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European Journal of Pharmacology 517 (2005) 51 – 58

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α_2-Adrenoceptor involvement in the in vitro inhibitory effect of citalopram on a subpopulation of rat locus coeruleus neurons

Laura Grandoso, María Torrecilla, Joseba Pineda, Luisa Ugedo*

Departamento de Farmacología, Universidad del País Vasco, E-48940 Leioa, Vizcaya, Spain

Received 6 December 2004; received in revised form 18 May 2005; accepted 24 May 2005

Abstract

The aim of the present study was to investigate the modulation of locus coeruleus neurons by the selective serotonin (5-HT) reuptake inhibitor citalopram using single-unit extracellular recordings in rat brain slices. Citalopram inhibited the activity of a subpopulation of locus coeruleus neurons; thus 10 μM citalopram inhibited neurons by 53 ± 17% (5 out of 15 cells), whereas the inhibition due to 100 μM was 64 ± 4% (32 out of 42 cells). This effect was partially reversed (47 ± 11%) by the α_2-adrenoceptor antagonist idazoxan (10 μM), whereas it was unaffected by antagonists for 5-HT_{1A}, 5-HT_2, and 5-HT_3 receptors, and μ opioid receptors. 5-HT (50 or 200 μM), the 5-HT_{1A} receptor agonist 8-OH-DPAT (±)-8-hydroxy-2-(DI-*n*-propyl-amino) tetralin hydrobromide, 10 μM) and the 5-HT_2 receptor agonist DOI ([±]-2,5-dimetoxy-4-iodoamphetamine) hydrochloride, 10 or 30 μM) also inhibited a subpopulation of locus coeruleus cells. In addition, citalopram but not 5-HT, enhanced by 1.7 fold the inhibitory effect of noradrenaline. Long-term treatment with citalopram (20 mg/kg/day) did not modify the effect of noradrenaline and bromoxidine. Taken together, our results indicate that citalopram exerts an inhibitory effect on locus coeruleus noradrenergic neurons. α_2-adrenoceptor activation may underlie this effect as a result of elevated levels of noradrenaline in the synaptic cleft.

Keywords: Firing activity; Antidepressant; 5-HT receptor; Idazoxan; Noradrenaline; α_2-Adrenoceptor

1. Introduction

Depression is a very important health problem for which pharmacological treatment is not yet entirely satisfactory. Most prescribed antidepressants enhance extracellular levels of monoamines by inhibition of serotonin (5-HT) and/or noradrenaline uptake transporters. Nowadays, selective serotonin reuptake inhibitors (SSRIs) are considered as a first-line therapeutic tool. As a consequence, the acute and chronic effects of this group of drugs on the serotonin system have been intensively studied in recent years. However, these drugs may also affect the noradrenergic system, since the etiopathology of depression is believed to involve deficiencies in both noradrenergic and serotoninergic central systems (Brunello et al., 2002). The main noradrenergic nucleus in the central nervous system is the locus coeruleus. It contains the highest density of noradrenergic neurons and projects to almost the entire neuroaxis (Maeda, 2000). The locus coeruleus is known to participate in neuropsychiatric disorders including depression (Harro and Oreland, 2001). Thus, recent post-mortem studies of the locus coeruleus have revealed elevated levels of tyrosine hydroxylase (Zhu et al., 1999) and reduced binding to norepinephrine transporters (Klimek et al., 1997). Locus coeruleus activity is modulated by somatodendritic α_2-adrenoceptors (Cedarbaum and Aghajanian, 1976). The levels of these receptors have also been found to be elevated in major depression (Ordway et al., 2003) and down-regulated by cyclic antidepressant treatments (García-Sevilla et al., 1990; Esteban et al., 1999).

The action of antidepressants which modulate the noradrenergic system in the locus coeruleus has been

* Corresponding author. Department of Pharmacology, Faculty of Medicine, University of the Basque Country, E-48940, Leioa, Vizcaya, Spain. Tel.: +34 94 601 5574; fax: +34 94 601 3220.
E-mail address: kfpugurl@lg.ehu.es (L. Ugedo).

