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Chronic fluoxetine induces opposite changes in G protein coupling at pre and postsynaptic 5-HT_{1A} receptors in rat brain

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Abstract

Chronic treatment with the antidepressant fluoxetine may lead to changes in the properties of pre- and postsynaptic 5-HT_{1A} receptors due to modifications in the receptor-G protein coupling process. We have evaluated, in rats, the effect of chronic fluoxetine (10 mg/kg/day) at brain 5-HT_{1A} receptors using different techniques. The density of 5-HT_{1A} receptors was unchanged in fluoxetine-treated rats vs. vehicle group. Stimulation of [^{35}S]GTPγS binding induced by (±)8-OH-DPAT was significantly attenuated in dorsal raphe nucleus after fluoxetine (+3.7 vs. +31.2% in vehicle). The inhibition of dorsal raphe firing by (±)8-OH-DPAT (ED_{50} in vehicle = 2.1 μg/kg, i.v.) was also attenuated in rats treated with fluoxetine (ED_{50} = 4.7 μg/kg). In contrast, a significant increase on (±)8-OH-DPAT-induced stimulation of [^{35}S]GTPγS binding was observed in CA_1 (+53.4 vs.+20.2% in vehicle) and dentate gyrus (+105.7 vs. +52.6% in vehicle) but not in entorhinal cortex. Our data demonstrate that fluoxetine-induced desensitization of 5-HT_{1A} autoreceptors occurs at G protein level. Moreover, a relevant finding is the region-specific hypersensitivity of postsynaptic 5-HT_{1A} receptors, in the hippocampus but not in entorhinal cortex, following chronic fluoxetine. These differential adaptive changes in brain 5-HT_{1A} receptors could underlie the mechanism of action of antidepressants and also contribute to their clinical effects.

Keywords: [^{35}S]GTPγS autoradiography; 5-HT_{1A} receptors; Fluoxetine; G proteins

1. Introduction

The effectiveness of the long-term administration of selective serotonin reuptake inhibitors (SSRIs) in the treatment of depression is well established. However, one of the major drawbacks is that the clinical improvement is only observed after 2–3 weeks following the initiation of treatment. This delay appears to be due to the time required for adaptive changes in 5-HT neurotransmission to occur. It has been suggested that one of such adaptations is the desensitization of 5-HT_{1A} autoreceptors (Blier and De Montigny, 1994).

5-HT_{1A} receptors are present in high densities in limbic brain areas, notably in hippocampus, lateral septum, cortical areas (particularly prefrontal and entorhinal cortex), and also in the mesencephalic raphe nuclei, both dorsal raphe nucleus (DRN) and median raphe nucleus (MRN) (Vergé et al., 1986). In DRN and MRN, the 5-HT_{1A} receptor subtype is localised on cell bodies and dendrites of serotonergic neurons where it functions as a somatodendritic autoreceptor (see Aghajanian et al., 1990); however in terminal areas, this receptor is localised postsynaptically (Vergé et al., 1986). This receptor belongs to the superfamily of G-protein-coupled receptors. It is well established that somatodendritic 5-HT_{1A} receptors cause neuronal hyperpolarization through G-protein coupled opening of K^+ channels (Innis and Aghajanian, 1987) and consequently 5-HT neuronal firing is reduced (Sprouse and Aghajanian, 1987). In addition, postsynaptic 5-HT_{1A} receptors mediate via G-proteins both neuronal hyperpolarization and inhibition of adenylate cyclase (De Vivo and Maayani, 1986; Andrade et al., 1986). In the last few years, new techniques that measure G-protein activity had become available. In this regard, the function of 5-HT_{1A} receptor can be measured by the agonist (±)8-hydroxy-2-dipropyl-aminotetralin (8-OH-DPAT) stimulated binding of the lab-

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elled GTP analogue [^{35}S]guanosine-5′-*O*-(3-thio)triphosphate ([^{35}S] GTPγS) to G-protein α–subunits to brain sections (Sim et al., 1995, 1997). The applicability of [^{35}S]GTPγS autoradiography to studies involving chronic drug treatments has previously been demonstrated in studies on the effect of chronic 5-HT_{1A} agonist (Sim-Selley et al., 2000; Hensler and Durgam, 2001).

