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Chronic citalopram administration desensitizes prefrontal cortex but not somatodendritic α_2-adrenoceptors in rat brain

Begoña Fernández-Pastor [a, 1], Jorge E. Ortega [a, b, d, *], Laura Grandoso [a, 2], Elena Castro [b, c], Luisa Ugedo [a], Ángel Pazos [b, c], J. Javier Meana [a, b, d]

[a] Department of Pharmacology, University of the Basque Country UPV/EHU, Leioa, Bizkaia, Spain
[b] Centro de Investigación Biomédica en Red de Salud Mental CIBERSAM, Spain
[c] Department of Physiology and Pharmacology, Institute of Biomedicine & Biotechnology of Cantabria (IBBTEC), University of Cantabria-CSIC-IDICAN, Santander, Cantabria, Spain
[d] BioCruces Health Research Institute, Bizkaia, Spain

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ABSTRACT

Selective serotonin reuptake inhibitors (SSRIs) regulate brain noradrenergic neurotransmission both at somatodendritic and nerve terminal areas. Previous studies have demonstrated that noradrenaline (NA) reuptake inhibitors are able to desensitize α_2-adrenoceptor-mediated responses. The present study was undertaken to elucidate the effects of repeated treatment with the SSRI citalopram on the α_2-adrenoceptor sensitivity in locus coeruleus (LC) and prefrontal cortex (PFC), by using *in vivo* microdialysis and electrophysiological techniques, and *in vitro* stimulation of [^{35}S]GTPγS binding autoradiography. Repeated, but not acute, treatment with citalopram (5 mg/kg, i.p., 14 days) increased extracellular NA concentration selectively in PFC. The α_2-adrenoceptor agonist clonidine (0.3 mg/kg, i.p.), administered to saline-treated animals (1 ml/kg i.p., 14 days) induced NA decrease in LC ($E_{max} = -44 \pm 4\%$; $p < 0.001$) and in PFC ($E_{max} = -61 \pm 5\%$, $p < 0.001$). In citalopram chronically-treated rats, clonidine administration exerted a lower decrease of NA ($E_{max} = -25 \pm 7\%$; $p < 0.001$) in PFC whereas the effect in LC was not different to controls ($E_{max} = -36 \pm 4\%$). Clonidine administration (0.625–20 μg/kg, i.v.) evoked a dose-dependent decrease of the firing activity of LC noradrenergic neurons in both citalopram-($ED_{50} = 3.2 \pm 0.4$ μg/kg) and saline-treated groups ($ED_{50} = 2.6 \pm 0.5$ μg/kg). No significant differences between groups were found in ED_{50} values. The α_2-adrenoceptor agonist UK14304 stimulated specific [^{35}S]GTPγS binding in brain sections containing LC (144 ± 14%) and PFC (194 ± 32%) of saline-treated animals. In citalopram-treated animals, this increase did not differ from controls in LC (146 ± 22%) but was lower in PFC (141 ± 8%; $p < 0.05$). Taken together, long-term citalopram treatment induces a desensitization of α_2-adrenoceptors acting as axon terminal autoreceptors in PFC without changes in somatodendritic α_2-adrenoceptor sensitivity.

1. Introduction

Most antidepressant drugs exert their pharmacological activity through modulation of monoaminergic systems. Brainstem noradrenergic neurons of locus coeruleus (LC), the main source of noradrenergic innervation in the brain, are thought to be involved in the pathophysiology of depression (Ressler and Nemeroff, 1999). In the LC, α_2-adrenoceptors exert an inhibitory role on somatodendritic noradrenaline (NA) release (Callado and Stamford, 1999; Mateo et al., 1998). In terminal noradrenergic areas, concentration of synaptic NA is under negative regulation of two different α_2-adrenoceptor populations, the α_2-adrenoceptors located in the LC controlling as autoreceptors the noradrenergic firing activity (Mateo et al., 1998; Van Gaalen et al., 1997) and the α_2-adrenoceptors located in terminals where they modulate NA release (Dalley and Stanford, 1995; van Veldhuizen et al., 1993). In this

Abbreviations: LC, Locus coeruleus; NA, Noradrenaline; NARI, NA reuptake inhibitor; NET, NA transporter; PFC, Prefrontal cortex; SERT, Serotonin transporter; SSRI, Selective serotonin reuptake inhibitor.

