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Fluoxetine-Induced Desensitization of Somatodendritic 5-HT$_{1A}$ Autoreceptors Is Independent of Glucocorticoid(s)

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ABSTRACT Previous in vitro studies showed that glucocorticoid receptor activation (notably by corticosterone) could induce a functional desensitization of somatodendritic 5-HT$_{1A}$ autoreceptors in the dorsal raphe nucleus [Laaris et al. (1995) Neuropharmacology 34:1201–1210], similar to that due to in vivo subchronic treatment with a 5-HT reuptake inhibitor, such as fluoxetine, in rats. In the present study, we investigated whether a link might exist between these effects, i.e., whether glucocorticoid receptor activation could be responsible for the fluoxetine-induced desensitization of 5-HT$_{1A}$ autoreceptors. In vitro recording in the dorsal raphe nucleus of brain-stem slices showed that subchronic treatment with fluoxetine (5 mg/kg intraperitoneally (i.p.), daily for 3–7 days) significantly reduced the potency of the 5-HT$_{1A}$ receptor agonist ipsapirone to inhibit the firing rate of serotoninergic neurons. Parallel experiments in adrenalectomized and sham-operated rats indicated that subchronic fluoxetine treatment produced a similar shift to the right of the ipsapirone inhibition curve in both groups of animals. Furthermore, the subchronic blockade of glucocorticoid receptors by RU 38486 (25 mg/kg subcutaneously (s.c.), daily) in intact rats treated with fluoxetine (5 mg/kg i.p., daily for 3 days) did not affect the ability of the latter treatment to reduce the potency of ipsapirone to inhibit the firing of serotoninergic neurons. These data suggest that glucocorticoid receptors (and their possible activation by corticosterone) are not involved in the functional desensitization of somatodendritic 5-HT$_{1A}$ autoreceptors, which occurs during long-term treatment with a serotonin reuptake inhibitor such as fluoxetine. **Synapse 27:303–312, 1997.**

INTRODUCTION

The antidepressant action of several classes of molecules is presumed to be linked to their ability to increase serotoninergic neurotransmission in the central nervous system (Blier et al., 1987; Delgado et al., 1990). This concept notably applies to the selective serotonin reuptake inhibitors (SSRIs), whose antidepressant properties are well-established (Hyttel, 1994).

Among the neurobiological mechanisms which may contribute to the facilitatory effect of chronic SSRI treatment on central serotoninergic neurotransmission, the functional desensitization of somatodendritic 5-HT$_{1A}$ autoreceptors (Hoyer et al., 1994), located in the dorsal raphe nucleus (DRN), seems to be particularly relevant (Blier and de Montigny, 1994). Indeed, direct inactivation of the latter receptors by pindolol was recently shown to significantly enhance the antidepressant action of SSRIs (Artigas et al., 1994; Blier and Bergeron, 1995). However, the mechanism(s) responsible for the desensitization of DRN 5-HT$_{1A}$ autoreceptors by chronic SSRI treatment is (are) still poorly understood. Because the latter phenomenon is not associated with a downregulation of 5-HT$_{1A}$ autoreceptors (Hensler et al., 1991; Jolas et al., 1994; Le Poul et al., 1995), other mechanisms can be postulated to occur, such as the uncoupling of the receptor binding subunit from its associated G protein (Albert et al., 1996; Raymond, 1991; Saitoh et al., 1995) or a decrease in the concentration of G proteins available for the functioning of 5-HT$_{1A}$ autoreceptors (Li et al., 1996).

Steroid hormones may play a key role in the functional desensitization of 5-HT$_{1A}$ autoreceptors, notably

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through their ability to affect the functions (Ffrench-Mullen, 1995) and levels (Lesch et al., 1992; Saito et al., 1989) of specific G proteins in the central nervous system. We recently found (Laaris et al., 1995) that corticosterone can affect 5-HT_{1A} autoreceptor functioning by activating glucocorticoid receptors in the cytoplasm of serotoninergic neurons (Aronsson et al., 1988; Härfstrand et al., 1986). Interestingly, acute administration of SSRIs produces a marked increase in the levels of circulating corticosterone (Duncan et al., 1996; Fuller, 1992; Gibbs and Vale, 1983), which may suggest that SSRI-induced functional desensitization of 5-HT_{1A} autoreceptors could be due, at least partly, to this hormonal change.

This hypothesis was addressed in the present study by investigating whether corticosterone removal by adrenalectomy could prevent the desensitization of 5-HT_{1A} autoreceptors due to subchronic administration of fluoxetine. In addition, possible alteration of the latter phenomenon by glucocorticoid receptor blockade during the whole treatment with fluoxetine was also examined. In all cases, electrophysiological experiments in brain-stem slices were performed in order to directly assess the functional state of 5-HT_{1A} autoreceptors within the DRN (Haj-Dahmane et al., 1991).

MATERIALS AND METHODS

Surgery

Experiments were performed on young adult male Sprague-Dawley rats (Centre d'Elevage René Janvier, Le Genest-Saint-Isle, France) weighing 150–200 g. All procedures involving animals and their care were conducted in conformity with institutional guidelines that are in compliance with national and international laws and policies (Council directive #87-848, October 19, 1987, Ministère de l'Agriculture et de la Fôret, Service Vétérinaire de la Santé et de la Protection Animale, permissions #0299 to M.H. and #6269 to L.L.).

Animals were maintained under standard laboratory conditions (22 ± 1°C, 60% relative humidity, 12-h light-dark cycle, food and water ad libitum) for 5–10 days before the experiments.

Bilateral adrenalectomy (ADX) was performed through a dorsal incision under pentobarbital (60 mg/kg, intraperitoneally (i.p.) anesthesia. Sham-operated rats were subjected to the same surgical procedure, except that the adrenals were left in place. After surgery, the rats were returned to their home cages where they were grouped at 6 animals per cage. Drinking water was replaced by saline (0.9% NaCl) for adrenalectomized rats.