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doi:10.1016/j.ejphar.2005.05.033

investigated in vivo (Svensson, 2000) and in vitro (Grandoso et al., 2004). However, little is known about the action of SSRIs on this nucleus, although a direct effect seems to exist. In fact, in functional in vitro assays, the SSRI fluvoxamine was found to inhibit the reuptake of noradrenaline by 5-HT nerve terminals in the locus coeruleus (Palij and Stamford, 1994). Thus Mateo et al., 2000 demonstrated that local administration of the SSRI citalopram in the locus coeruleus decreases the release of noradrenaline and the firing rate of locus coeruleus neurons. Adaptive changes in the locus coeruleus have also been reported following repeated administration of SSRIs such as a progressive decrease in neuron firing activity in the rat (Béïque et al., 1999; Grant and Weiss, 2001; Szabo et al., 1999, 2000).

One of the big problems with current pharmacological treatment of depression is the long time to onset of therapeutic effects, often taking 2–3 weeks. The precise mechanism underlying this delay is still not understood (see Holsboer, 2001). In an attempt to hasten the antidepressant response, 5-HT_{1A} somatodendritic receptor antagonists together with SSRI has been tested, on the base of their effect on 5-HT neurons (see Artigas et al., 1996). And more recently, an α_2-adrenoceptor antagonist, yohimbine, in combination with the SSRI fluoxetine has also been tested (Sanacora et al., 2004). However, these therapeutic strategies of combining autoreceptor antagonists will require further in-depth exploration (Svensson, 2000).

The aim of this study was to characterize the direct effects of the SSRI citalopram on locus coeruleus neurons and the participation of noradrenaline and 5 HT in these effects. Adaptive changes in somatodendritic α_2-adrenoceptors on locus coeruleus neurons associated with prolonged treatment with citalopram were also studied. To this end, single unit extracellular recordings of locus coeruleus neurons were carried out on rat brain slices in vitro.

2. Methods

2.1. Animals and treatments

Experiments were performed using male Sprague–Dawley rats weighing 250–300 g. The animals were housed under standard laboratory conditions (22 °C, 12 h light/dark cycles, food and water ad libitum). The rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and decapitated. The brains were carefully dissected and subsequently sliced. Electrophysiological recordings were subsequently performed as described below. Experimental procedures were carried out in compliance with the relevant Spanish Legislation and the European Community Council Directive on "Protection of Animals Used in Experimental and Other Scientific Purposes" of 24 November 1986 (86/609/EEC).

The rats were treated with citalopram (20 mg/kg/day), duloxetine (20 mg/kg/day) which is a dual 5-HT and noradrenaline reuptake inhibitor or saline for 14 days, delivered by osmotic minipumps. Rats were anesthetized with ether for subcutaneous implantation of the minipumps (ALZET, Palo Alto, USA). The skin was shaved and sterilized with an antiseptic solution (Betadine). An incision of about 2 cm was made between the scapulae and the filled pump was inserted, stitched and supported in place with three clips. Doses of citalopram and duloxetine were calculated for a mean body weight of 250 g. Another group of rats was treated with the 5-HT synthesis inhibitor *p*-chlorophenylalanine (PCPA, 400 mg/kg, i.p).

2.2. Brain slice preparation

Immediately after death, the brain was removed and placed in ice-cold artificial cerebrospinal fluid (aCSF) made up of (in mM): NaCl 129, KCl 3, NaH_2PO_4 1.25, $MgCl_2$ 2, $CaCl_2$ 2, $NaHCO_3$ 21 and glucose 10 (pH 7.4). A block of tissue containing the locus coeruleus was immersed in ice-cold aCSF and cut into 500–600 μm thick coronal sections using a vibratome. A single slice was positioned on a nylon mesh and transferred to an interface chamber which provided an excellent perfusion to the slice. The slice was continuously perfused with aCSF at rate of 1.5 ml/min, saturated with 95% O_2 plus 5% CO_2 and maintained at 32–33 °C for at least 1 h.

2.3. Identification of locus coeruleus neurons and extracellular recording

Extracellular recordings were carried out as previously described (Ugedo et al., 1998). The firing rate recording electrode was an Omegadot glass micropipette filled with 0.05 M NaCl. The electrode was positioned in the locus coeruleus which was identified under a binocular microscope as a dark oval area in the upper pons on the lateral borders of the central gray and the fourth ventricle, just anterior to the genu of the facial nerve.