A number of studies have analysed the effects of chronic treatment with SSRIs on 5-HT_{1A} receptors, at both pre- and postsynaptic levels. Regarding raphe 5-HT_{1A} autoreceptors, it has been shown that chronic SSRIs treatment leads to a desensitization, demonstrated by single extracellular recordings (Blier and De Montigny, 1994) and microdialysis (Invernizzi et al., 1994). The molecular nature of this modification remains unknown. With respect to the effects on postsynaptic 5-HT_{1A} receptors, contradictory results have been reported in electrophysiological (Chaput et al., 1991; Haddjeri et al., 1998, Le Poul et al., 2000) and binding assays (Le Poul et al., 2000; Hervás et al., 2001). Long-term antidepressant treatment has been shown to increase extracellular 5-HT in several brain structures including the hippocampus (Bel and Artigas, 1993). Thus, an enhancement of 5-HT neurotransmission at these postsynaptic areas could be envisaged, although only few papers reported functional evidence of this facilitation after chronic antidepressant treatment.

Therefore, it is deemed of interest to understand, using the same model, both the pre- and post-receptor changes following chronic treatment with SSRIs. In this study we report the effects of chronic treatment with fluoxetine on the stimulation of [^{35}S]GTPγS binding induced by (±)-8-OH-DPAT associated to the activation of 5-HT_{1A} receptors, at the different synaptic levels. Preliminary data from this study were presented at Society for Neuroscience 31st Meeting, San Diego, USA (Castro et al., 2001).

2. Materials and methods

2.1. Animals

Male Wistar rats weighing 200–250 g were group-housed and maintained on 12/12 h light/dark cycle, with access to food and water ad libitum. All experimental procedures were done according to the Spanish legislation and the European Communities Council Directive on ‘Protection of Animals Used in Experimental and Other Scientific Purposes’ (86/609/EEC).

2.2. Drug treatment

Rats were anaesthetised with ether and implanted with an osmotic minipump Alzet 2002 (Alza Corp., Palo Alto, CA) which delivered 0.5 μl per hour during 14 days. Six to twelve animals per group were treated for 14 days with the vehicle used to dissolve fluoxetine (50% propylenglycol, 10% ethanol and 40% distilled water) or fluoxetine HCl (10 mg/kg/day) and minipumps were removed after 14 days of treatment. Twenty-four hours later, a group of animals were either killed by decapitation for autoradiography or used in the electrophysiological experiments.

2.3. Tissue preparation

Rat brains were rapidly removed, frozen immediately in isopentane and then stored at −80 °C until sectioning. Coronal sections of 20 μm thickness were cut at −20 °C using a microtome cryostat and thaw-mounted in gelatinised slides and stored at −20 °C until use.

2.4. [^{3}H]8-OH-DPAT autoradiography

5-HT_{1A} receptor binding was determined using [^{3}H]8-OH-DPAT as radioligand (Pazos and Palacios, 1985). The sections were preincubated at room temperature for 30 min in 170 mM Tris–HCl buffer (pH 7.5) containing $CaCl_2$ 4 mM and ascorbic acid (0.01%). Sections were then incubated at room temperature for 1 h in the same buffer containing 10 μM pargyline with 2 nM [^{3}H]8-OH-DPAT. Non-specific binding was determined using 10 μM 5-hydroxytryptamine (5-HT). Following incubations, sections were washed twice for 5 min in ice-cold buffer, briefly dipped in desionised water at 4 °C, and then cold air-dried. Autoradiograms were generated by apposing the slides to [^{3}H]Hyperfilm (Amersham International, UK) with tritium labelled standards for 6 weeks at 4 °C.

2.5. [^{35}S]GTPγS autoradiography

Labelling of brain sections with [^{35}S]GTPγS was carried out as described previously (Sim et al., 1995) with some modifications. Slide-mounted sections were preincubated for 30 min at room temperature in a buffer containing 50 mM Tris–HCl, 0.2 mM EGTA, 3 mM $MgCl_2$, 100 mM NaCl, 1 mM DL-dithiothreitol and 2 mM GDP at pH 7.7. Slides were subsequently incubated, for 2 h, in the same buffer containing adenosine deaminase (3 mU/ml) with [^{35}S]GTPγS (0.04 nM) and consecutive sections were coincubated with (±)-8-OH-DPAT(10^{-4}–10^{-9} M) alone or in the presence of [*N*-[2-(4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinyl-cyclohexanecarboxamide)-maleate (WAY100635, 10^{-5} M). Non-specific binding was determined in the presence of 10 μM guanosine-5-*O*-(3-thio)triphosphate (GTPγS).

After the incubation, the sections were washed twice for 15 min in cold 50 mM Tris–HCl buffer (pH 7.4) at 4 °C, rinsed in distilled cold water and then dried under a cold air stream. Sections were exposed to autoradio-

graphic film (Hyperfilm β–max, Amersham, UK) together with ^{14}C microscales (Amersham) at 4 °C for 4 days.