Treatments

Fluoxetine

Fluoxetine was dissolved in saline (0.9% NaCl) and injected i.p. (5 mg/kg daily, at 9:00 AM) for 3 or 7 days. Control rats received the same volume of saline (5 ml/kg i.p.) under the same time conditions. These treatments started 40–42 h after surgery in both adrenalectomized and sham-operated rats.

RU 38486 + fluoxetine

The specific glucocorticoid receptor antagonist RU 38486 (Moguilewski and Philibert, 1984) was suspended in 40% polyethyleneglycol (see Ratka et al., 1989) and injected at the dose of 25 mg/kg subcutaneously (s.c.) immediately after the i.p. administration of fluoxetine (5 mg/kg i.p.). This combined treatment was applied daily for 3 days to intact rats. Other animals received the vehicle (40% polyethyleneglycol) plus fluoxetine under the same time conditions.

Corticosterone assay

Rats were killed by decapitation 24 h after the last injection of saline or fluoxetine. Blood from trunk vessels was collected in chilled tubes and allowed to clot for 12 h at 4°C. Samples were centrifuged at 4,000*g* for 20 min, and the serum was collected to be frozen at −20°C until corticosterone determination. Corticosterone was quantified by radioimmunoassay (RIA) after extraction in ethanol (Grino et al., 1987). Anti-corticosterone antiserum was generously donated by F. Héry (INSERM U.297, Marseille, France). Corticosterone was used as standard, and [1,2,6,7-^{3}H]-corticosterone as radiotracer.

In vitro extracellular recording of dorsal raphe serotoninergic neurons

Immediately after sacrifice, the brain was rapidly removed from the skull and immersed in an ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl, 126; KCl, 3.5; NaH_2PO_4, 1.2; $MgCl_2$, 1.3; $CaCl_2$, 2.0; $NaHCO_3$, 25; glucose, 11. Bubbling the ACSF with a mixture of 95% O_2 and 5% CO_2 yielded a pH of 7.3–7.4. A block of tissue containing the DRN was cut into frontal sections (350 µm thick) in ACSF using a vibratome (Haj-Dahmane et al., 1991). After sectioning, slices were kept in ACSF for at least 1 h at room temperature. They were then transferred to a recording chamber continuously superfused with oxygenated ACSF (2 ml/min at 34°C).

Extracellular recordings were made using a single-barrel micropipette (filled with 2 M NaCl; impedance, 12–15 MΩ) introduced into the DRN area (Haj-Dahmane et al., 1991). In all experiments, the otherwise silent serotoninergic neurons were induced to fire by adding 3 µM phenylephrine (α_1-adrenoreceptor agonist) into the superfusing ACSF (VanderMaelen and Aghajanian, 1983). When a cell was recorded, it was identified on line as a 5-HT neuron according to the following criteria: biphasic action potentials of 2–3 msec duration, and a slow (0.5–2.0 Hz) and regular pattern of discharge (VanderMaelen and Aghajanian, 1983).

Baseline activity was recorded for 5–10 min before infusion of the 5-HT$_{1A}$ receptor agonist ipsapirone (Schechter et al., 1990) into the chamber via a three-way tap system. Complete exchange of fluids occurred within 2 min of the arrival of a new solution into the chamber. The electrical signals were fed into a high-input impedance amplifier, an oscilloscope, and an electronic ratemeter triggered by single-action potentials. The integrated firing rate was computed and recorded graphically as consecutive 10-sec samples. The effects of each concentration of ipsapirone were evaluated by comparing the mean discharge frequency during 2 min prior to its addition to the superfusing ACSF and 2–3 min after the end of drug infusion, when the resulting changes in firing frequency reached their maximal amplitude. Nonlinear regression fitting of concentration-response curves was carried out using Inplot 4 (Graph Pad Software Inc., San Diego, CA) software facilities.

In vitro application of the glucocorticoid receptor agonist RU 28362

Immediately after their preparation, brain-stem slices were incubated for 20 min in oxygenated-ACSF containing either 30 nM RU 28362 (Hermann et al., 1987) or its vehicle (0.005% ethanol), and then for 1–3 h in drug-free oxygenated ACSF prior to the recording session (see Laaris et al., 1995).

Statistical analyses

Data are expressed as percentages of the baseline firing rate ± SEM. For cells recorded in brain-stem slices from fluoxetine-treated rats, only desensitized cells were taken into account, i.e., cells for which the firing inhibition induced by 100 nM ipsapirone was <50%, vs. 100% in saline-treated rats (see Le Poul et al., 1995). Data were analyzed by one-way ANOVA, and, in case of significance ($P < 0.05$), the F-test for significant treatment effects was followed by a two-tailed t-test to compare the experimental groups with their controls (Snedecor and Cochran, 1967).

Chemicals

The drugs used in the present study were: ipsapirone (Troponwerke-Bayer, Cologne, Germany), phenylephrine and corticosterone (Sigma, St. Quentin-Fallavier, France), fluoxetine (Eli Lilly, Indianapolis, IN), RU 28362 (11β, 17β-dihydroxy-6-methyl-17 alpha (prop-1-ynyl) androsta-1,4,6-trien-3-one) and RU 38486 (11β-(4-dimethyl-amino-phenyl)-17β-hydroxy-17 alpha-(prop-1-ynyl) estra-4,9-dien-3-one) (Roussel-Uclaf, Romainville, France).

[1,2,6,7-^{3}H]corticosterone (87 Ci/mmole) was from Amersham (Les Ulis, France).

TABLE I Serum corticosterone levels after subchronic fluoxetine treatment in adrenalectomized (ADX) or sham-operated rats[1]

	Corticosterone (µg/100 ml serum)			
	Sham		ADX	
	Saline	Fluoxetine	Saline	Fluoxetine
3days	6.37 ± 1.27 (9)	32.69 ± 5.88* (8)	<1 (9)	<1 (10)
7days	7.72 ± 1.05 (11)	23.88 ± 3.61* (10)	<1 (7)	<1 (9)

[1]Corticosterone levels were measured in trunk blood samples collected from rats sacrificed 24 h after the last i.p. injection of a 3- or 7-day treatment with fluoxetine (5 mg/kg/day) or saline. Each value is the mean ± SEM of the number of independent determinations indicated in parentheses.
*$P < 0.001$, as compared to respective values in saline-treated rats.