Electrical signals recorded by the electrode were passed through a high-input impedance amplifier and continuously monitored with an audiomonitor and also with an oscilloscope. Single-unit spikes were discriminated, fed into a computer and analyzed using a custom-made computer program which generated firing rate histograms (creating 10 s consecutive samples). Noradrenergic neurons were identified by a steady spontaneous firing rate at ~0.5 Hz and long-lasting positive–negative spikes (Andrade et al., 1983). Baseline activity was recorded for 2 min before application of drugs and for the last 2 min of drug application (when firing rate was stable) which lasted 10–15 min. Concentration–effect curves for α_2-adrenoceptor agonists (noradrenaline and bromoxidine) were constructed by applying increasing concentrations of these drugs (at 10 min intervals for bromoxidine and at 1 min intervals for noradrenaline). The inhibition of locus coeruleus neurons induced by these drugs was quantified as the percentage reduction from the basal firing rate.

Experimental data from each animal were analyzed for the best non-linear fit to the logistic three parameters equation using the computer program Microsoft Excel for Windows 98: $E = E_{max}/\{(1+EC_{50}^{n}/[C]^{n})\}$, where $[C]$ is the concentration of the drug, E is the effect on the firing rate induced by C, E_{max} is the maximal effect, EC_{50} is the effective concentration for eliciting 50% of E_{max} and n is the slope factor of the concentration–effect curve. EC_{50}, E_{max} and n were estimated by this analysis. The theoretical curves were generated with the GraphPad program.

2.4. Analysis of data

All data are expressed as mean values ± S.E.M. The *n* values represent the number of neurons recorded in each group. Statistical comparisons were performed using either the paired or unpaired Student *t*-test or Fisher's exact test or the one-way repeated measures analysis of variance (ANOVA) followed by the Bonferroni's test. The significance level was set at $P = 0.05$.

2.5. Drugs

Naloxone HCl and UK 14,304 (bromoxidine) were obtained from RBI. The following drugs were obtained from Sigma-Aldrich: *p*-chlorophenylalanine (PCPA); (±)-2,5-dimetoxy-4-iodoamphetamine) hydrochloride (DOI); (±)-8-hydroxy-2-(DI-*n*-propyl-amino) tetralin hydrobromide (8-OH-DPAT); MDL 11.939; methiothepin mesylate; 2-methyl-5-hydoxytryptamine maleate; noradrenaline; ((±)-*N*-(1-Azabicyclo[2.2.2]oct-3-yl)-6-chloro-4-methyl-3-oxo-3,4-dihydro-2H-1,4-benzoxazine-8-carboxamide hydrochloride (Y-25130); *N*-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-*N*-(2-pyridinyl) cyclohexane carboxamide (WAY 100,635) and serotonin HCl (5-HT). A 5-carboxyamidotryptamine maleate was obtained from Tocris. Citalopram HBr, duloxetine and idazoxan were generously provided by Lundbeck (Denmark) Lilly Corporate Center (Indianapolis) and Lasa laboratories (Spain), respectively. All drugs were dissolved in aCSF for bath application.

3. Results

3.1. Effect of citalopram on the firing activity of locus coeruleus neurons in control and PCPA-treated rats

Bath application of citalopram (10 or 100 μM) affected a subpopulation of locus coeruleus neurons. Thus, when only effects larger than 20% with respect to the basal firing rate value were considered, 10 μM citalopram inhibited 5 out of 15 cells (33% of registered neurons), reaching a maximal inhibition of 53 ± 17% ($P < 0.01$) (Fig. 1A). A higher concentration (100 μM) of citalopram also decreased the firing rate of 32 out of 42 locus coeruleus neurons (76% of registered neurons) with a maximum inhibitory effect of 64 ± 4% ($P < 0.001$) (Fig. 1B). In both cases, the inhibitory effect lasted more than 20 min. No excitatory effect was observed. It is important to note that the lack of effect was not due to methodological factors, since all preparations were pre-tested with a single test concentration of noradrenaline (10 or 100 μM) at the beginning of each experiment. As shown in Fig 1A, noradrenaline produced a complete and reversible inhibition, which demonstrated that the preparation was well perfused.

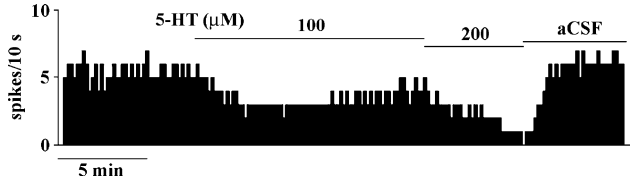

Fig. 2. Effect of 5-HT on locus coeruleus neuron activity. Representative integrated firing rate histograms showing the inhibitory effect of 5-HT and the rapid reversibility of the effect, since drug withdrawal during artificial cerebrospinal fluid (aCSF) application leads to the prompt return to basal conditions. Drugs were bath administrated at the indicated concentrations, for the time indicated by the horizontal bars. Vertical lines representing the extracellularly recorded firing rates were displayed as integrated time histograms (spikes per 10 s).