2.6. Electrophysiological studies

Animals were initially anaesthetised with 400 mg kg^{-1} i.p. chloral-hydrate and maintained under full general anaesthesia with supplementary i.v. doses. The jugular vein was cannulated for drug or anaesthesia administration. Rats were mounted in a stereotaxic frame and the dura and sagital sinus removed. Single-barrelled glass micropipettes, filled with 2 M NaCl containing 2% Pontamine Sky Blue (3–8 MΩ impedance in vitro), were implanted in DRN (ML 0 mm and AP 1 mm to lambda) with the aid of a microdriver. Serotonergic cell firing in DRN was measured using standard extracellular recording methods (Aghajanian et al., 1970). 5-HT neurons, encountered over 5.6 mm DV, were identified according to their electrophysiological characteristics: wide-duration action potential (0.8–1.2 ms) and a spontaneous regular pattern of firing (0.5–2.5 Hz). Signals were amplified, filtered and the integrated firing rates were computed and stored in 10 s bins (Spike2, Cambridge Electronic Design, Cambridge, UK). The mean baseline firing rate was determined for at least 5 min before administering cumulative doses of (±)8-OH-DPAT (0.5–10 μg/kg, i.v). The inhibitory effect of each dose was followed for 1 min, quantified (spikes/10 s) and expressed as a percentage of baseline value. Only one neurone per animal was studied.

2.7. Data analysis

Autoradiograms were analysed and quantified using a computerised image analysis system (Microm-IP, Microm, Barcelona, Spain). Receptor density values (B_{max}) were calculated considering a K_d value of 2 nM for [^{3}H]8-OH-DPAT at 5-HT_{1A} receptors (Hoyer et al., 1985). Individual dose–response curves were obtained by non-linear regression analysis. The theoretical maximal effect (E_{max}) and the potency (pEC_{50}) for specific (±)-8-OH-DPAT-stimulated [^{35}S]GTPγS binding, as well as the ED_{50} in the electrophysiological studies were calculated using the program GraphPad Prism (GraphPad Software, 1998). Statistical comparison between experimental groups was made using non-paired Student *t*-test, with a level of significance set at $p < 0.05$.

2.8. Drugs

[^{3}H]8-OH-DPAT (135 Ci/mmol) and [^{35}S]GTPγS (1250 Ci/mmol) were purchased from DuPont NEN (Brussels, Belgium). DL-dithiothreitol, GDP, GTP, GTPγS and WAY100635 were obtained from Sigma-Aldrich (Madrid, Spain). (±)-8-OH-DPAT and 5-HT were obtained from RBI (Madrid, Spain). Fluoxetine-HCl was kindly donated by Lilly (Barcelona, Spain). Fluoxetine was dissolved in a mixture of 50% propylenglycol, 10% ethanol and 40% distilled water. All other chemicals used were analytical grade.

3. Results

3.1. Effect of chronic fluoxetine on the density of 5-HT_{1A} receptors

Quantitative receptor studies with 2 nM of [^{3}H]8-OH-DPAT indicated that chronic administration of fluoxetine (10 mg/kg/day) did not alter the density of pre- and postsynaptic 5-HT_{1A} receptors in any of the brain areas studied. Table 1 summarises the density values observed in those brain regions highly enriched in 5-HT_{1A} receptors (Fig. 1), in which 8-OH-DPAT-mediated binding responses were assessed (see below). In all the other brain areas analysed (cingulate, frontal and striatal cortices, thalamus and hypothalamus) no significant changes in the density of 5-HT_{1A} receptors were observed after the chronic administration of fluoxetine (data not shown).

3.2. Effect of chronic fluoxetine in (±)8-OH-DPAT stimulated [^{35}S]GTPγS binding

Hippocampus (dentate gyrus and CA_1 field), entorhinal cortex and dorsal raphe nucleus showed the highest levels of 5-HT_{1A} receptor stimulated [^{35}S]GTPγS binding, as previously reported (Sim et al., 1997). Therefore, these brain regions were selected for densitometric analysis of the changes induced in 5-HT_{1A} receptor-activated G-proteins by the chronic administration of the antidepressant drug.

Basal [^{35}S]GTPγS binding values in DRN were: 183.1 ± 11.6 nCi/g tissue in the vehicle group and 227.6 ± 4.3 nCi/g tissue in fluoxetine-treated group ($p < 0.01$). In vehicle rats, (±)8-OH-DPAT stimulated specific [^{35}S]GTPγS binding in a concentration-dependent manner, with an E_{max} of + 31.2 ± 4.2% above basal and pEC_{50} of 7.4 ± 0.3 (Table 2; Figs. 2B′ and 3A). In contrast, the (±)8-OH-DPAT-induced stimulation of specific [^{35}S]GTPγS binding in DRN was completely abolished after 14 days treatment with fluoxetine (E_{max} = 103.7 ± 3.8%, $p < 0.01$; $pEC_{50} < 4$) (Table 2; Figs. 2C′ and 3A).