* Corresponding author. Department of Pharmacology, University of the Basque Country UPV/EHU, E-48940 Leioa, Bizkaia, Spain.
E-mail address: jorge.ortega@ehu.eus (J.E. Ortega).
[1] Present address: Laboratorios Dr. Esteve, Barcelona, Spain.
[2] Present address: Progenika Biopharma, Derio, Bizkaia, Spain.

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sense, it has been previously described that local administration into the LC of the NA reuptake inhibitor (NARI) antidepressant desipramine increases NA in the area and induces a decrease of noradrenergic firing activity trough α_2-adrenoceptor activation, leading to a subsequent decrease of NA release in the prefrontal cortex (PFC) (Mateo et al., 1998).

Early concepts suggested that the selective regulation of serotonergic function accounts for the antidepressant effect of selective serotonin reuptake inhibitors (SSRIs). However, reciprocal anatomo-physiological interactions between noradrenergic and serotonergic systems make possible that even if their pharmacological activity is mediated by inhibition of serotonin reuptake, SSRIs might also act through modulation of the brain noradrenergic system. Thus, it has been described a confluence between noradrenergic and serotonergic systems in the LC (Cedarbaum and Aghajanian, 1978; Maeda et al., 1991) and in terminal areas, such as the PFC. It has been previously described that endogenous serotonin release in the LC is able to regulate NA in the area (Singewald and Philippu, 1998) and, subsequently, modulates noradrenergic firing activity (Mateo et al., 2000). Acute administration of the SSRI citalopram induces a dose-dependent increase of serotonin synaptic concentrations in both LC and PFC areas (Millan et al., 1999; Ortega et al., 2013). In accordance, acute administration of the SSRIs citalopram and paroxetine enhances NA in the LC but decreases the firing activity of LC neurons and the NA release in terminal areas (Fernández-Pastor et al., 2013; Mateo et al., 2000; Szabo et al., 1999). This inhibitory effect of SSRIs involves α_2-adrenoceptors located on LC neurons (Mateo et al., 2000) in a similar way to the inhibition induced by acute administration of the NARI desipramine (Mateo et al., 1998). Therefore, the acute *in vivo* inhibitory effect of antidepressant drugs on brain noradrenergic neurons seems to be independent of their intrinsic pharmacological *in vitro* mechanism (NA or serotonin reuptake inhibition).

A drawback of all marketed antidepressants, regardless of their mechanisms of action, is the long delay necessary to achieve therapeutic efficacy. This lag time is believed to reflect, to a large extent, the time required for desensitization of the inhibitory autoreceptors regulating monoamine release (Artigas et al., 1996). Consistent with this hypothesis, several studies have demonstrated α_2-adrenoceptor up-regulation and supersensitivity in postmortem brain of depressed subjects and in platelets of depressed patients (for a review, see Cottingham and Wang, 2012). Interestingly, antidepressant treatment induces α_2-adrenoceptor down-regulation that results in reduced functionality (García-Sevilla et al., 1990; Rivero et al., 2014; reviewed in Cottingham and Wang, 2012). In agreement with these findings in humans, α_2-adrenoceptor down-regulation and desensitization in the rat central nervous system are common responses to the chronic treatment with antidepressant drugs that increase synaptic NA (reviewed in Cottingham and Wang, 2012). In fact, the sustained increase of extracellular NA following chronic administration of the NARIs desipramine, reboxetine or the MAO inhibitor clorgyline has been proposed as the mechanism of α_2-adrenoceptor desensitization observed by *in vivo* microdialysis (Invernizzi et al., 2001; Mateo et al., 2001; Page and Lucki, 2002; Parini et al., 2005; Sacchetti et al., 2001).

