Abstract Electrophysiological and autoradiographic approaches were used to assess possible changes in 5-hydroxytryptamine (serotonin) 5-HT_{1A} receptors in the rat dorsal raphe nucleus after a subchronic treatment with fluoxetine or paroxetine, two specific serotonin reuptake inhibitors with antidepressant properties. Fluoxetine or paroxetine were injected daily (5 mg/kg, i.p.) for various time periods up to 21 days. Electrophysiological recordings performed 24 h after the last injection showed that the potency of the 5-HT_{1A} receptor agonist, 8-OH-DPAT, to depress the firing of serotoninergic neurons in the dorsal raphe nucleus within brain stem slices was significantly reduced as early as after a 3-day treatment with either drug. The proportion of recorded neurons showing desensitization of somatodendritic 5-HT_{1A} autoreceptors increased along the treatment from ~40% on the 3rd day to 60–80% on the 21st day. At no time during the treatment, was the specific binding of [^3H]8-OH-DPAT (agonist radioligand) or [^3H]WAY-100635 (antagonist radioligand) to 5-HT_{1A} receptors modified in the dorsal raphe nucleus or in other brain areas, suggesting that neither the density nor the coupling of these receptors to G-proteins were probably altered in rats injected with fluoxetine or paroxetine for up to 21 days.

These results show that adaptive desensitization of somatodendritic 5-HT_{1A} autoreceptors within the dorsal raphe nucleus can already be detected after a 3-day treatment with selective serotonin reuptake inhibitors. Rather than the desensitization per se, it may be the progressive increase in the number of serotoninergic neurons with desensitized 5-HT_{1A} autoreceptors which plays a critical role in the (slowly developing) antidepressant action of these drugs.

Key words Fluoxetine • Paroxetine • Serotonin • 5-HT_{1A} autoreceptors • Dorsal raphe nucleus • Firing • Autoradiography

Introduction

The involvement of serotonin (5-hydroxytryptamine, 5-HT) in the pathogenesis of depressive disorders and in the mechanisms of action of antidepressant drugs has been convincingly established (Delgado et al. 1990; Briley and Moret 1993). One of the most direct lines of evidence attesting to the dysfunction of serotonergic neurotransmission in depression is the clinical efficacy of selective serotonin reuptake inhibitors for its treatment (Fuller et al. 1991; Nemeroff 1993; Tignol 1993).

Drugs belonging to this group, such as fluoxetine and paroxetine, apparently exert their antidepressant activity by increasing the concentration of 5-HT in the extracellular compartment, thereby enhancing serotonergic neurotransmission (Hyttel 1994). This effect has been shown to occur under acute conditions, as expected from the fact that blockade of the 5-HT transporter by selective reuptake inhibitors is achieved without any delay (see Fuller 1994). However, two to three weeks of treatment with these drugs have been reported to be necessary to produce the first signs of clinical efficacy (Nemeroff 1993; Tignol 1993).

Several hypothetical mechanisms have been proposed to account for this delay. Pharmacokinetics, neurotransmitter metabolism, and/or adaptive regulation of various pre- and/or postsynaptic receptors, including those of the 5-HT family (Hoyer et al. 1994), have thus been considered to be important parameters which may play a critical role in the slowly developing therapeutic action of antidepressants (see Briley and...
Nevertheless, in vivo and in vitro electrophysiological investigations have clearly demonstrated that chronic stimulation of 5-HT$_{1A}$ receptors by 5-HT after long-term treatment with a 5-HT reuptake inhibitor can produce a functional desensitization of the presynaptic (i.e. somato-dendritic) 5-HT$_{1A}$ autoreceptors in the dorsal raphe nucleus (DRN) (De Montigny et al. 1990; Jolais et al. 1994). This phenomenon has been reported to be highly significant after two weeks of treatment (Chaput et al. 1988), but the kinetics of 5-HT$_{1A}$ autoreceptor desensitization in rats treated with selective 5-HT reuptake inhibitors have not been studied in detail.

The present study was aimed at investigating the time course of the adaptive changes in somato-dendritic 5-HT$_{1A}$ autoreceptors during treatment with fluoxetine and paroxetine, two selective 5-HT reuptake inhibitors with different affinities for the 5-HT transporter (Wong et al. 1991; Hyttel 1994). Each drug was injected daily for 3, 7, 14 and 21 days and in vitro electrophysiological experiments were performed to directly assess the functional status of somato-dendritic 5-HT$_{1A}$ autoreceptors in the DRN. Furthermore, an autoradiographic approach was used to examine the binding characteristics of central 5-HT$_{1A}$ sites after both treatments.

