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Additive effect of rimonabant and citalopram on extracellular serotonin levels monitored with in vivo microdialysis in rat brain

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ABSTRACT

Current pharmacological therapies for depression, including selective serotonin reuptake inhibitors (SSRI), are far from ideal. The cannabinoid system has been implicated in control of mood and neural processing of emotional information, and the modulation of serotonin (5-HT) release in the synaptic clefts. The aim of the present study was to evaluate whether the combination of a selective SSRI (citalopram) with a selective cannabinoid CB_1 receptor antagonist (rimonabant) represents a more effective strategy than the antidepressant alone to enhance serotonergic transmission. For this purpose extracellular 5-HT levels were monitored with microdialysis in forebrain (prefrontal cortex, PFC) and mesencephalic (locus coeruleus, LC) serotonergic terminal areas in freely awake rats. Rimonabant at 10 mg/kg, i.p., but not at 3 mg/kg i.p. increased 5-HT in both areas. Citalopram at 3, 5 and 10 mg/kg i.p. increased 5-HT both in PFC and LC in a dose-dependent manner. The effect of citalopram (5 mg/kg, i.p.) on 5-HT levels was significantly enhanced by rimonabant at 10 mg/kg, i.p. but not at 3 mg/kg i.p. in both areas. The present results demonstrate that the cannabinoid CB_1 receptor antagonist rimonabant is able to enhance in an additive manner the citalopram-induced increase of 5-HT concentrations in serotonergic terminal areas. The combination of a cannabinoid antagonist and a SSRI may provide a novel strategy to increase 5-HT availability, reducing the dose of SSRIs, and potentially decreasing the time lag for the clinical onset of the antidepressant effect.

1. Introduction

Serotonergic and noradrenergic systems have been classically linked to the control of emotional responses. The increase of serotonin (5-HT) and noradrenaline in the extracellular space of specific brain regions has been associated with antidepressant effects. However, there is a large number of treatment resistant patients and a delay of weeks before the onset of a therapeutic action of available drugs. It has been suggested that this delay could be due to activation of inhibitory autoreceptors by the increase of 5-HT levels in somatodendritic areas at the beginning of treatment, condition that could imply short lasting increase of 5-HT release in terminal areas as the prefrontal cortex (PFC) (Adell and Artigas, 1991; Invernizzi et al., 1992). In this context, it is of great interest to identify new agents and therapeutic strategies that might offer a faster onset of action and better efficacy in a larger proportion of patients. Several approaches are actually under investigation. Among others, combinations of antidepressants with serotonin $5\text{-}HT_{1A}$, $5\text{-}HT_2$ and $5\text{-}HT_3$ receptor or α_2-adrenoceptor antagonists have been studied on the basis that the blockage of the feedback inhibition mediated by presynaptic receptors might potentiate the antidepressant effect (Ortega et al., 2010; Rajkumar and Mahesh, 2010; Sharp et al., 2007).

Previous studies have revealed that the endocannabinoid system contributes to maintain the homeostasis of mood and emotion (Valverde, 2005). Endogenous cannabinoids act as retrograde signaling molecules to homeostatically inhibit presynaptic activity and neurotransmitter release (Diana and Marty, 2004). Several studies have provided evidence for functional cannabinoid-5-HT interactions (Egertova et al., 2003; Moldrich and Wenger, 2000). Indeed, cannabinoid CB_1 receptors are abundantly expressed in serotonergic terminal areas, such as the PFC (Moldrich and Wenger, 2000) and locus coeruleus (LC) (Herkenham et al., 1991). Furthermore, upregulation of cannabinoid CB_1 receptors has been observed in different animal models of depression (Hill et al., 2008; Rodriguez-Gaztelumendi et al., 2009), and in the neocortex of suicidal patients (Hungund et al., 2004). In addition, functional interactions between

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endocannabinoid and monoaminergic systems have been recently described in various psychiatric disorders, including major depression (Esteban and Garcia-Sevilla, 2012). Interestingly, the CB_1-cannabinoid antagonist rimonabant increases 5-HT efflux in the PFC of rats (Tzavara et al., 2003), producing antidepressant-like effects in various animal models of depression (ElBatsh et al., 2012; Griebel et al., 2005; Shearman et al., 2003).

The use of cannabinoid CB_1 receptor antagonists might represent a yet unexplored strategy for the treatment of depression, exhibiting a synergism with selective serotonin reuptake inhibitors (SSRIs). Indeed, it has been reported that co-administration of sub-threshold doses of SSRIs and cannabinoid CB_1 receptor antagonists have additive effects in forced swimming and tail suspension tests (Takahashi et al., 2008). The aim of the present study is to evaluate whether the combination of citalopram, a serotonin selective reuptake inhibitor, and rimonabant, a selective cannabinoid CB_1 receptor antagonist represents an effective strategy to enhance serotonergic transmission in the brain. For this purpose a microdialysis approach was performed for monitoring 5-HT concentrations simultaneously in PFC and LC of freely awake rats.