Overall, application of 10 or 100 μM citalopram decreased the locus coeruleus firing rate by 23 ± 8% ($n = 15$) and by 50 ± 5% ($n = 42$), respectively (Fig. 1). A concentration-dependent inhibition with respect to the maximum effect reached ($P < 0.006$) and the number of neurons inhibited (Fisher's exact test $P = 0.0046$) was detected upon analyzing all the results together.

In order to assess the role of 5-HT in this inhibitory effect of citalopram, the synthesis of 5-HT was inhibited by administering PCPA (400 mg/kg i.p.) 24 h before the experiment. This protocol has been shown strongly reduce the 5-HT content of locus coeruleus tissue (Mateo et al., 2000). Under these conditions, the effect of bath application of citalopram (10 or 100 μM) was unaltered, either with respect to the number of neurons which responded to citalopram (2 out of 6, i.e. 33% of registered neurons

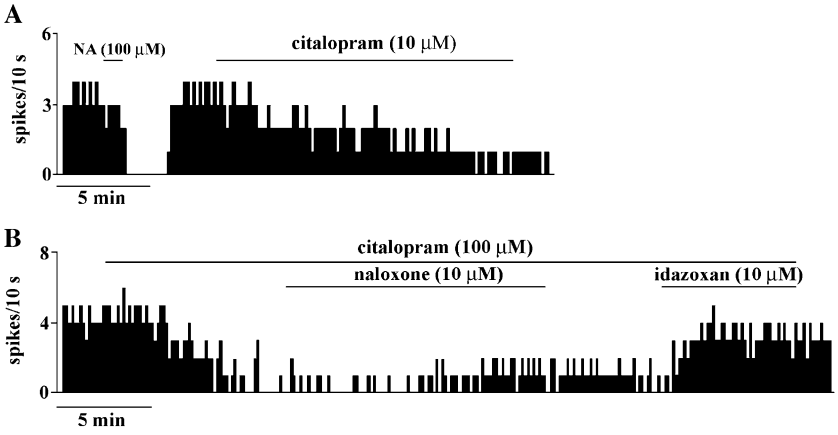

Fig. 1. Effect of citalopram on locus coeruleus neuron activity. Representative integrated firing rate histograms showing the inhibitory effect of (A) noradrenaline (NA) and 10 μM and (B) 100 μM of citalopram and its reversion by application of the α_2-adrenoceptor antagonist idazoxan, but not by the μ opioid receptor antagonist naloxone. Drugs were bath administrated at the indicated concentrations, for the time indicated by the horizontal bars. Vertical lines representing the extracellularly recorded firing rates were displayed as integrated time histograms (spikes per 10 s).

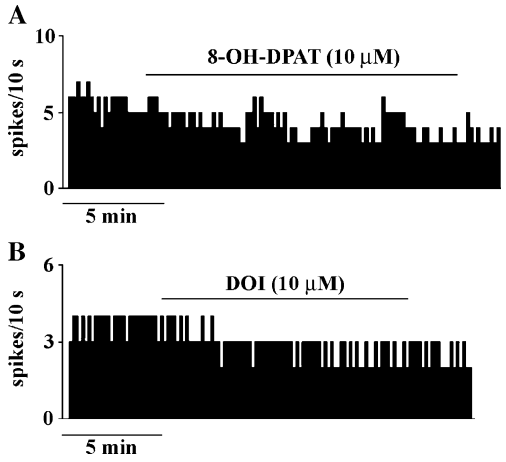

Fig. 3. Effect of 8-OH-DPAT and DOI, 5-HT_{1A} and 5-HT_2 receptor agonists respectively, on locus coeruleus neuron activity. Representative integrated firing rate histograms showing the inhibitory effect of (A) 8-OH-DPAT and (B) DOI. Drugs were bath administered for the time indicated by the horizontal bars. Vertical lines representing extracellularly recorded firing rates were displayed as integrated time histograms (spikes per 10 s).

and 4 out of 6, i.e. 67% of registered neurons respectively), or to the maximum effect presented by these neurons (64±35% and 52±17%; results not shown). Overall, application of 10 or 100 μM citalopram decreased the locus coeruleus firing rate by 26±15% ($n=6$) and by 39±14% ($n=6$), respectively, magnitudes which are similar to those of the control group. As previously shown in vivo (Ruiz-Ortega and Ugedo, 1997), the dose of PCPA used in that study did not modify the basal firing rate of locus coeruleus neurons (mean basal firing rate values were 7.7±0.4 spikes/10 s, $n=47$ and 7.2±1.3 spikes/10 s, $n=6$ in control and PCPA pretreated groups respectively).