RESULTS

Serum corticosterone levels

In sham-operated rats, the serum levels of corticosterone measured 24 h after the last injection of a 3-day treatment with fluoxetine (5 mg/kg i.p., daily) were ~5 times higher ($P < 0.001$) than those found in rats treated with saline under the same schedule conditions (Table I). A significant increase in serum corticosterone levels was still observed 24 h after a 7-day treatment with fluoxetine, but this effect was less pronounced than that noted earlier during treatment. As shown in Table I, the hormone levels were increased by ~200% only ($P < 0.001$) in sham-operated rats treated for 7 days with fluoxetine as compared to saline-treated controls.

Adrenalectomy was associated with corticosterone levels below the limit of detection of the RIA (1 µg/100 ml of serum). Similar findings were made whether adrenalectomized rats were treated with fluoxetine or saline for 3 or 7 days (Table I).

Effects of adrenalectomy on ipsapirone-induced inhibition of electrical activity of DRN serotoninergic neurons in rats treated for 3 or 7 days with fluoxetine

No significant differences between adrenalectomized and sham-operated animals, treated with fluoxetine or saline, were observed in the baseline firing rate of DRN serotoninergic neurons (Table II). In all treatment groups, the mean discharge rate of these neurons ranged between 1.07–1.41 spikes/sec (Table II), which corresponded to that usually found in experiments performed on brain slices from untreated rats (Haj-Dahmane et al., 1991). Furthermore, the combined in vivo administration of fluoxetine plus the glucocorticoid receptor antagonist RU 38486 to intact rats for 3 days also exerted no significant influence on the baseline firing rate of DRN serotoninergic neurons recorded under in vitro conditions (mean ± SEM = 1.19 ± 0.12 Hz, n = 8). Similarly, in vitro application of the glucocorticoid receptor agonist RU 28362 (30 nM) onto brain-stem slices from intact rats that were treated with fluoxetine for 3 days did not significantly affect the

TABLE II Effects of adrenalectomy and/or fluoxetine treatment for 3 or 7 days on the baseline firing rate of DRN serotoninergic neurons[1]

Treatment	Firing frequency (Hz)	
	Sham	ADX
Saline, 3 days	1.21 ± 0.08 (11)	1.25 ± 0.09 (9)
Saline, 7 days	1.16 ± 0.06 (32)	1.07 ± 0.08 (13)
Fluoxetine, 3 days	1.33 ± 0.08 (11)	1.08 ± 0.08 (15)
Fluoxetine, 7 days	1.41 ± 0.18 (11)	1.20 ± 0.08 (28)

[1]Fluoxetine (5 mg/kg) or saline was injected i.p. at 9:00 AM each day for 3 or 7 days. Adrenalectomy (ADX) or sham-surgery was performed ~40 h before the first injection. Animals were killed 24 h after the last injection, and the electrical discharge of DRN serotoninergic neurons was recorded in brain-stem slices, as described in Materials and Methods. Each value is the mean ± SEM of the spontaneous firing frequency (Hz) of the number of recorded cells indicated in parentheses. No significant differences were found between the different treatment groups.

spontaneous discharge of DRN serotoninergic neurons (mean ± SEM = 1.17 ± 0.27 Hz, n = 4).

As expected of the stimulation of somatodendritic 5-HT_{1A} autoreceptors (see Haj-Dahmane et al., 1991), addition of ipsapirone to the ACSF superfusing brain-stem slices from saline-treated rats resulted in a concentration-dependent reduction in the firing rate of DRN serotoninergic neurons (Figs. 1 and 3). This effect was at its maximum within 2–3 min after a 2.5-min application of ipsapirone (10–300 nM) and then progressively disappeared. Nearly complete recovery of the baseline firing rate was usually observed 15–20 min after removal of ipsapirone from the superfusing ACSF. As shown in Figures 1–3, the time-course changes and the potency of the 5-HT_{1A} receptor agonist were similar in ADX and sham-operated rats. In both cases, the IC_{50} value of ipsapirone was close to 60 nM (Figs. 2 and 4).

Like that previously observed in intact rats (Le Poul et al., 1995), the subchronic administration of fluoxetine to sham-operated rats resulted in a decreased efficiency of ipsapirone to inhibit the firing of DRN serotoninergic neurons (Figs. 2 and 4). In contrast to the cells recorded in tissues from saline-treated rats, on which 100 nM ipsapirone always produced a complete (but reversible) inhibition of discharge, a proportion of cells in fluoxetine-treated rats was markedly less affected by the 5-HT_{1A} receptor agonist. Thus, the percentage of recorded DRN serotoninergic neurons for which 100 nM ipsapirone produced a reduction in firing rate lower than 50% (as compared to baseline) increased from 30% (3 out of a total of 10 cells) to 62% (5 out of a total of 8 cells) in rats that were treated with fluoxetine for 3 or 7 days, respectively. These two treatment conditions were chosen for a comparison between sham-operated and ADX rats.

As shown in Figure 1, a 3-day treatment with fluoxetine also markedly reduced the potency of ipsapirone to decrease the firing rate of DRN serotoninergic neurons in ADX animals. This change involved 50% (4 out of a total of 8 cells) of neurons recorded in this treatment-group. The resulting shift to the right of the respective ipsapirone-concentration curves indicated that fluoxetine treatment increased the IC_{50} value of the 5-HT_{1A} receptor agonist by 217% in sham-operated rats and 343% in ADX rats (Fig. 2).

Similar results were found in rats that were treated with fluoxetine for 7 days. In 86% (20 out of a total of 23 cells) of cells recorded in ADX rats, this treatment produced a decrease in the potency of ipsapirone to inhibit the firing of DRN serotoninergic neurons, as also observed in sham-operated animals (Fig. 3). Thus, the IC_{50} values of the 5-HT_{1A} receptor agonist increased by 344% in ADX rats and 293% in sham-operated rats after a 7-day treatment with fluoxetine (Fig. 4).