In agreement with previous studies, the selective 5-HT_{1A} antagonist WAY100635 (10 μM) blocked the effect of (±)8-OH-DPAT in all brain areas (data not shown).

At the postsynaptic level, basal values of specific [^{35}S]GTPγS binding in entorhinal cortex were 175.0 ± 7.6 and 175.5 ± 16.7 nCi/g tissue in vehicle and

Table 1
Effect of chronic fluoxetine treatment on the specific [^{3}H]8-OH-DPAT binding in the rat brain. Data are expressed in B_{max} (fmol/ mg tissue)

Brain region	Vehicle $n = 6$	Fluoxetine $n = 6$
Fronto-parietal cortex		
Layers I–III	22.5±1.9	23.7±2.9
Layers IV–VI	49.9±3.0	49.2±4.4
Entorhinal cortex	137.9±11.4	109.2±7.9
Hippocampus		
CA1oriens	121.1±14.9	117.9±14.4
CA1radiatum	177.7±15.8	166.9±17.0
CA3	79.5±7.6	67.1±5.9
DG molecular	247.0±16.2	274.3±24.8
DG granular	144.4±10.7	128.3±15.7
DRN	315.9±39.4	383.6±61.4

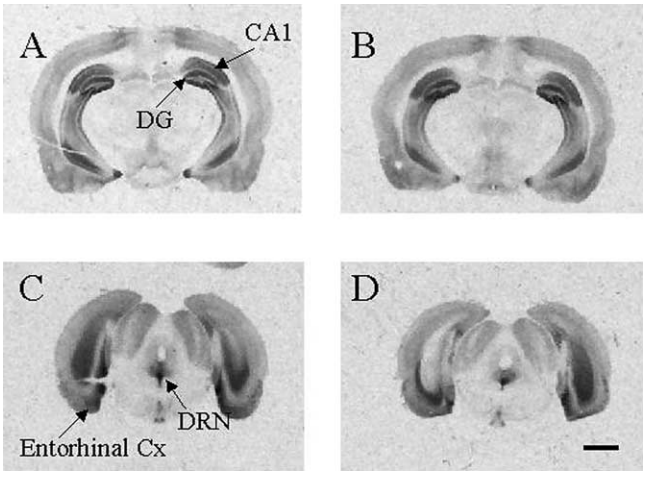

Fig. 1. Autoradiographic illustration of [^{3}H]8-OH-DPAT binding at the level of posterior hippocampus and midbrain. (A) and (C), vehicle-treated rats; (B) and (D), fluoxetine-treated rats (10 mg/kg/day, 14 days). CA1, CA1 field of hippocampus; DG, dentate gyrus; DRN, dorsal raphe nucleus. Bar = 2 mm.

fluoxetine-treated groups, respectively. In hippocampus, basal [^{35}S]GTPγS binding yielded values of 181.7 ± 9.3 nCi/g tissue in dentate gyrus and 225.2 ± 12.2 nCi/g tissue in CA_1 of Ammon's horn in the vehicle-treated group. After 14 days of treatment with fluoxetine, the basal values in these regions ranged from 136.7 ± 11.8 nCi/g tissue in dentate gyrus ($p < 0.01$) to 199.1 ± 14.9 nCi/g tissue in CA_1 field. As shown in Table 2, in vehicle rats (±)8-OH-DPAT stimulated, concentration-dependently, [^{35}S]GTPγS binding in these brain areas with E_{max} values above basal of +20.2 to +52.6% and yielding similar pEC_{50} (Figs. 2B, 2B′, 3B–D). When compared with the vehicle-treated rats, stimulation of [^{35}S]GTPγS binding by (±)8-OH-DPAT was greatly increased after chronic administration of fluoxetine in hippocampus (+105.7% in dentate gyrus, $p < 0.01$ and +53.4% in CA_1 field, $p < 0.05$) but not in entorhinal cortex (Table 2; Figs. 2C, 2C′; 3B–D).