2. Materials and methods

2.1. Animals and drug administration

Male Sprague–Dawley rats (250–300 g) were obtained from Harlan Interfauna Ibérica, SA (Barcelona, Spain), housed in an l2 h light-dark cycle and maintained at room temperature with free access to food and water. Animal care and experimental protocols were performed in agreement with European Union Regulations (O.J. of E.C. L 358/1 18/12/1986) and approved by the UPV/EHU Ethical Board for Animal Welfare (CEBA).

Drug effects were evaluated following acute systemic administration of rimonabant (SR141716A: *N*-(Piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide hydrochloride) (i.p., diluted in vehicle: DMSO/saline 20%) (donated by Sanofi-Synthelabo, France) or 1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydro-5-isobenzofurancarbonitrile hydrobromide (citalopram) (i.p., diluted in saline) (Tocris Cookson, UK). Control animals received the same volume of vehicle or saline that treated groups.

2.2. Surgery and microdialysis procedure

Animals were anesthetized with chloral hydrate (400 mg/kg i.p.) and placed in a David Kopf stereotaxic frame. Two microdialysis probes were implanted in rat brain with the incisor bar lowered to a 15° angle, selecting coordinates according to the atlas of Paxinos and Watson (1986). One of the probes (exposed tip 2.0 mm × 0.25 mm) was implanted in the proximity of the right LC (AP −3.7, L +1.3, V −8.2, taken from the lambda suture point) and the other one (exposed tip 4.0 mm × 0.25 mm) in the ipsilateral PFC (AP +2.8, L +1, V −5, taken from Bregma). Animals recovered from surgery for approximately 20 h in their individual cages.

Around 20 h after probe implantation; rats were placed in a CMA/120 microdialysis arena (CMA Microdialysis, Solna, Sweden) for freely moving animals. Modified cerebrospinal fluid (CSF) was perfused through the microdialysis probes (148 mM NaCl, 2.7 mM KCl, 1.2 mM $CaCl_2$ and 0.85 mM $MgCl_2$; pH 7.4) at a flow rate of 1 μl/min that was constant along the experiment. After 1 h for stabilization, dialysate samples were collected every 35 min in proper vials. The initial four samples were used for estimating basal concentrations.

At the end of the experiments, animals were killed and brains were dissected for the verification of the correct intracerebral implantation of the microdialysis probes. In vitro recovery for 5-HT was in the 10–15% range.

2.3. Chromatographic analysis

5-HT concentrations were measured immediately after collection of the dialysate samples by HPLC coupled to an electrochemical detector. The mobile phase (12 mM of citric acid, 1 mM of ethylenediaminetetraacetic acid (EDTA), 0.7 mM of sodium octyl sulfate, pH = 5 and 10% methanol) was filtered, degassed (Hewlett–Packard 1100 degasser), and delivered at a flow rate of 0.2 ml/min by a Hewlett–Packard 1100 pump. Stationary phase was a column BDS-Hypersil 3 μ C18, 2 × 150 mm (Thermo Electron, USA). Samples (injection volume 30 μl) were injected and 5-HT measured by amperometric detection with a Hewlett–Packard 1049 A detector at an oxidizing potential of +650 mV. Solutions of standard 5-HT were injected every working day to create a new calibration table.

2.4. Statistical analyses

The mean value of the four initial dialysate samples was taken as the 100% value. Measures of extracellular 5-HT concentrations are expressed as percentages of the baseline values. Drugs effects were compared to their respective vehicle effects by two-way ANOVA of repeated measures followed by Bonferroni's test. In these analyses all the experimental points, including basal values, were considered. F values were expressed as F_{tr} (treatment; between-groups), F_t (time; within-groups) or F_i (treatment x time; interaction). Maximal effects (E_{max}) were expressed as percentage over basal values. The area under the curve (AUC) values were also calculated by the addition of the percent change from baseline over the entire period after drug treatment. One-way ANOVA followed by Bonferroni's test was used to compare the AUC of different groups of treatment. Results are expressed as mean ± S.E.M. values. All statistical procedures were performed using GrahPad Prism™ (GraphPad Sofware, San Diego, CA, USA).

3. Results

3.1. Steady-state 5-HT levels

Basal 5-HT levels were 0.82 ± 0.09 nM in the PFC ($n = 76$) and 0.53 ± 0.03 nM in the LC ($n = 77$). No differences were found between different treated groups.

3.2. Effect of systemic rimonabant (3 and 10 mg/kg, i.p.) administration on 5-HT levels monitored in PFC and LC

3.2.1. PFC

In PFC, rimonabant increased 5-HT levels only following 10 mg/kg i.p. ($E_{max} = 187 \pm 8\%$, $P < 0.001$; vs vehicle) (3 mg/kg i.p., $E_{max} = 113 \pm 9\%$, $P > 0.05$; vs vehicle) (Fig. 1A; Table 1). One-way ANOVA of 5-HT outflow measured from AUC values (280 min post-treatment period) revealed significant effects of the treatment ($F[2,14] = 18.61$; $P < 0.0001$) at the 10 mg/kg dose (Fig. 1B).