Effects of in vivo administration of the glucocorticoid receptor antagonist RU 38486 on ipsapirone-induced inhibition of electrical activity of DRN serotoninergic neurons in fluoxetine-treated rats

As also observed in sham-operated rats, the administration of fluoxetine (5 mg/kg i.p., daily) for 3 days decreased the potency of ipsapirone to inhibit the firing of DRN serotoninergic neurons in intact rats (IC_{50} = 60 ± 11 nM in saline-treated rats, and 221 ± 27 nM in fluoxetine-treated rats). In contrast, a 3-day treatment with RU 38486 (25 mg/kg s.c., daily) exerted no influence on the effect of ipsapirone in both control (not shown) and fluoxetine-treated rats. Thus, a similar reduction in potency of ipsapirone to inhibit the electrical discharge of DRN serotoninergic neurons was observed in rats that received fluoxetine alone or fluoxetine + RU 38486 (Fig. 5).

In vitro effect of RU 28362 on ipsapirone-induced inhibition of DRN serotoninergic neuron firing in fluoxetine-treated rats

Experiments were performed with brain-stem slices from intact rats that were treated with fluoxetine (5 mg/kg i.p., daily) for 3 days. Incubation of tissues with the glucocorticoid agonist RU 28362 (at 30 nM for 20 min) exerted no significant effect on ipsapirone-induced inhibition of the electrical activity of DRN serotoninergic neurons. This applied to all concentrations of ipsapirone tested, so that the concentration-curves allowed the calculation of similar IC_{50} values for the 5-HT_{1A} receptor agonist in experiments with slices exposed to RU 28362 or its vehicle (Fig. 6).

DISCUSSION

Comparison of the functional desensitization of somatodendritic 5-HT_{1A} autoreceptors in the DRN of rats treated repeatedly with SSRIs (de Montigny et al., 1990; Jolas et al., 1994; Le Poul et al., 1995) with that due to activation of glucocorticoid receptors (Laaris et al., 1995, 1997) in the serotoninergic neurons of this structure (Aronsson et al., 1988; Härfstrand et al., 1986) revealed striking similarities. Thus, in both

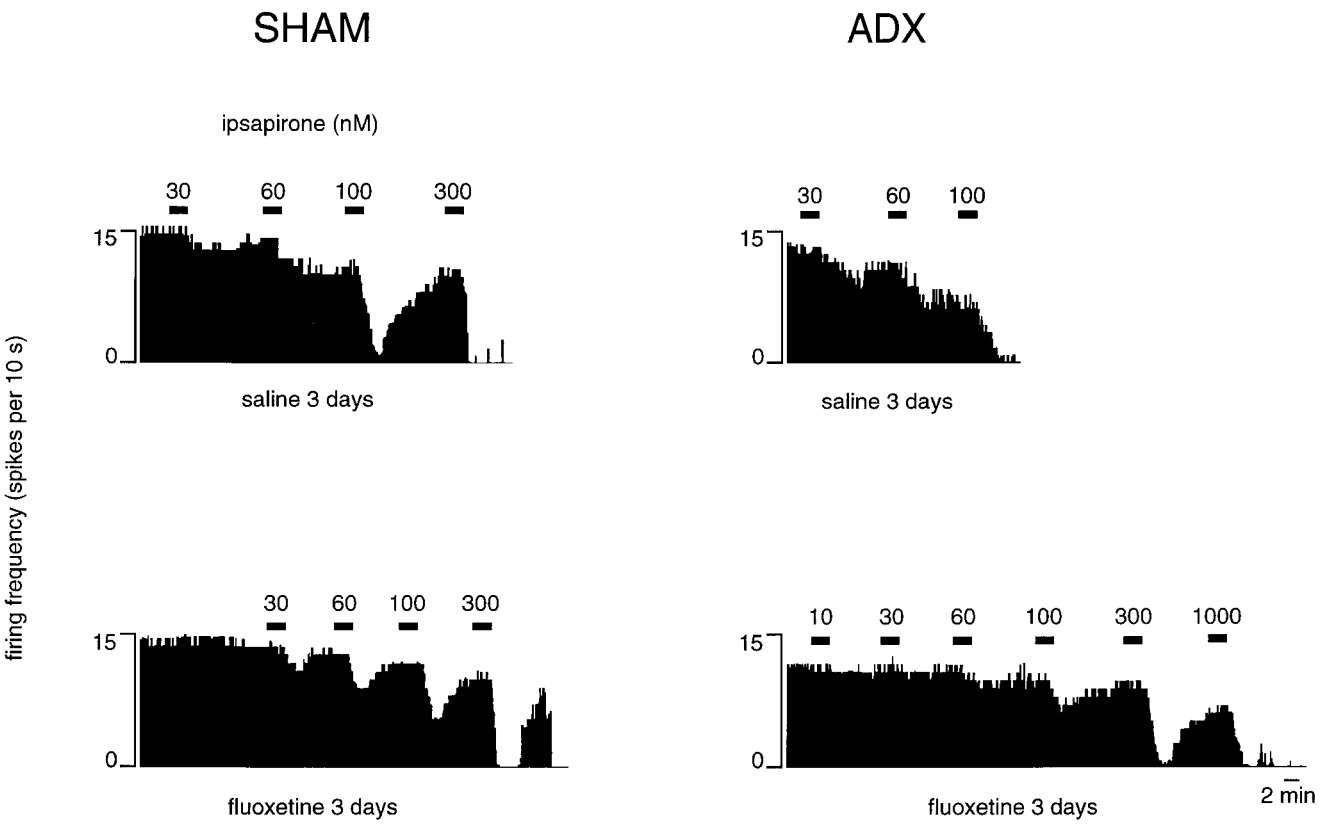

Fig. 1. Effects of a 3-day treatment with fluoxetine on the potency of ipsapirone to depress the firing frequency of DRN 5-HT neurons in adrenalectomized and sham-operated rats. Integrated firing rate histograms of DRN 5-HT neurons exposed to ipsapirone (10–1,000 nM) from sham-operated (**left**) and ADX (**right**) rats treated with saline or fluoxetine (5 mg/kg/day, i.p.) for 3 days. Each horizontal bar represents the bath application of ipsapirone for 2.5 min at the indicated concentration.