3.2. Effects of 5-HT, α_2-adrenoceptor and μ-opioid receptor antagonists on the inhibition of the firing activity of locus coeruleus neurons by citalopram

To investigate the nature of the receptor or receptors involved in the inhibitory effect of citalopram, several 5-HT receptor antagonists were applied in those experiments in which citalopram produced more than a 20% inhibition of the firing rate of locus coeruleus neurons. The selective 5-HT_{1A} receptor antagonist WAY 100635 (10 μM), the 5-HT_3 receptor antagonist Y-25130 (10 μM) and the 5-HT_{2C} receptor antagonist MDL 11.939 (10 μM) did not reverse the inhibition induced by citalopram (100 μM) ($n=3$, for each receptor antagonist, data not shown). This inhibitory effect was also unaltered by the non-selective 5-HT_2 receptor antagonist methiothepin (10 μM; $n=3$) and the μ-opioid receptor antagonist naloxone (10 μM; $n=4$; Fig 1B). The α_2-adrenoceptor antagonist idazoxan (1–10 μM) was the only one which partially reversed (47±11%; $n=4$; $P<0.05$, one-way repeated measures ANOVA followed by Bonferroni's test) the inhibitory effect of citalopram (Fig. 1B).

3.3. Effects of 5-HT and 5-HT agonists on the locus coeruleus activity

To further investigate the participation of 5-HT in the citalopram effect, we applied 5-HT to locus coeruleus slices. As with citalopram application, bath application of 5-HT (50–200 μM) resulted in a decrease in the firing rate of locus coeruleus neurons, although not all neurons were affected. Thus, 32 out of 81 cells showed more than 20% inhibition with respect to their basal firing rate, with a maximum effect of 45±5% ($P<0.001$) (Fig. 2). This inhibitory effect of 5-HT was rapidly reversible (Fig. 2).

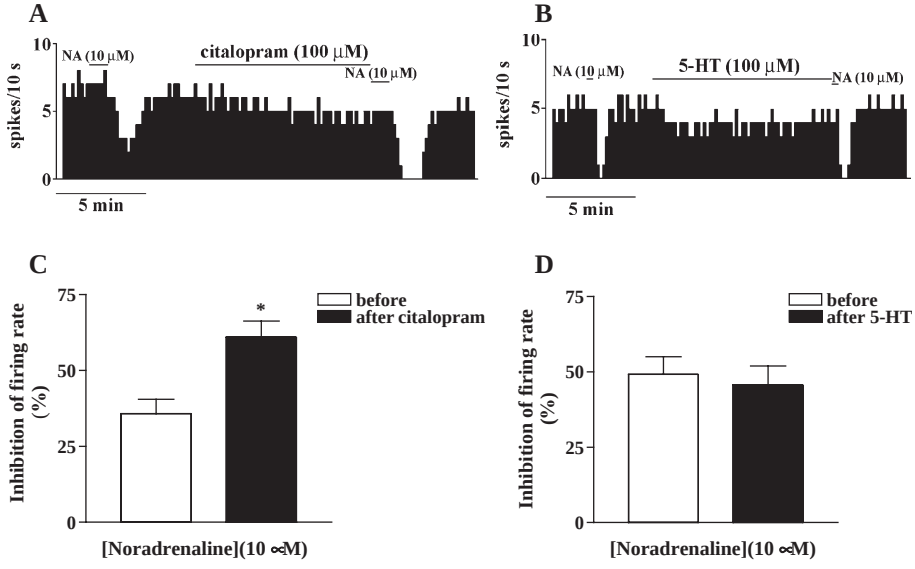

Fig. 4. Enhancement of the inhibitory effect of noradrenaline (NA) on locus coeruleus neuron activity following application of citalopram and lack of effect of application of 5-HT. Representative integrated firing rate histograms showing the inhibitory effect of noradrenaline before and after application of citalopram (A) and 5-HT (B). Drugs were bath administrated at the indicated concentrations, for the time indicated by the horizontal bars. Vertical lines representing extracellularly recorded firing rates were displayed as integrated time histograms (spikes per 10 s). Bar histograms represent the mean±S.E.M. ($n=5$) of the percentage inhibitory effect of noradrenaline with respect to the basal firing rate, before and after application of citalopram (C) *$P<0.05$ (paired Student t-test) and 5-HT (D).