3.2.2. LC

Rimonabant increased 5-HT levels in LC following 10 mg/kg i.p. administration ($E_{max} = 214 \pm 38\%$, $P < 0.001$; vs vehicle), but not at 3 mg/kg i.p. ($E_{max} = 119 \pm 24\%$, $P > 0.05$; vs vehicle) (Fig. 1C; Table 1). The effect of rimonabant returned rapidly to basal values after 140 min. One-way ANOVA of AUC values (280 min post-treatment period) also showed significant effects of the treatment ($F[2,18] = 4.26$; $P < 0.05$) at 10 mg/kg (Fig. 1D).

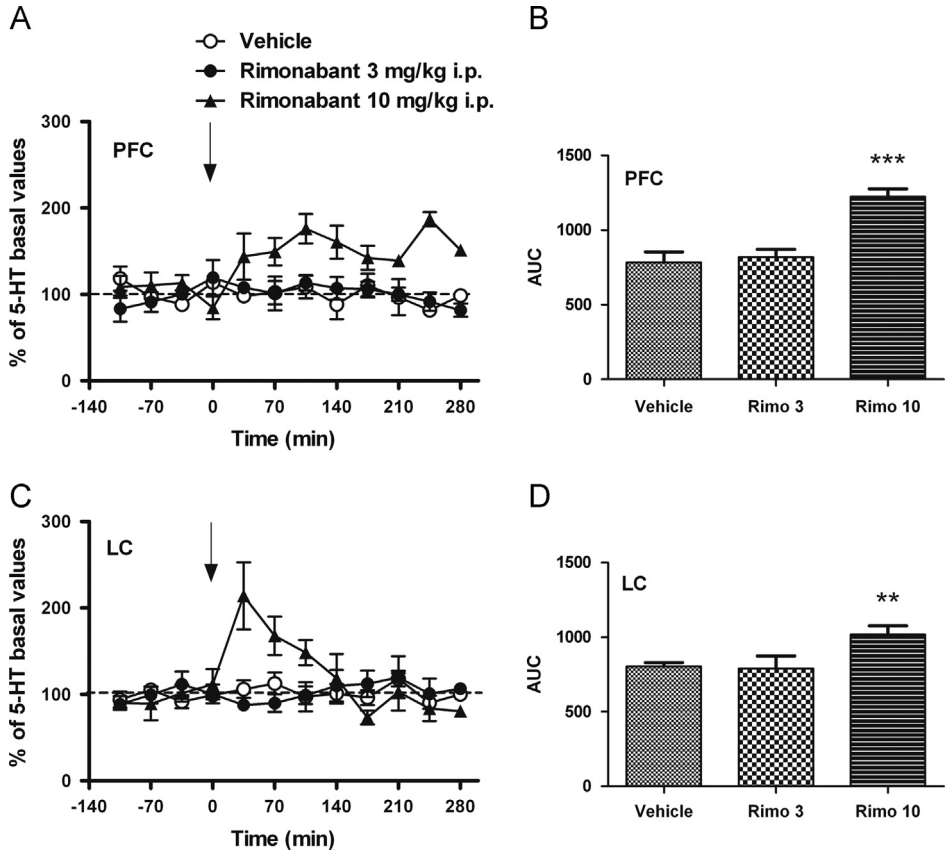

Fig. 1. Effect of systemic administration of rimonabant on extracellular 5-HT levels simultaneously monitored in PFC (A) and LC (C) of rats. Data are mean ± S.E.M. values from 5 to 6 experiments, expressed as percentages of the corresponding basal values. The arrow represents administration of the drug or vehicle (0.5 ml/kg). (B and D) Raw area under each 280-min time course curve. $^{**}P < 0.001$; $^{***}P < 0.0001$ compared with vehicle (one-way ANOVA followed Bonferroni's test).

Table 1
Maximal effects (E_{max}) observed after administration of different doses of rimonabant or citalopram. Data are expressed as the percentage (%) over their respective 5-HT basal levels. *F* values are for data obtained from the comparison of each treatment with their respective control group (vehicle administration) by two-way ANOVA of repeated measures. *F* values are expressed as F_{tr} (treatment; between-groups), F_t (time; within-groups) or F_i (treatment × time; interaction).