cases, this adaptive phenomenon resulted in a similar shift to the right of the concentration-curves of 5-HT$_{1A}$ receptor agonist-induced inhibition of the firing of DRN serotoninergic neurons. Consequently, the IC$_{50}$ values of 8-OH-DPAT and ipsapirone increased by 2–3-fold after repeated treatment with SSRIs (such as fluoxetine and paroxetine; see Le Poul et al., 1995, and this paper) as well as after glucocorticoid receptor activation by corticosterone or a selective agonist such as RU 28362 (Laaris et al., 1995, 1997). In addition, the functional desensitization of somatodendritic 5-HT$_{1A}$ autoreceptors due to glucocorticoid receptor activation occurred without any change in the density and/or affinity of DRN 5-HT$_{1A}$ binding sites (Laaris et al., 1995), such as observed after repeated treatment with 5-HT reuptake inhibitors (Hensler et al., 1991; Le Poul et al., 1995). However, this observation does not apply to all brain areas, since corticosterone is well-known to affect the density of the 5-HT$_{1A}$ receptor and the concentration of 5-HT$_{1A}$ receptor mRNA in selected subregions of the rat hippocampus (Burnet et al., 1992; Chalmers et al., 1993; Chaouloff, 1995; Mendelson and McEwen, 1990).

This parallelism between the characteristics of 5-HT$_{1A}$ autoreceptor desensitization due to two different pharmacological conditions led us to address the question of the existence of a possible link between the effect of SSRIs on the one hand and that of glucocorticoid receptor activation on the other hand. Indeed, SSRIs are known to enhance the secretion of corticosterone in rats (Fuller, 1992). Although this effect adapts to some extent upon repeated administration of these drugs (Duncan et al., 1996), increased serum levels of the hormone were still observed 24 h after the last injection of fluoxetine in the 3–7-day treatments of the present study (See Table I). Therefore, such subchronic treatments with fluoxetine produced a rather sustained increase in the circulating levels of corticosterone, similar to that evoked by various stressful conditions in rats (Laaris et al., 1997). Because stress was shown to trigger 5-HT$_{1A}$ autoreceptor desensitization through the stimulation of glucocorticoid receptors by endogenous corticosterone (Laaris et al., 1997), it was of interest to investigate whether fluoxetine-induced elevation in the serum levels of this hormone could also contribute to 5-HT$_{1A}$ autoreceptor desensitization in rats treated with this drug.

Indeed, this question was especially relevant in the case of treatment with antidepressants such as fluoxetine, because chronic administration of these drugs

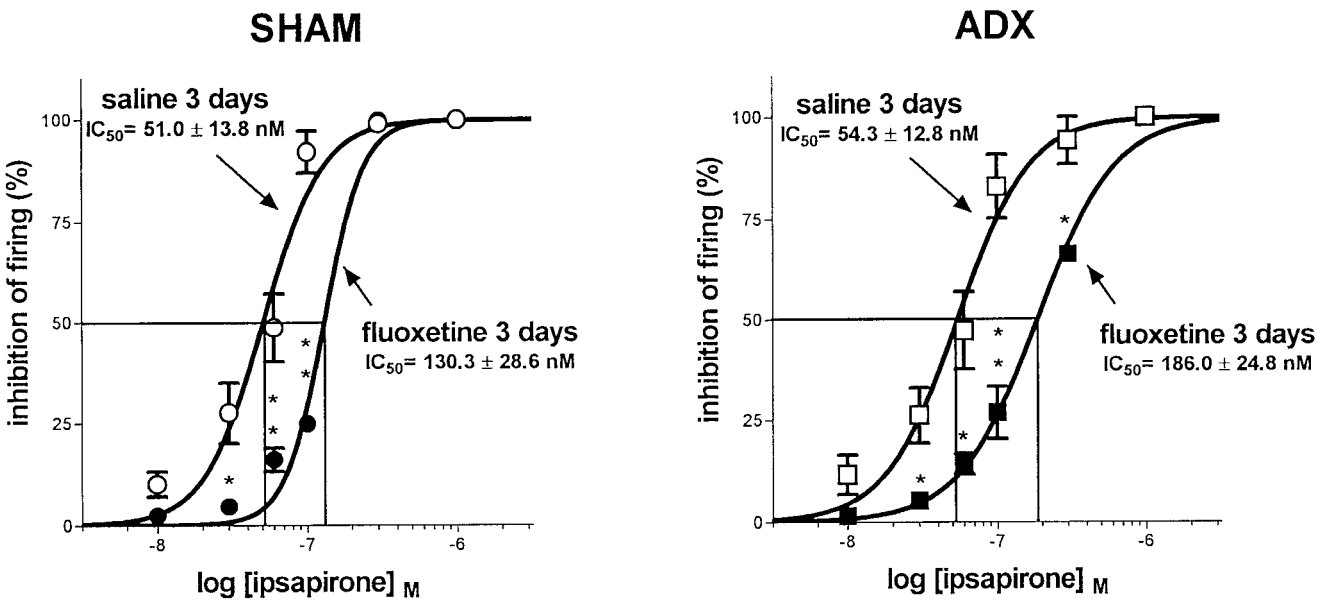

Fig. 2. Concentration-response curves of ipsapirone-induced inhibition of the firing of DRN 5-HT neurons in brain-stem slices from sham-operated or adrenalectomized rats treated with saline or fluoxetine for 3 days. Ipsapirone-induced inhibition is expressed as a percentage of the baseline firing rate in sham-operated (curves at left; open and solid circles for saline- and fluoxetine-treated rats, respectively) or ADX (curves at right; open and solid squares for saline- and fluoxetine-treated rats, respectively) rats. Each point is the mean ± SEM of data obtained from 9–18 cells (1–3 cells per rat; 7–10 rats per group). IC_{50} ± SEM values (in nM) were calculated by nonlinear regression analysis, using Inplot 4. $^{*}P < 0.05$, $^{**}P < 0.02$ as compared to the respective inhibition in saline-treated rats.