3.3. Effect of chronic treatment with fluoxetine on the sensitivity of somatodendritic 5-HT$_{1A}$ autoreceptors

There were no significant differences between the mean firing activity of vehicle and fluoxetine-treated rats (1.5 vs.1.4 Hz, respectively). The degree of inhibition of the firing activity of 5-HT neurons induced by the acute i.v. administration of cumulative doses of (±)8-OH-DPAT (0.5–10 μg/kg) was determined. In vehicle treated rats, (±)8-OH-DPAT suppressed the neuronal firing of

Table 2
Effect of chronic fluoxetine treatment on (±)-8-OH-DPAT stimulated [^{35}S]GTPγS binding in the rat brain[a]

Region[b]	Vehicle E_{max}[c] (mean±s.e.m)	pEC_{50} (mean±s.e.m)	Fluoxetine E_{max} (mean±s.e.m)	pEC_{50} (mean±s.e.m)
DRN	131.2±4.3	7.4±0.3 (6)	103.7±3.8**[d]	<4 (6)
CA_1	120.2±3.6	7.4±0.2 (8)	153.4±8.6*[d]	6.8±0.3 (9)
DG	152.6±5.3	7.0±0.2 (11)	205.7±15.8**	7.1±0.2 (7)
Entorhinal Cx	159.9±11.1	6.9±0.1 (9)	161.9±7.8	6.9±0.4 (6)

[a] Number of animals between brackets.
[b] DRN, Dorsal raphe nucleus; CA_1, CA_1 field of hippocampus and DG, dentate gyrus.
[c] E_{max} values are expressed as a percentage of basal values (100%) of [^{35}S]GTPγS binding. $pEC_{50} = -\log EC_{50}$.
[d] $*p < 0.05$ and $**p < 0.01$ vs vehicle-treated rats.

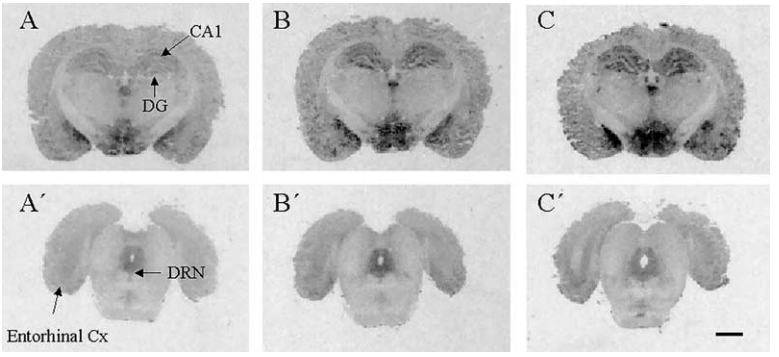

Fig. 2. Brain sections showing basal (A,A′) and (±)8-OH-DPAT (10 μM) stimulated [^{35}S]GTPγS binding in vehicle- (B,B′) and fluoxetine-treated rats (C,C′). CA1, CA1 field of hippocampus; DG, dentate gyrus; DRN, dorsal raphe nucleus. Bar = 2 mm.

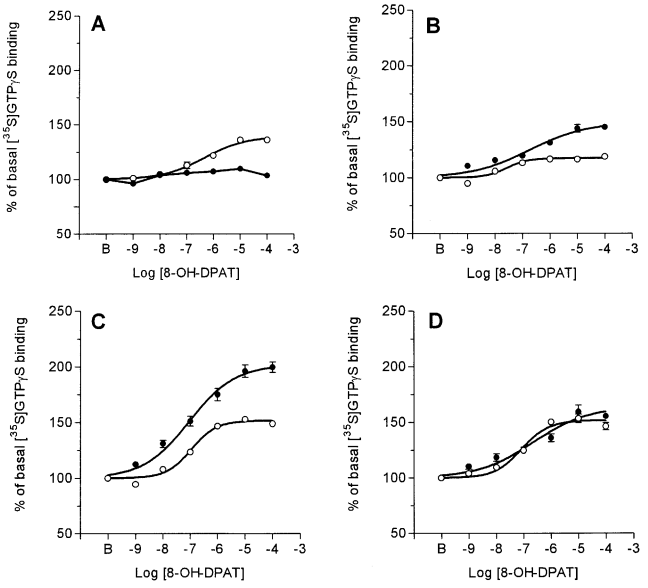

Fig. 3. Dose–response curves for (±)8-OH-DPAT stimulated [^{35}S]GTPγS binding in rats treated with vehicle (O) and fluoxetine (●). (A) Dorsal raphe nucleus; (B) CA_1 field of hippocampus; (C) dentate gyrus; and (D) entorhinal cortex. Note that chronic fluoxetine treatment totally abolished (±)8-OH-DPAT-induced stimulation of [^{35}S]GTPγS binding in DRN and increased this response in hippocampus.

dorsal raphe 5-HT neurones with an ED_{50} of 2.1 ± 0.3 μg/kg whereas, in fluoxetine-treated rats the ED_{50} value of (±)8-OH-DPAT was significantly higher (4.7 ± 0.3 μg/kg, $p < 0.01$ vs. vehicle group) (Fig. 4).

