835 – 841.

GOBERT, A. & MILLAN, M.J. (1999). Modulation of dialysate levels of dopamine, noradrenaline, and serotonin (5-HT) in the frontal cortex of freely-moving rats by (−)-pindolol alone and in association with 5-HT reuptake inhibitors: comparative roles of beta-adrenergic, 5-HT1A, and 5-HT1B receptors. *Neuropsychopharmacology,* **21,** 268 – 284.

GOZLAN, H., THIBAULT, S., LAPORTE, A.M., LIMA, L. & HAMON, M. (1995). The selective 5-HT_{1A} antagonist radioligand [3H]WAY 100,635 labels both G-protein-coupled and free 5-HT_{1A} receptors in rat brain membranes. *Eur. J. Pharmacol.,* **288,** 173 – 186.

HERVAS, I. & ARTIGAS, F. (1998). Effect of fluoxetine on extracellular 5-hydroxytryptamine in rat brain. Role of 5-HT autoreceptors. *Eur. J. Pharmacol.,* **358,** 9 – 18.

HJORTH, S. (1993). Serotonin 5-HT_{1A} autoreceptor blockade potentiates the ability of the 5-HT reuptake inhibitor citalopram to increase nerve terminal output of 5-HT in vivo: a microdialysis study. *J. Neurochem.,* **60,** 776 – 779.

INVERNIZZY, R., BELLI, S. & SAMANIN, R. (1992). Citolopram's ability to increase the extracellular concentrations of serotonin in the dorsal raphe prevents drug's effect in the frontal cortex. *Brain Res.,* **584,** 322 – 324.

LE POUL, E., LAARIS, N., DOUCET, E., LAPORTE, A.M., HAMON, M. & LANFUMEY, L. (1995). Early desensitization of somato-dendritic 5-HT_{1A} auto receptors in rats treated with fluoxetine or paroxetine. *Naunyn Schmiedebergs Arch. Pharmacol.,* **352,** 141 – 148.

MAGNI, G. (1991). The use of antidepressants in the treatment of chronic pain. A review of the current evidence. *Drugs,* **42,** 730 – 748.

MCQUAY, H.J., TRAMER, M., NYE, B.A., CARROLL, D., WIFFEN, P.J. & MOORE, R.A. (1996). A systematic review of antidepressants in neuropathic pain. *Pain,* **68,** 217 – 227.

MILLAN, M.J. (1994). Serotonin and pain: evidence that activation of 5-HT1A receptors does not elicit antinociception against noxious thermal, mechanical and chemical stimuli in mice. *Pain,* **58,** 45 – 61.

MILLAN, M.J., BROCCO, M., VEIGA, S., CISTARELLI, L., MELON, C. & GOBERT, A. (1998). WAY 100,635 enhances both the 'antidepressant' actions of duloxetine and its influence on dialysate levels of serotonin in frontal cortex. *Eur. J. Pharmacol.,* **341,** 165 – 167.

MILLAN, M.J., SEGUIN, L., HONORE, P., GIRARDON, S. & BERVOETS, K. (1996). Pro- and antinociceptive actions of serotonin (5-HT)1A agonists and antagonists in rodents: relationship to algesiometric paradigm. *Behav. Brain Res.,* **73,** 69 – 77.

MITCHELL, P.J. & REDFERN, P.H. (1997). Potentiation of the time-dependent, antidepressant-induced changes in the agonistic behaviour of resident rats by the 5-HT_{1A} receptor antagonist, WAY-100,635. *Behav. Pharmacol.,* **8,** 585 – 606.

MURPHY, R.M. & ZEMLAN, F.P. (1990). Selective serotonin1A/1B agonists differentially affect spinal nociceptive reflexes. *Neuropharmacology,* **29,** 463 – 468.

ONGHENA, P. & VAN HOUDENOVE, B. (1992). Antidepressant-induced analgesia in chronic non-malignant pain: a meta-analysis of 39 placebo-controlled studies. *Pain,* **49,** 205 – 219.

PAZOS, A. & PALACIOS, J.M. (1985). Quantitative autoradiographic mapping of serotonin receptors in the rat brain. I. Serotonin-1 receptors. *Brain. Res.,* **346,** 205 – 230.

PEREZ, V., GILABERTE, I., FARIES, D., ALVAREZ, E. & ARTIGAS, F. (1997). Randomised, double-blind, placebo-controlled trial of pindolol in combination with fluoxetine antidepressant treatment. *Lancet,* **349,** 1594 – 1597.

RANDALL, L.O. & SELITTO, J.J. (1957). A method for measurement of analgesic activity on inflammed tissue. *Arch. Int. Pharmacodyn.,* **61,** 409 – 419.

REICHENBERG, K., GAILLARD PLAZA, G. & MONTASTRUC, J.L. (1985). Influence of nalaoxone on the antinociceptive effects of some antideprssants drugs. *Arch. Int. Pharmacodyn. Ther.,* **275,** 78 – 85.

ROBLES, L.I., BARRIOS, M., DEL POZO, E., DORDAL, A. & BAEYENS, J.M. (1996). Effects of K + channel blockers and openers on antinociception induced by agonists of 5-HT1A receptors. *Eur. J. Pharmacol.,* **295,** 181 – 188.

ROMERO, L. & ARTIGAS, F. (1997). Preferential potentiation of the effects of serotonin uptake inhibitors by 5-HT1A receptor antagonists in the dorsal raphe pathway: role of somatodendritic autoreceptors. *J. Neurochem.,* **68,** 2593 – 2603.

SINDRUP, S.H., GRAM, L.F., BROSEN, K., ESHOJ, O. & MOGENSEN, E.F. (1990). The selective serotonin reuptake inhibitor paroxetine is effective in the treatment of diabetic neuropathy symptoms. *Pain,* **42,** 135 – 144.

TOME, M.B., ISAAC, M.T., HARTE, R. & HOLLAND, C. (1997). A randomized trial of serotonergic autoreceptor blockade in the reduction of antidepressant latency. *Int. Clin. Psychopharmacol.,* **12,** 81 – 89.

TRILLAT, A.C., MALAGIE, I., MATHE-ALLAINMAT, M., ANMELLA, M.C., JACQUOT, C., LANGLOIS, M. & GARDIER, A.M. (1998). Synergistic neurochemical and behavioral effects of fluoxetine and 5-HT_{1A} receptor antagonists. *Eur. J. Pharmacol.,* **357,** 179 – 184.

VERGE, D., DAVAL, G., MARCINKIEWICZ, M., PATEY, A., EL MESTIKAWY, S., GOZLAN, H. & HAMON, M. (1986). Quantitative autoradiography of multiple 5-HT1 receptor subtypes in the brain of control or 5,7-dihydroxytryptamine-treated rats. *J. Neurosci.,* **6,** 3474 – 3482.

WILLIS, W.D. & WESTLUND, K.N. (1997). Neuroanatomy of the pain system and of the pathways that modulate pain. *J. Clin. Neurphysiol.,* **14,** 2 – 31.

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