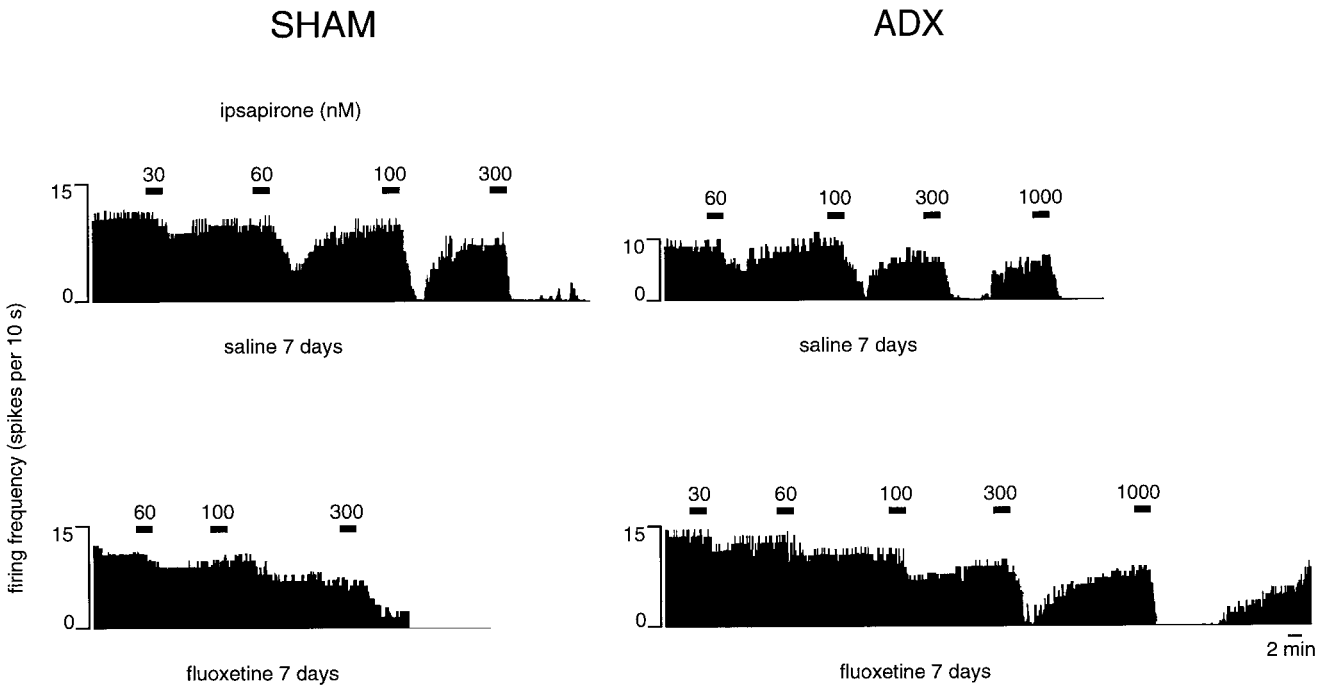

Fig. 3. Effects of a 7-day treatment with fluoxetine on the potency of ipsapirone to depress the firing frequency of DRN 5-HT neurons in adrenalectomized and sham-operated rats. Integrated firing-rate histograms of DRN 5-HT neurons exposed to ipsapirone (10–1,000 nM) from sham-operated (**left**) and ADX (**right**) rats treated with saline or fluoxetine (5 mg/kg/day, i.p.) for 7 days. Each horizontal bar represents the bath application of ipsapirone for 2.5 min at the indicated concentration.

has also been shown to increase the synthesis of glucocorticoid receptors, resulting both in an enhanced density of these receptors and in a facilitated response to endogenous corticosterone in the rat brain (Barden, 1996; Peiffer et al., 1991; Przegaliñski and Budziszewska, 1993; Przegaliñski et al., 1993).