4. Discussion

We demonstrate herein that chronic treatment with the selective 5-HT reuptake inhibitor fluoxetine leads to a regionally different regulation in 5-HT_{1A} receptor-activated G-proteins. Our results show an increase in (±)8-OH-DPAT stimulated [^{35}S]GTPγS binding in hippocampus, whereas this agonist-induced response is completely abolished in DRN after 14 days treatment with fluoxetine. Interestingly, these changes at G-protein level occur with no concurrent modification in the number of either pre- or postsynaptic 5-HT_{1A} receptors. Moreover, the impaired [^{35}S]GTPγS binding response to (±)8-OH-DPAT in the DRN is paralleled with a desensitisation of the somatodendritic 5-HT_{1A} autoreceptors measured as a loss of sensitivity to the suppressant effect of (±)8-OH-DPAT upon the neuronal firing activity.

Labelling of 5-HT_{1A} receptors with the 5-HT_{1A} agonist [^{3}H]8-OH-DPAT indicated that chronic treatment with fluoxetine did not alter the density of either pre- or postsynaptic 5-HT_{1A} receptors in any of the brain areas examined. Previous studies on the regulation of 5-HT_{1A} receptors after chronic antidepressant treatment have drawn controversial results. Depending on the type and the dose of drug administered, the duration and route of administration, as well as the brain area analysed, different authors have reported no changes, up- or down-regulation of 5-HT_{1A} receptors (Klimek et al., 1994; Le Poul et al., 1995; Subhash et al., 2000). However, it can be suggested that, in good agreement with our results, the general consensus is that chronic administration of SSRIs, such as fluoxetine, is not followed by significant adaptive modifications in the density of 5-HT_{1A} receptors (Le Poul et al., 2000; Hervás et al., 2001). These results are in good correlation with most of the studies carried out in post-mortem samples from depressed patients. They have not demonstrated consistent changes in 5-HT_{1A} receptor densities (see Sargent et al., 2000), although the possible up-regulation of presynaptic receptors in the dorsal raphe nucleus is a matter of debate (Stockmeier et al., 1998; Arango et al., 2001).

An unexpected finding was that the basal values of [^{35}S]GTPγS binding in fluoxetine-treated rats were significantly different to those observed in vehicle-treated rats. At the moment we do not have a plausible explanation for this change. It could be speculated that chronic inhibition of 5-HT reuptake might also alter the basal (constitutive) activity of the different G-protein coupled receptors, not only 5-HT receptors. This observation raises an interesting question in the understanding of the exact adaptive changes after chronic SSRI treatment and needs further investigation.