**Materials and methods**

**Animals.** All the experiments were performed using male Sprague-Dawley rats (Centre d’Elevage R. Janvier, 53940 Le Genest-St Isle, France) of 200–250 g body weight. Rats were housed in groups of nine and maintained under standard laboratory conditions (22 ± 1°C, 60% relative humidity, 12:12 h light-dark cycle, food and water ad libitum) for at least 1 week before being used for the experiments.

Procedures involving animals and their care were all conducted in conformity with the 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 Forêt, Service Vétérinaire de la Santé et de la Protection Animale, permissions # 0299 to M.H. and # 6269 to L.L.)

**Electrophysiology — In vitro recording of the electrical activity of DRN serotoninergic neurons in brain stem slices.** Rats were decapitated, and the brains were rapidly removed and immersed in an ice-cold Krebs’ solution, bubbled continuously with an O$_2$/CO$_2$ mixture (95/5%). A block of tissue containing the DRN was cut into frontal sections (350 μm thick) in the same ice-cold Krebs’ solution using a vibratome (Haj-Dahmane et al. 1991). Brain stem slices were immediately incubated at room temperature for at least 1 h in an artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl 126; KCl 3.5; NaH$_2$PO$_4$, 1.2; MgCl$_2$, 1.3; CaCl$_2$, 2.0; NaHCO$_3$, 25; glucose, 11. Equilibrating the ACSF with 95% O$_2$ and 5% CO$_2$ yielded a pH of 7.3–7.4. A single slice was then transferred to a recording chamber in which it was continuously superfused with oxygenated ACSF (2 ml/min) at 34°C (Haj-Dahmane et al. 1991).

Extracellular recordings were obtained using a single barrel micropipette (filled with 2 M NaCl; impedance = 15 MΩ) introduced into the DRN area, which could be located easily in the midline of the slice, between the medial longitudinal fasciculi extending dorsally toward the aqueduct. In all cases, the otherwise silent serotoninergic neurons in the brain stem slice were induced to fire by adding 3 μM phenylephrine (an α$_2$ adrenoceptor agonist) to the superfusing ACSF (Vandermaelen and Aghajanian 1983). When a cell was recorded, it was identified on line as serotoninergic using the following criteria: biphasic action potentials and slow (0.5 to 2 spikes/s) and regular pattern of discharge (Vandermaelen and Aghajanian 1983).

Baseline activity was recorded for 5 to 10 min before application of the different drugs via a three-way tap system that allowed immediate exchange of fluids within 2 min of the arrival of a new solution. Two to three min infusion of the 5-HT$_{1A}$ agonist, 8-hydroxy-2-(di-n-propylamino)tetratin (8-OH-DPAT, Hall et al. 1985), was enough to induce a maximal effect. The 5-HT$_{1A}$ antagonist, N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridyl)cyclohexane carboxamide (WAY-100635, Fletcher et al. 1994) was added to the superfusing fluid for 6–8 min before the addition of the agonist, and application of the antagonist then proceeded for 5–8 min. Three to five min of infusion with fluoxetine or paroxetine were needed to induce a notable effect on the electrical activity of the DRN 5-HT cells (see Results).

The electrical signals were fed into a high-input impedance amplifier, an oscilloscope and an electronic ratemeter triggered by individual neuronal spikes (Haj-Dahmane et al. 1991). The integrated firing rate was computed, and recorded graphically as consecutive 10-s samples. The effect of a given drug was evaluated by comparing the mean discharge frequency during the 2 min prior to its addition to the superfusing ACSF and the mean discharge frequency 2 to 10 min after the end of the drug infusion, when the resulting changes in firing frequency reached their maximal amplitude. When two drugs were infused simultaneously, the resulting discharge frequency was compared to the baseline firing rate and to the frequency recorded upon application of only one drug. Data are presented in percentage of baseline firing rate ± SEM.

**Quantitative autoradiography.** Protocols for the measurement of $[^{3}H]$8-OH-DPAT and $[^{3}H]$WAY-100635 binding to 5-HT$_{1A}$ receptors in rat brain sections were adapted from previously described procedures (Gozlan et al. 1995). Briefly, treated and control rats were decapitated, and the brains quickly removed and frozen in...