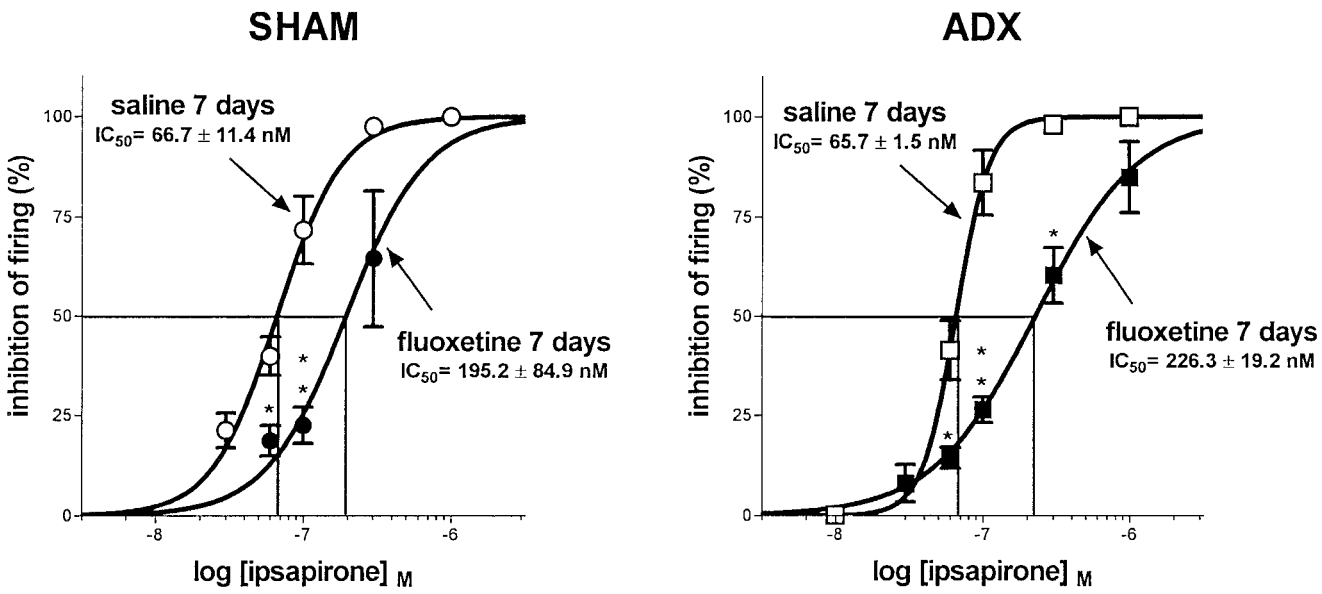

Fig. 4. Concentration-response curves of ipsapirone-induced inhibition of the firing of DRN 5-HT neurons in brain-stem slices from sham-operated or adrenalectomized rats treated with saline or fluoxetine for 7 days. Ipsapirone-induced inhibition is expressed as a percentage of the baseline firing rate in sham-operated (curves at left; open and solid circles for saline- and fluoxetine-treated rats, respectively) or ADX (curves at right; open and solid squares for saline- and fluoxetine-treated rats, respectively) rats. Each point is the mean ± SEM of data obtained from 5–23 cells (1–3 cells per rat; 5–12 rats per group). IC_{50} ± SEM (in nM) values were calculated by nonlinear regression analysis, using Inplot 4. *$P < 0.05$, **$P < 0.02$ as compared to the respective inhibition in saline-treated rats.

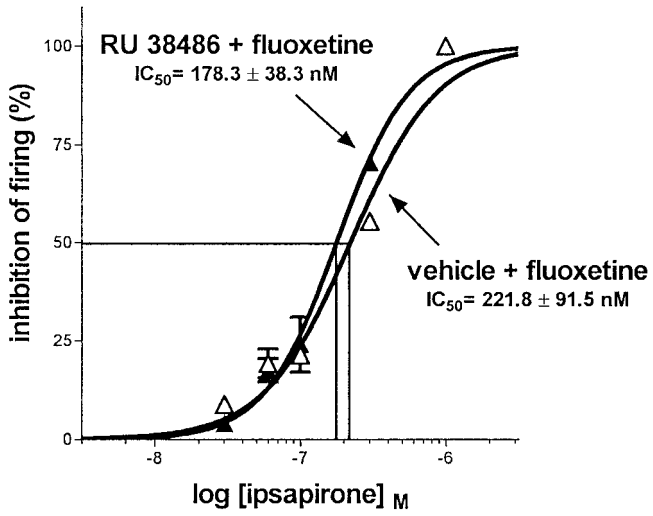

Fig. 5. Concentration-response curves of ipsapirone-induced inhibition of the firing of DRN 5-HT neurons in brain-stem slices from intact rats treated with fluoxetine and RU 38486 or vehicle for 3 days. Fluoxetine (5 mg/kg i.p., daily) was administered together with RU 38486 (25 mg/kg s.c., daily) or its vehicle (0.5 ml of 40% polyethyleneglycol s.c., daily) for 3 days, and rats were killed 24 h after the last injection. Electrical recordings (see Figs. 1 and 3) were performed in slices exposed to various concentrations of ipsapirone (abscissa). Inhibition due to the 5-HT$_{1A}$ receptor agonist is expressed as a percentage of the baseline firing rate. Each point is the mean ± SEM of data obtained from 3–8 cells (1–3 cells per rat; 4–5 rats per group). IC_{50} ± SEM (in nM) values were calculated by nonlinear regression analysis, using Inplot 4.

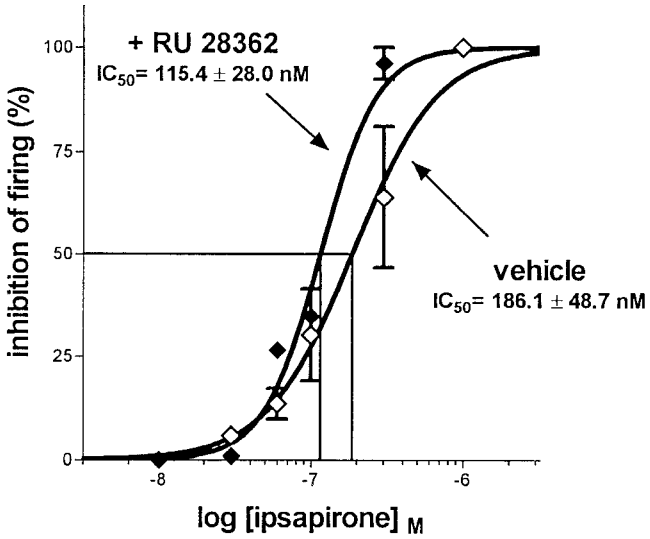

Fig. 6. Concentration-response curves of ipsapirone-induced inhibition of the firing of DRN 5-HT neurons in brain-stem slices from fluoxetine-treated rats. Effects of in vitro exposure to RU 28362. Rats were treated for 3 days with fluoxetine (5 mg/kg i.p., daily). Brain-stem slices were prepared 24 h after the last injection, and exposed to 30 nM RU 28362 or its vehicle (0.005% ethanol) for 20 min prior to the recording session. Ipsapirone-induced inhibition is expressed as a percentage of the baseline firing rate. Each point is the mean ± SEM of data obtained from 3–8 cells (1–3 cells per rat; 4–5 rats per group). IC_{50} ± SEM (in nM) values were calculated by nonlinear regression analysis, using Inplot 4.