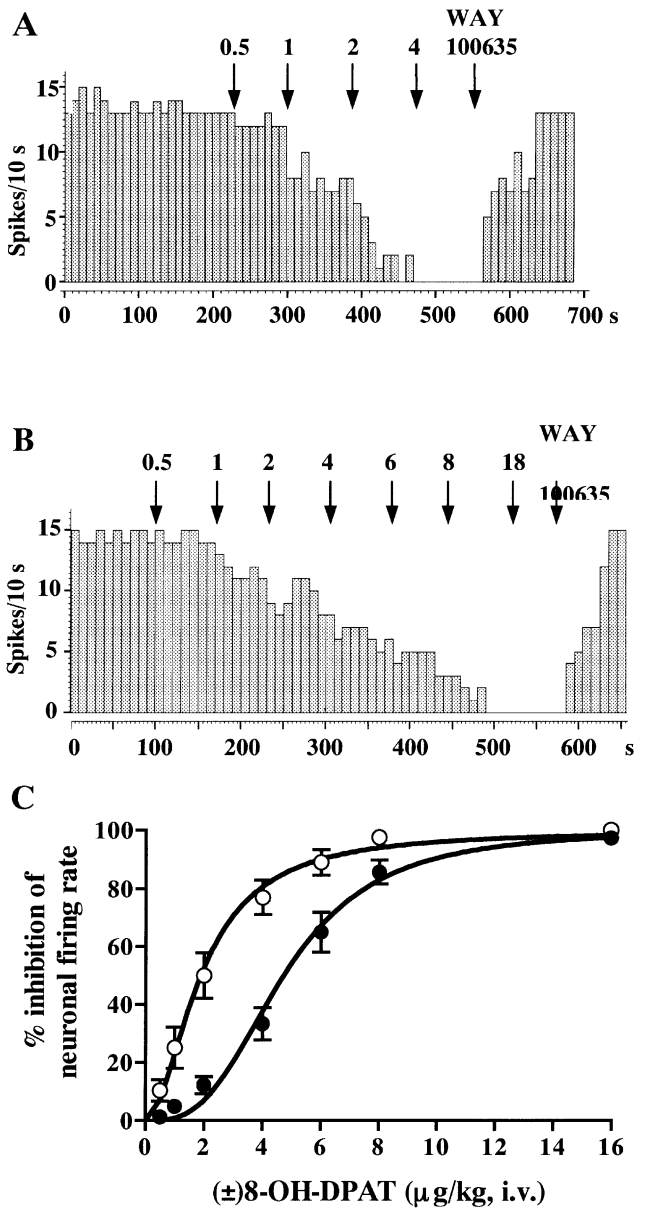

Fig. 4. Effect of (±)8-OH-DPAT in DRN neurones. Representative histograms of the inhibitory response of a single 5-HT neurone to cumulative doses of (±)8-OH-DPAT in rats treated with vehicle (A) and fluoxetine (B). Note that the selective 5-HT_{1A} antagonist WAY100635 (100 μg/kg, i.v.) completely reverted (±)8-OH-DPAT induced inhibition of 5-HT neuronal firing. (C) Dose–response curve of the inhibitory effect of (±)8-OH-DPAT upon the 5-HT neuronal firing. Note the right shift of the curve after chronic fluoxetine treatment (●) ($p < 0.01$ vs. vehicle, O).

It is noteworthy that the density of 5-HT_{1A} receptors remained unchanged in those brain areas in which the sensitivity to the 5-HT_{1A} agonist-mediated responses was altered. In DRN, chronic fluoxetine treatment did produce a clear desensitisation of somatodendritic 5-HT_{1A} autoreceptors measured using two different experimental approaches with the same design of drug administration. First, (±)8-OH-DPAT-induced stimulation of [^{35}S]GTPγS binding in DRN was totally abolished after the chronic administration of fluoxetine. Second, in agreement with previous studies, we also observed a reduced inhibitory response to (±)8-OH-DPAT upon the firing activity of serotonergic neurones. It is clear that both functional responses are 5-HT_{1A} receptor-mediated since WAY100635 antagonised (±)8-OH-DPAT-induced stimulation of [^{35}S]GTPγS binding as well as reverting the suppressant effect of (±)8-OH-DPAT in 5-HT neuronal firing. Our data demonstrate a reduction in the efficacy of the coupling of somatodendritic 5-HT_{1A} receptors to their transductional systems and confirm that G proteins are involved in the adaptive changes to antidepressant treatment. In this regard, Li et al. (1996) have found a reduction in the levels of Gi and Go proteins in rat midbrain after chronic SSRI treatment. Since it is well known that Go proteins are coupled to somatodendritic 5-HT_{1A} receptors (see Barnes and Sharp, 1999), the decrease in their levels might be related to the desensitisation observed in our electrophysiological and [^{35}S]GTPγS assays, although the implication of Gi proteins could not be ruled out.

The most outstanding finding in our study is the great increase in (±)8-OH-DPAT stimulated [^{35}S]GTPγS binding found in the hippocampus (dentate gyrus, CA_1 field), an area where the density of 5-HT_{1A} receptors remained unchanged. The consequences of chronic antidepressant treatment on the sensitivity of postsynaptic 5-HT_{1A} receptors constitute a matter of debate. Nevertheless, there is a lot of evidence, from different experimental approaches, in support of a hypersensitivity of these receptors following the administration of several drugs, including fluoxetine.

Firstly, at a transduction level, increased levels of Gi1 and Gi2 alpha proteins in the hippocampus have been reported following subchronic administration of some antidepressants (Dwivedi and Pandey, 1997). Tentatively, if this occurred after chronic fluoxetine it could be related to the hypersensitivity we observe at postsynaptic 5-HT_{1A} receptors in the hippocampus.