	PFC		LC	
Rimonabant				
3 mg/kg i.p.	$E_{max}=113\pm9\%$	$F_{tr}[1,9]=0.002$, $P=0.98$; $n=11$ $F_t[11,99]=1.18$, $P=0.30$; $n=11$ $F_i[11,99]=0.82$, $P=0.61$; $n=11$	$E_{max}=120\pm24\%$	$F_{tr}[1,9]==0.018$, $P=0.89$; $n=11$ $F_t[11,99]=0.83$, $P=0.60$; $n=11$ $F_i[11,99]=0.73$, $P=0.70$; $n=11$
10 mg/kg i.p.	$E_{max}=187\pm8\%$	$F_{tr}[1,9]=28.64$, $P<0.001$; $n=11$ $F_t[11,99]=2.01$, $P<0.05$; $n=11$ $F_i[11,99]=3.81$, $P<0.001$; $n=11$	$E_{max}=214\pm38\%$	$F_{tr}[1,8]=3.23$, $P=0.10$; $n=10$ $F_t[11,88]=3.50$, $P<0.001$; $n=10$ $F_i[11,88]=3.50$, $P<0.001$; $n=10$
Citalopram				
3 mg/kg i.p.	$E_{max}=199\pm28\%$	$F_{tr}[1,17]=14.46$, $P<0.01$; $n=19$ $F_t[10,170]=2.64$, $P<0.01$; $n=19$ $F_i[10,170]=1.97$, $P<0.05$; $n=19$	$E_{max}=169\pm8\%$	$F_{tr}[1,12]=51.79$, $P<0.0001$; $n=14$ $F_t[10,120]=5.824$, $P<0.0001$; $n=14$ $F_i[10,120]=7.97$, $P<0.0001$; $n=14$
5 mg/kg i.p.	$E_{max}=362\pm44\%$,	$F_{tr}[1,15]=22.20$, $P<0.001$; $n=17$ $F_t[10,150]=8.45$, $P<0.0001$; $n=17$ $F_i[10,150]=8.25$, $P<0.0001$; $n=17$	$E_{max}=328\pm36\%$	$F_{tr}[1,20]=104.5$, $P<0.0001$; $n=22$ $F_t[10,200]=12.11$, $P<0.0001$; $n=22$ $F_i[10,200]=12.74$, $P<0.0001$; $n=22$
10 mg/kg i.p.	$E_{max}=927\pm293\%$	$F_{tr}[1,9]=93.67$, $P<0.0001$; $n=11$ $F_t[10,90]=4.59$, $P<0.0001$; $n=11$ $F_i[10,90]=4.46$, $P<0.0001$; $n=11$	$E_{max}=945\pm195\%$	$F_{tr}[1,10]=51.58$, $P<0.0001$; $n=12$ $F_t[10,100]=12.09$, $P<0.0001$; $n=12$ $F_i[10,100]=12.31$, $P<0.0001$; $n=12$

3.3. Effect of systemic citalopram (3, 5 and 10 mg/kg, i.p.) administration on extracellular 5-HT levels monitored in PFC and LC

3.3.1. PFC

In PFC, citalopram increased extracellular 5-HT levels in a dose-dependent manner, reaching the highest effect after 10 mg/kg i.p. of citalopram (3 mg/kg i.p., $E_{max}=199\pm28\%$, $P<0.01$; vs saline; 5 mg/kg i.p., $E_{max}=362\pm44\%$, $P<0.001$; vs saline; 10 mg/kg i.p., $E_{max}=927\pm293\%$, $P<0.001$; vs saline) (Fig. 2A; Table 1). The comparison of AUC values measured during the 245 min post-treatment period by one-way ANOVA revealed significant dose-dependent effects of citalopram treatment ($F[3,31]=67.22$; $P<0.0001$) (Fig. 2B).

3.3.2. LC

Citalopram increased extracellular 5-HT levels in LC in a dose-dependent manner, reaching the highest effect after 10 mg/kg i.p.

