European Journal of Pharmacology 709 (2013) 13–19

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Behavioural pharmacology

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

Jorge Emilio Ortega [a,b,d,*], Victor Gonzalez-Lira [c], Igor Horrillo [a,b], Mario Herrera-Marschitz [c], Luis Felipe Callado [a,b,d], J. Javier Meana [a,b,d]

[a] Department of Pharmacology, University of the Basque Country UPV/EHU, Spain
[b] Centro de Investigación Biomédica en Red de Salud Mental, CIBERSAM, Spain
[c] Millenium Institute BNI and Programme of Molecular and Clinical Pharmacology, Faculty of Medicine, University of Chile, Chile
[d] BioCruces Health Research Institute, Spain

ARTICLE INFO

Article history:
Received 22 January 2013
Received in revised form
13 March 2013
Accepted 24 March 2013
Available online 3 April 2013

Keywords:
Rimonabant
Citalopram
Microdialysis
Serotonin
Locus coeruleus
Prefrontal cortex

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

* Corresponding author at: Department of Pharmacology, University of the Basque Country (UPV/EHU), 48940 Leioa, Bizkaia, Spain. Tel.: +34 946015674; fax: +34 946013220.
E-mail address: jorge.ortega@ehu.es (J.E. Ortega).

0014-2999/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ejphar.2013.03.043

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).