Thus, both the increase in the serum concentration of endogenous corticosterone and an elevated density of glucocorticoid receptors in the brain might have contributed to a possible glucocorticoid-mediated desensitization of somatodendritic 5-HT$_{1A}$ autoreceptors in rats treated subchronically with fluoxetine. This hypothesis

was presently tested under the same conditions as those which allowed the conclusion of the involvement of endogenous corticosterone in the desensitization of somatodendritic 5-HT_{1A} autoreceptors in stressed rats (Laaris et al., 1997).

Interestingly, the glucocorticoid receptor agonist RU 28362 (Hermann et al., 1987), at a concentration of 30 nM which, on its own, produced a desensitization of somatodendritic 5-HT_{1A} autoreceptors in tissues from untreated intact rats (Laaris et al., 1995), exerted no influence on the potency of ipsapirone to inhibit the firing of DRN serotoninergic neurons in rats which had been pretreated with fluoxetine for 3 days (Fig. 6). Because the desensitizing effect of RU 28362 was not additive with that due to repeated administration of fluoxetine, it could be inferred that the latter treatment already affected 5-HT_{1A} receptor functioning, possibly through the activation of glucocorticoid receptors. However, the other experimental series that we performed did not support this interpretation. First, the repeated administration of the selective glucocorticoid receptor antagonist RU 38486 (Moguilewski and Philibert, 1984), to prevent any glucocorticoid receptor activation by corticosterone secreted after each administration of fluoxetine during the 3- or 7-day treatment, did not affect the capacity of these treatments to reduce the potency of ipsapirone to inhibit the electrical discharge of DRN serotoninergic neurons. Second, the removal of corticosterone by adrenalectomy, which renders impossible any glucocorticoid receptor activation by this hormone, did not prevent the ability of fluoxetine treatment to reduce the potency of ipsapirone to inhibit the electrical activity of DRN serotoninergic neurons. Indeed, the fluoxetine-induced shift to the right of ipsapirone concentration-curves involved a higher proportion of recorded cells and was slightly (but not significantly) larger in adrenalectomized rats than in sham-operated animals (see Figs. 1 and 3). In this respect, the desensitization of somatodendritic 5-HT_{1A} autoreceptors due to subchronic treatment with fluoxetine differed markedly from that observed in stressed rats, because adrenalectomy was found to protect these receptors from stress-induced desensitization, as expected of the mediation of the latter phenomenon by endogenous corticosterone.

All these data clearly indicate that the functional desensitization of somatodendritic 5-HT_{1A} autoreceptors due to subchronic administration of fluoxetine occurred independently of the activation (and possibly induction) of glucocorticoid receptors that might be triggered by this treatment. In this context, the fact that the effect of in vitro exposure to RU 28362 did not add with that due to in vivo treatment with fluoxetine suggests that the functional desensitization of somatodendritic 5-HT_{1A} autoreceptors might already be maximal after one treatment, preventing any further effect by the second treatment. Accordingly, although different triggering mechanisms appear to be responsible for 5-HT_{1A} autoreceptor desensitization due to glucocorticoid receptor activation on the one hand and subchronic treatment with fluoxetine on the other, some common features might finally occur in both types of desensitization. Previous studies on SSRI-induced 5-HT_{1A} autoreceptor desensitization have shown that this effect develops rather slowly during treatment with these drugs (Blier et al., 1987; Blier and de Montigny, 1994; de Montigny et al., 1990; Le Poul et al., 1995). Similarly, under both in vitro and in vivo conditions, we noted that 5-HT_{1A} autoreceptor desensitization due to corticosterone only occurred several hours after the cessation of activation of glucocorticoid receptors by this hormone (Laaris et al., 1995, 1997, and unpublished observations). This would suggest that glucocorticoid receptor activation is permissive for the development of 5-HT_{1A} autoreceptor desensitization. Whether the resulting changes in 5-HT_{1A} autoreceptor structure and/or function finally resemble those due to subchronic treatment with fluoxetine is an open question to be addressed in future investigations.

Interestingly, previous studies on 5-HT_2 receptors also showed that the reduced response to selective 5-HT_2 agonists after repeated treatment with these drugs did not involve glucocorticoids, because adrenalectomy did not prevent this phenomenon (Chaouloff et al., 1993). In that case, extensive studies have shown that chronic stimulation of 5-HT_2 receptors produces a direct adaptive mechanism ending with the downregulation of these receptors (Ivins and Molinoff, 1991; Rahman and Neuman, 1993).

Similarly, studies on 5-HT_{1A} receptors in various transfected cell lines have shown that chronic activation of these receptors results in their functional desensitization, probably through the phosphorylation by protein kinases A and/or C of threonine and serine residues in the second and third intracellular loops of the receptor protein (Albert et al., 1996; Lembo and Albert, 1995; Nebigil et al., 1995; Raymond, 1991; Raymond and Olsen, 1994). Investigations on the possible involvement of such a mechanism in the desensitization of somatodendritic 5-HT_{1A} autoreceptors in fluoxetine-treated rats will require the performance of Western blot experiments with specific antibodies (El Mestikawy et al., 1990) to directly assess the phosphorylation status of the receptor. Furthermore, whether selective inhibitors of protein kinases A and/or C can prevent the desensitization due to fluoxetine treatment is also a key question to be addressed in future investigations.

In conclusion, the present data demonstrate that the functional desensitization of somatodendritic 5-HT_{1A} autoreceptors, which probably plays a key role in the antidepressant action of chronic treatment with fluoxetine (and other SSRIs; Artigas et al., 1994; Blier and de Montigny, 1994), occurs independently of the activation

of glucocorticoid receptors during this treatment. More probably, direct tonic stimulation of these autoreceptors by 5-HT in excess in the extracellular space within the raphe area, because of the chronic blockade of 5-HT reuptake (Bel and Artigas, 1992; Gardier et al., 1996), might account for the desensitization phenomenon. Whether similar molecular mechanisms as those described for 5-HT_{1A} receptor desensitization in various transfected cell lines (Albert et al., 1996; Raymond and Olsen, 1994) contribute to the desensitization of somatodendritic 5-HT_{1A} autoreceptors during chronic administration of SSRIs remains a working hypothesis to be addressed in future investigations.

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