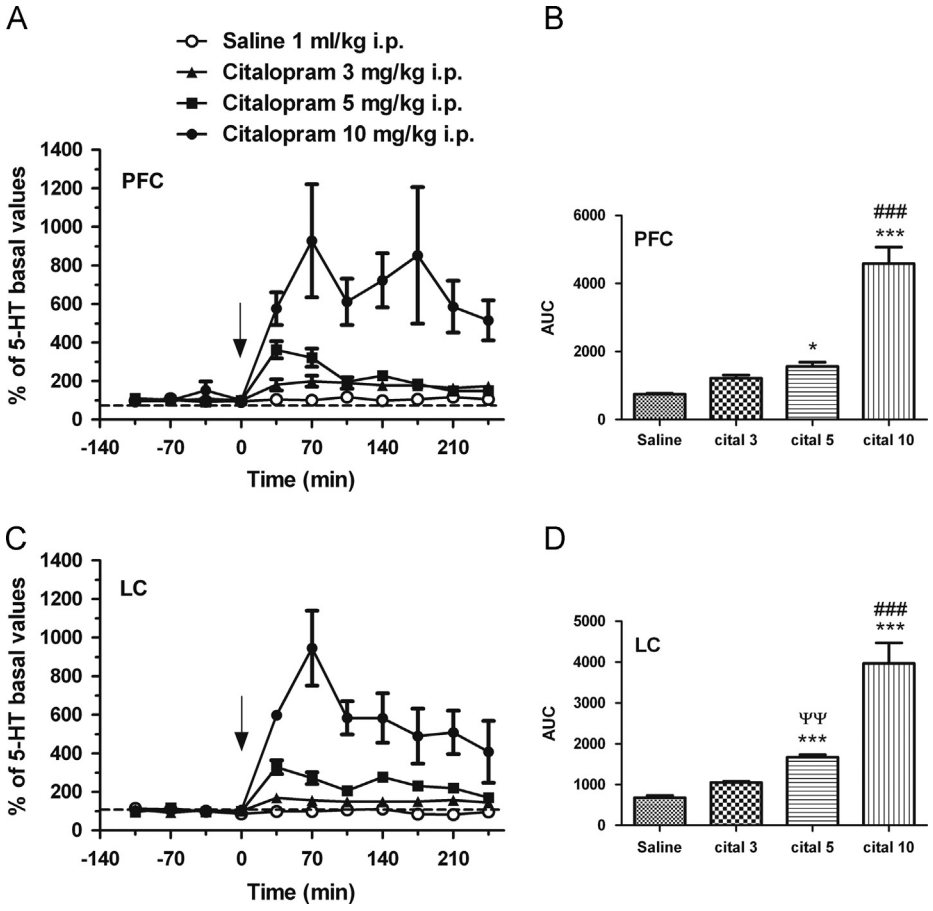

Fig. 2. Effects of systemic citalopram administration on extracellular 5-HT levels simultaneously monitored in PFC (A) and LC (C) of rats. Data are mean ± S.E.M. values from 5 to 15 experiments, expressed as percentages of the corresponding basal values. The arrow indicates administration of citalopram or saline (1 ml/kg). (B and D) Raw area under each 245-min time course curve. * $P<0.05$, *** $P<0.0001$ compared with saline; $^{\Psi\Psi}$ $P<0.001$ compared with citalopram 3; $^{\#\#\#}$ compared with citalopram 5 (one-way ANOVA followed Bonferroni's test).

(3 mg/kg i.p., $E_{max}=169\pm8\%$, $P<0.001$; vs saline; 5 mg/kg i.p., $E_{max}=328\pm36\%$, $P<0.001$; vs saline; 10 mg/kg i.p., $E_{max}=945\pm195\%$, $P<0.001$; vs saline) (Fig. 2C; Table 1). As observed in the PFC, one-way ANOVA of AUC values measured during the 245 min post-treatment period revealed significant dose-dependent effects of citalopram treatment in the LC ($F[3,34]=68.99$; $P<0.0001$) (Fig. 2D).

3.4. Effect of systemic rimonabant administration in citalopram pretreated animals on extracellular 5-HT levels evaluated in PFC and LC

In order to evaluate the effect of rimonabant (3 or 10 mg/kg i.p.) administration on citalopram-treated rats (5 mg/kg i.p.), the CB_1-cannabinoid antagonist was administered 105 min after the administration of the SSRI drug.

3.4.1. PFC

The administration of a subtreshold dose of rimonabant (3 mg/kg i.p.) 105 min after citalopram administration (5 mg/kg i.p.) did not significantly enhance or prolong the effect of the antidepressant in the PFC (Fig. 3A and B). Two-way ANOVA did not reveal differences between citalopram (5 mg/kg)+rimonabant (3 mg/kg) versus citalopram (5 mg/kg)+vehicle administration ($F_{tr}[1,14]=0.038$, $P=0.84$; $F_t[11,154]=12.19$, $P<0.0001$; $F_i[11,154]=1.54$, $P=0.12$; $n=16$) (Fig. 3A). However, rimonabant (10 mg/kg) administered 105 min after citalopram (5 mg/kg) increased 5-HT levels in the PFC (Fig. 3A and B). The effect reached a maximum of 649 ± 37% ($P<0.001$), approximately 140 min after citalopram administration (vs E_{max} of citalopram+vehicle = 247 ± 33%) ($F_{tr}[1,16]=12.62$, $P<0.01$; $F_t[11,176]=22.40$, $P<0.0001$; $F_i[11,176]=8.45$, $P<0.0001$; $n=18$) (Fig. 3A). The effect of rimonabant in citalopram pretreated animals returned to control values 70 min after acute cannabinoid CB_1 receptor antagonist administration. In agreement, one-way ANOVA of AUC values (280 min post-treatment period) revealed significant differences between the treatment conditions ($F[3,31]=28.24$; $P<0.0001$). Thus, Bonferroni's posthoc test showed statistical differences between citalopram+vehicle vs citalopram+rimonabant 10 mg/kg ($t=3.68$; $P<0.001$) but not between citalopram+vehicle vs citalopram+rimonabant 3 mg/kg ($t=0.02$; $P>0.05$) (Fig. 3B).