Secondly, previous studies in the literature, at the level of second messengers, draw inconclusive results. Some studies have shown down-regulation of 5-HT_{1A} receptor-dependent modulation of adenylate cyclase in limbic areas when assessing ex vivo the 5-HT_{1A} receptor inhibition of forskolin-stimulated cAMP after chronic SSRI treatment. Using this approach, Newman et al. (1992) and Varrault et al. (1991) have found a decrease of 5-HT_{1A} receptor-mediated inhibition and lack of effect, respectively. In contrast, it has been demonstrated that administration of (±)8-OH-DPAT to living rats resulted in an increase in extracellular hippocampal cAMP levels in vivo, as measured by microdialysis (Cadogan et al., 1994). Indeed Newman et al. (2000), using this technique, demonstrated that postsynaptic 5-HT_{1A} receptor sensitivity in the hippocampus was increased after

chronic clomipramine, thus indicating a postsynaptic 5-HT_{1A} receptor hypersensitivity in rat hippocampus after chronic SSRI administration. Moreover, studies of post-receptor elements of the cAMP pathway (PKA activity, expression of CREB, expression of BDNF and gene transcription) indicate that the net effect of antidepressant treatment is up-regulation of this second messenger system (see Duman, 1998). Thus, it is not unlikely that the hypersensitivity observed in our study could be closely related to the above phenomena.

Thirdly, the postsynaptic hypersensitivity of 5-HT_{1A} receptors that we have observed in the hippocampus can also have functional consequences. It is well known that activation of 5-HT_{1A} receptor is implicated in the neurogenesis process (Whitaker-Azmitia, 1991; Duman, 1998). Since chronic antidepressant treatment is reported to increase 5-HT_{1A} function (Duman, 1998; Haddjeri et al., 1998), it is possible that the antidepressant treatment may increase neurogenesis in part by activation of the 5-HT_{1A} receptor. In this regard, two studies (Jacobs and Fornal, 1999; Jacobs et al., 2000) have reported an increase in cell proliferation in the dentate gyrus after a 3-week treatment with fluoxetine. Interestingly, this is the hippocampal area in which we did detect a marked functional 5-HT_{1A} receptor supersensitivity. Although the participation of glucocorticoids and/or a putative mitogenic action of serotonine have been suggested, the exact receptor-mediated mechanism underlying this process remains unknown.

Finally, also at a functional level, there are several electrophysiological studies describing postsynaptic 5-HT_{1A} hypersensitivity in hippocampus after chronic antidepressant administration. Early studies with chronic clomipramine (De Montigny and Aghajanian, 1978) showed an increase in the response of forebrain neurones to 5-HT. Similar results have been found with SSRIs such as paroxetine and fluoxetine. Tokarski et al. (1996) have described a significant increase in the inhibitory effect of 5-HT and (±)8-OH-DPAT upon the amplitude of population spikes evoked in CA_1 cell layer by electrical stimulation of the stratum radiatum following 2-week treatment with paroxetine. It has also been demonstrated that long-term fluoxetine treatment increases the potency of 5-HT for the 5-HT_{1A} receptor-mediated hyperpolarisation in CA_1 (Beck et al., 1997).

In a very recent report Hensler (2002) has found an attenuation (~50%) of 5-HT_{1A} receptor-stimulated [^{35}S]GTPγS binding in rat DRN, in agreement with our results, without significant changes in postsynaptic forebrain areas. A possible explanation for the latter discrepancy could be the type of drug delivery in both studies. In the current work, since fluoxetine was given by chronic subcutaneous infusion using osmotic minipumps, it is conceivable that this type of administration may produce a more sustained chronic blockade of the 5-HT reuptake process. Although speculative, this particular pharmacokinetic feature might explain the sensitisation of the 5-HT_{1A} receptor mediated [^{35}S]GTPγS binding response we have found in dentate gyrus and CA1. Indeed, it may also account for the total abolishment that we observed in the DRN which is higher that than reported by Hensler (2002).

We found a hypersensitivity of hippocampal 5-HT_{1A} receptors, without detecting any change in the sensitivity of postsynaptic 5-HT_{1A} receptors in entorhinal cortex. These region-specific findings have also been observed by other authors. Li et al. (1996) have observed a functional desensitisation of postsynaptic 5-HT_{1A} receptors in hypothalamus after chronic SSRI and Sim-Selley et al. (2000) reported a decrease in (±)8-OH-DPAT-stimulated [^{35}S]GTPγS binding in DRN, but no change in hippocampus after chronic buspirone. Obviously this phenomenon may underlie the multiple effects of the drug on different brain functions (mood, emotion, appetite...).

In conclusion, the present data demonstrate regional specific changes in 5-HT_{1A} receptor-stimulated [^{35}S]GTPγS binding following 14 days of fluoxetine administration. Fluoxetine-induced desensitization of 5-HT_{1A} autoreceptors occurs at the G protein level. In addition, the postsynaptic hypersensitivity of 5-HT_{1A} receptors observed in the hippocampus following chronic fluoxetine treatment could contribute to the clinical effects of this drug.

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