Currently, it is not clear whether antidepressant response to SSRIs involves the regulation of α_2-adrenoceptor-mediated functions. The aim of the present study was to evaluate the effect of chronic citalopram treatment on α_2-adrenoceptor sensitivity by using *in vivo* microdialysis and electrophysiological assays and by *in vitro* [^{35}S]GTPγS binding autoradiography. These approaches evaluate the contribution of somatodendritic and terminal α_2-adrenoceptor subpopulations to the long-term citalopram modulation of noradrenergic transmission.

2. Materials and methods

2.1. Animals and treatments

Experiments were performed on male Sprague-Dawley rats (SGIker facilities, University of the Basque Country, UPV/EHU, Spain). Animals were housed 4/5 per cage in a 12 h light-dark cycle at room temperature (22 °C) with food and water *ad libitum*. Animal care and experimental protocols were in agreement with European Union regulations and approved by the UPV/EHU Ethical Board for Animal Welfare (CEEA).

Animals weighed 175–200 g at the start of treatment and 275–310 g when the experiments were carried out. Rats were chronically treated with citalopram 5 mg/kg i.p., every 24 h for 14 days (diluted in saline 0.9%, 5 mg/ml). Control group received saline under similar conditions (0.9%, 1 ml/kg i.p., every 24 h for 14 days).

2.2. Drugs and reagents

Citalopram HBr, 5-bromo-6-(2-imidazolin-2-ylamino) quinoxaline (UK14304) and 2-methoxyidazoxan (RX821002) were provided by Tocris Cookson Ltd. (Bristol, UK); clonidine HCl was purchased from Sigma Co. (St. Louis, MO, USA); chloral hydrate was provided by Fluka Chemie AG (Buchs, Switzerland). [^{35}S]GTPγS was purchased from DuPont NEN (Brussels, Belgium). All reagents were of the highest purity available and were obtained in the standard commercial sources.

2.3. Microdialysis

Two sets of experiments were carried out. In the first one, two dialysis probes were implanted (13th day) in the rat brain under anesthesia (chloral hydrate 400 mg/kg i.p.). Twenty four hours after implantation, a challenge dose of citalopram (5 mg/kg i.p.) was administered (14th day) both to citalopram- and saline-pretreated groups. Basal NA concentrations were evaluated between 35 min and 140 min (three 35 μl dialysate fractions) and 48 h (three 35 μl dialysate fractions) after the last dose administration. A challenge dose of saline vehicle was also performed in a third group of rats that was used as injection control.

In the second set of experiments, the functional sensitivity of α_2-adrenoceptors after chronic citalopram or saline treatments was assessed by clonidine systemic administration after a 48 h washout period. This time has been described as a suitable period to avoid residual effects of different antidepressants (Mateo et al., 2001; Muguruza et al., 2014; Sacchetti et al., 2001). Clonidine was selected as α_2-adrenoceptor agonist because of the existence of previous validated data of its activity by *in vivo* microdialysis (Horrillo et al., 2016; Mateo et al., 2001) and its good solubility in saline solution. For this purpose, rats were anesthetized with chloral hydrate (400 mg/kg i.p.) on the 15th day for microdialysis probe implantation and clonidine (0.3 mg/kg, i.p.) or vehicle (saline 1 ml/kg, i.p.) were injected on the 16th day.

2.3.1. Probe implantation and microdialysis

Microdialysis experiments were carried out as described (Ortega et al., 2010). Two concentric Cuprophan microdialysis probes were stereotaxically implanted choosing coordinates according to Paxinos and Watson (1986). One (exposed tip 2.0 mm × 0.25 mm) was implanted in the vicinity of the right LC (AP−3.7, L+1.3, V−8.2, taken from λ suture point and the incisor bar lowered to a 15° angle) and the other (exposed tip 4.0 mm × 0.25 mm) in the ipsilateral PFC (AP+2.8, L+1.0, V−5, taken from bregma).

Microdialysis experiments were performed one day after