3.4.2. LC

As observed in the PFC, the administration of rimonabant (3 mg/kg i.p.) after citalopram (5 mg/kg i.p.) administration did not significantly enhance the effect of the antidepressant in the LC (Fig. 3C and D). Two-way ANOVA did not reveal differences between citalopram (5 mg/kg)+rimonabant (3 mg/kg) versus citalopram (5 mg/kg)+vehicle administration ($F_{tr}[1,19]=0.69$, $P=0.41$; $n=15$; $F_t[11,209]=20.42$, $P<0.001$; $n=15$; $F_i[11,209]=1.01$, $P=0.43$; $n=21$) (Fig. 3C). Again, rimonabant (10 mg/kg) administered 105 min after citalopram (5 mg/kg) increased 5-HT levels in the LC (Fig. 3C and D). The effect reached a maximum of 780 ± 250% ($P<0.001$) approximately 200 min after citalopram administration (vs E_{max} of citalopram+vehicle = 257 ± 35%) ($F_{tr}[1,18]=5.91$, $P<0.05$; $n=15$; $F_t[11,198]=10.51$, $P<0.0001$; $n=15$; $F_i[11,198]=9.07$,

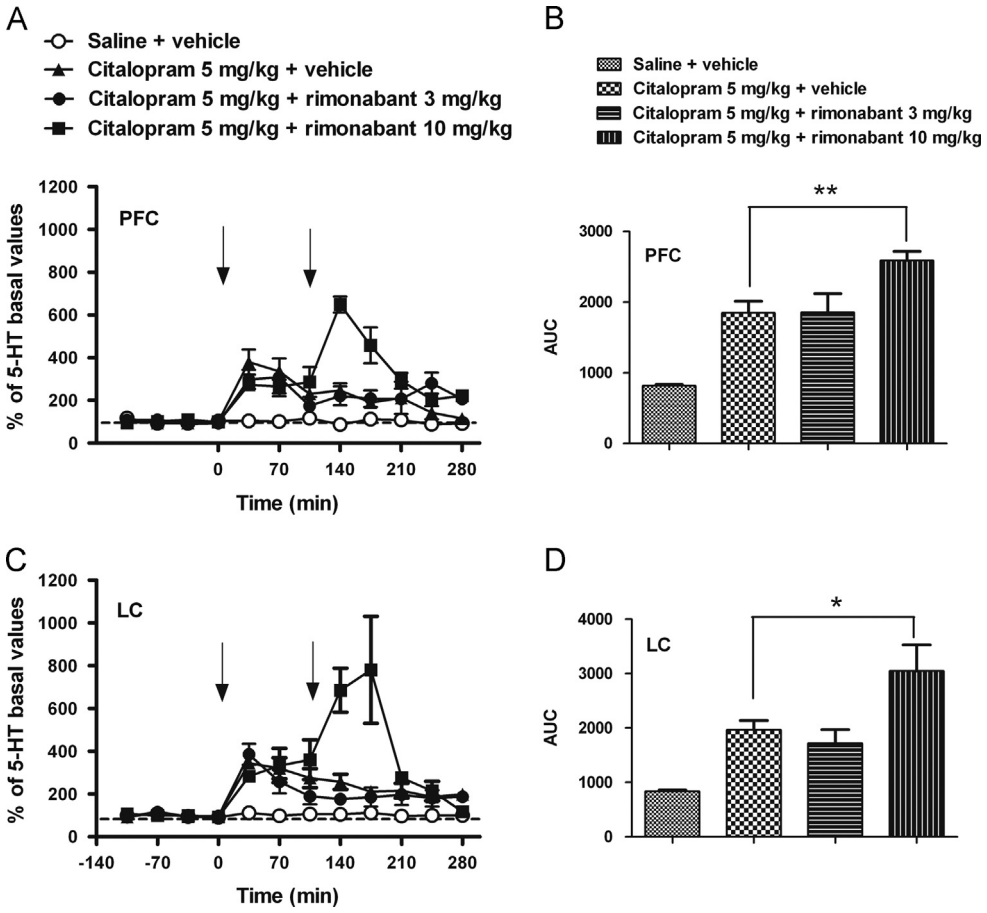

Fig. 3. Effects of systemic citalopram administration on extracellular 5-HT levels simultaneously monitored in PFC (A) and LC (C). Data are mean ± S.E.M. values from 6 to 13 experiments, expressed as percentages of the corresponding basal values. First arrow shows the time of administration of citalopram 5 mg/kg i.p. or saline (1 ml/kg). Second arrow shows administration of rimonabant (3 mg/kg and 10 mg/kg) or vehicle (0.5 ml/kg). (B and D) Raw area under each 280-min time course curve. $^{*}P<0.05$, $^{**}P<0.001$ (one-way ANOVA followed Bonferroni's test).

$P<0.0001$; $n=20$) (Fig. 3C). The effect of rimonabant in citalopram pretreated animals returned to control values 70 min after acute cannabinoid CB_1 receptor antagonist administration. One-way ANOVA of AUC values calculated during the 280 min post-treatment period revealed significant effects of the different treatment conditions ($F[3,36]=14.92$; $P<0.0001$). The Bonferroni's posthoc test showed statistical differences between citalopram+vehicle vs citalopram+rimonabant 10 mg/kg ($t=3.26$; $P<0.05$) but not for citalopram+vehicle vs citalopram+rimonabant 3 mg/kg ($t=0.77$; $P>0.05$) (Fig. 3D).

4. Discussion

The present study shows that the cannabinoid CB_1 receptor antagonist rimonabant increases 5-HT levels in PFC and in LC areas and thus demonstrates that there is an inhibitory feedback control of the endocannabinoid system on 5-HT release, both in brainstem and forebrain areas. Complementary, the SSRI citalopram exerts a dose-dependent increase of 5-HT levels in both PFC and LC. The combination of citalopram and rimonabant enhances the increase of 5-HT elicited by the antidepressant alone in both studied brain areas. Thus, the blockade of the cannabinoid CB_1 receptor-mediated inhibitory tone is able to increase the acute citalopram effect on extracellular 5-HT levels.

There are contrasting results pertaining to cannabinoid CB_1 receptor-mediated neurotransmitter release. Doses and brain regions evaluated are important contributing factors to this discrepancy. Nevertheless, an excitatory role mediated by cannabinoid CB_1 receptor antagonism has been demonstrated on different neurotransmitter systems by several in vivo studies. Thus, it has been reported that rimonabant administration increases noradrenaline, dopamine and 5-HT in different brain areas (Tzavara et al., 2001, 2003). Additionally, the genetic and/or the pharmacological blockade of cannabinoid CB_1 receptors increase extracellular 5-HT levels in PFC (Aso et al., 2009). It has also been reported that administration of the cannabinoid CB_1 receptor agonist delta-9-tetrahydrocannabinol decreases 5-HT release in nucleus accumbens, hippocampus and cortex (Egashira et al., 2002; Nakazi et al., 2000; Sano et al., 2008). In agreement, the synthetic agonist WIN55,212-2 is able to suppress the effect of citalopram on 5-HT release in PFC (Kleijn et al., 2011). Surprisingly, opposite effects have also been shown. Indeed, both blockade and increase of endocannabinoid tone have been shown to enhance the firing activity of serotonergic neurones in dorsal raphe nucleus (Bambico et al., 2007; Gobbi et al., 2005; Mendiguren and Pineda, 2009). In addition, antidepressant-like behavioral properties of both delta-9-tetrahydrocannabinol and rimonabant in an animal model of depression (olfactory bulbectomised rats) have been recently demonstrated (ElBatsh et al., 2012). Biphasic effects of cannabinoids on different neurotransmitter release related to the expression of cannabinoid CB_1 receptors on different subpopulations of neurones could explain this apparent discrepancy. Thus, it has been reported that brain cannabinoid CB_1 receptors are localized

on monoaminergic neurones (Lau and Schloss, 2008; Oropeza et al., 2007), but also on glutamatergic and GABAergic neurones (Katona et al., 2000; Robbe et al., 2001), controlling monoaminergic tone. A direct action of rimonabant on presynaptic cannabinoid CB_1 receptors located on serotonergic terminals of PFC and LC seems to be a plausible mechanism of action of rimonabant on 5-HT transmission. However, the effects could also be indirect, involving interneurones supplying an input to serotonergic terminals.

The classical hypothesis of depression is linked to reduced 5-HT activity. SSRIs enhance 5-HT availability in the synaptic cleft and this pharmacological mechanism could contribute to their therapeutical antidepressant activity. The present findings could suggest a putative antidepressant activity mediated by blockade of cannabinoid CB_1 receptors. The hypothesis is supported by evidence showing that chronic exposure to cannabinoid agonists is associated with mood disorders, including anxiety and depression-like symptoms in rats (Pattij et al., 2008; Schneider et al., 2008). In agreement with behavioral experiments, neurochemical studies have shown that administration of the cannabinoid CB_1 receptor inverse agonist AM251 reduces immobility in the tail suspension and forced swimming tests, effects also produced by antidepressant drugs (Griebel et al., 2005).

At the beginning of the treatment with citalopram and other SSRIs, an autoinhibitory effect mediated by somatodendritic receptors seems to limit the release of 5-HT by nerve terminals (Adell and Artigas, 1991; Invernizzi et al., 1992). In this context, the blockage of cannabinoid CB_1 receptors during the initial phases of SSRI treatment could contribute to overcome this undesirable effect. According to the proposed hypothesis, experiments were designed to evaluate whether the citalopram and rimonabant associated treatment is able to generate an additive or even a potentiation effect on 5-HT release under acute administration when compared to single citalopram administration. The dose-dependent responses produced by systemic citalopram were evaluated in two serotonergic terminal areas. These two areas were selected based on their main role in depressive disorders and antidepressant mechanisms. PFC is a complex collection of neuronal systems with extensive afferents from serotonergic and noradrenergic neurones (Lindvall et al., 1978; Van Bockstaele et al., 1993). The LC is the major noradrenergic cell-body group in the brain and represents the most important source of noradrenergic inputs to the PFC (Levitt and Moore, 1978). The treatment schedule, with citalopram as the first drug to be administered, was designed on the basis of previous findings for serotonergic and noradrenergic systems (Kleijn et al., 2011; Ortega et al., 2010; Taber et al., 2000). Rimonabant was administered 105 min after citalopram for evaluating the role of cannabinoid CB_1 receptor antagonism on already released endocannabinoids, assuming an inhibitory basal effect on 5-HT levels produced by cannabinoid CB_1 receptor activation. For testing the hypothesis of a cannabinoid CB_1 modulation, citalopram had to be administered at low doses, making possible to assess an inhibitory tone by endogenous CB_1 modulation that could be antagonized by a pharmacological treatment, leading to a further increase of 5-HT levels. Indeed, a too high increase of 5-HT levels produced by high doses of citalopram would be out of the control provided by a cannabinoid-dependent inhibitory feedback. Thus, taking this into account, a dose of citalopram (5 mg/kg i.p.) was chosen. Rimonabant at a sub-threshold dose (3 mg/kg, i.p.) did not modify the citalopram-induced increase of 5-HT, but it did it at the dose of 10 mg/kg i.p., both in PFC and LC. In PFC, when maximal effects of 10 mg/kg of rimonabant alone (1.87 fold increase over vehicle; Fig. 1) or in combination with citalopram (6.49 fold increase over saline+vehicle; Fig. 3) were compared, a 3.47-fold increase was obtained, a value very similar to that observed after single citalopram administration (3.62-fold increase over saline; Fig. 2) suggesting additive effects of both drugs. Very similar conclusions could be obtained for the LC area. According to the present data, additive effects following co-administration of antidepressants and cannabinoid CB_1 receptor antagonists in the forced swimming- and tail suspension tests have also been demonstrated (Takahashi et al., 2008). The additive effect on 5-HT concentrations observed after acute administration was transient in the two areas studied. The pharmacokinetic properties of rimonabant could differentiate the effect after acute or repeated administration. Thus, due to its accumulation in adipose tissue (Barna et al., 2009), chronic combinational effects could result in a more prolonged effect after co-administration of both drugs, supporting the mechanism proposed in the present work.

Rimonabant has been clinically used for the treatment of obesity and associated metabolic dysregulation. However, license of rimonabant was withdrawn both in North America and Europe, principally due to its occasionally association with negative affective symptoms and suicidality (Christensen et al., 2007; Després et al., 2005; Van Gaal et al., 2008). There are several reasons that could explain the adverse effects observed. It is possible that long-term treatment with rimonabant may have some impact especially if it accumulates in fatty tissues, such as the brain. Moreover, it has been reported that rimonabant elicits a significant agonist response via GPR55 (Henstridge et al., 2010) a recently de-orphanized G protein-coupled receptor activated by the endogenous lipid signaling molecule L-α-lysophosphatidylinositol (LPI). Additionally, a functionally active pharmacological target other than the cannabinoid CB_1 receptor orthosteric binding site has been demonstrated for rimonabant but not for other CB_1 receptor antagonists in human brain (Erdozain et al., 2012). Therefore, non-cannabinoid CB_1 actions might contribute to the adverse effects observed after chronic treatment with rimonabant, especially if it accumulates in brain tissue. The use of low doses or short-term treatments of rimonabant along the first stage of antidepressant therapy could lack of the adverse effects derived from high brain rimonabant levels after prolonged use in anti-obesity treatments. Recently, development of anti-obesity drugs targeting cannabinoid CB_1 receptors has been relaunched. In this sense, new neutral cannabinoid CB_1 receptors antagonists have been shown to potentially lack of adverse effects in anxiety and depression models (Kirilly et al., 2012; McLaughlin, 2012). Thus, a large number of possibilities to develop new antidepressant treatments based on combinations of different neutral cannabinoid CB_1 receptors antagonists in addition to SSRIs may be possible.

Taken together, these findings suggest that cannabinoid CB_1 receptor antagonism could play a role to overcome the delay in the development of therapeutic activity of the current antidepressant treatments based on the enhancement of 5-HT levels in terminal areas. It is sound to assume that cannabinoid CB_1 antagonists would have their main action associated to SSRIs during a short-term period at the beginning of the antidepressant treatment. In order to evaluate a putative hasten clinical response to SSRIs, the timing window for the onset of antidepressant-like properties of citalopram alone or in combination with a cannabinoid CB_1 antagonist needs to be compared.

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