We subsequently employed 5-HT receptor agonists to determinate the receptor type implicated in the inhibitory effect of 5-HT on the spontaneous firing rate of locus coeruleus neurons. As illustrated in Fig. 3A, the 5-HT_{1A} receptor agonist 8-OH-DPAT (10 μM) caused an inhibition of more than 20% of the basal firing of 3 out of 5 cells recorded, with a maximum effect of 45±2% ($P<0.05$). The 5-HT_2 receptor agonist DOI (10–30 μM) also inhibited 3 out of 5 cells by more than 20% (Fig. 3B) with a maximum effect of 32±3% of the basal value ($P<0.01$). On the other hand, the 5-HT_3 receptor agonist 2-methyl-5-hydroxytryptamine (10 μM) and the 5-$HT_{1,5,7}$ receptor agonist 5-carboxyamidotryptamine (300 nM) did not modify the spontaneous firing activity of locus coeruleus neurons (data not shown).

3.4. Effect of citalopram and 5-HT on the inhibitory effect of noradrenaline in locus coeruleus neurons

To determine if citalopram or 5-HT modified the inhibitory effect of exogenous noradrenaline, we evaluated the inhibition induced by a non-saturating concentration of noradrenaline (10 μM) before and after citalopram (100 μM) or 5-HT (100 μM) application. In order to compare the effect of noradrenaline more accurately, only experiments in which citalopram or 5-HT did not induce a large change (less than 30%) of the basal firing rate were considered. Bath application of noradrenaline rapidly inhibited ($E_{max}=36\pm5\%$, $n=5$) the firing rate of locus coeruleus neurons and this effect was enhanced 1.7 fold ($n=5$; $P<0.05$) after citalopram application (Fig. 4 A, C). However, 5-HT did not modify noradrenaline inhibitory effect (Fig. 4 B,D).

3.5. Effect of long-term citalopram and duloxetine treatments on the inhibitory effect of noradrenaline and bromoxidine in locus coeruleus neurons

Since the clinical benefits of antidepressants become evident only after long-term exposure to these drugs, we evaluated the effects of long-term treatment with citalopram on the sensitivity of locus coeruleus α_2-adrenoceptors. To this end, we compared the effects of increasing concentrations of the α_2-adrenoceptor agonists noradrenaline and bromoxidine on locus coeruleus neurons in slices from control rats and rats treated with citalopram (20 mg/kg, minipumps for 14 days). After citalopram treatment, locus coeruleus neurons fired at the same frequency as neurons from control animals (Table 1). Thus, the concentration–effect curves and the EC_{50} values for noradrenaline (0.1–100 μM) and for bromoxidine (0.1–100 nM) were unaltered with respect to corresponding values from the control group (Fig. 5, A,B; Table 1). Similarly, when the dual 5-HT and noradrenaline reuptake inhibitor duloxetine (see Kasamo et al., 1996; 20 mg/kg, minipumps for 14 days) was administrated, no changes in the parameters of the concentration–effect curves for noradrenaline and bromoxidine were found (Table 1).

Table 1
Effect of long-term treatment with citalopram or duloxetine on the parameters of the concentration–effect curves for noradrenaline and bromoxidine

Treatment	Noradrenaline			Bromoxidine		
	Basal	EC_{50} (μM)	(n)	Basal	EC_{50} (nM)	(n)
Control	6.4±1.0	9.2±3.9	(5)	5.7±0.8	3.3±1.1	(4)
Citalopram (20 mg/kg 14 d)	7.1±1.4	12.4±4.9	(5)	6.4±0.6	4.1±1.9	(5)
Duloxetine (20 mg/kg 14 d)	6.7±1.1	15.0±6.0	(5)	6.1±0.7	5.9±3.3	(5)

Animals were treated with vehicle, citalopram or duloxetine for 14 days, delivered by osmotic minipumps. Parameters of concentration–effect curves for noradrenaline and bromoxidine were estimated in each experiment using the Parker and Waud equation. EC_{50} values are shown as means±S.E.M. of (n) experiments.

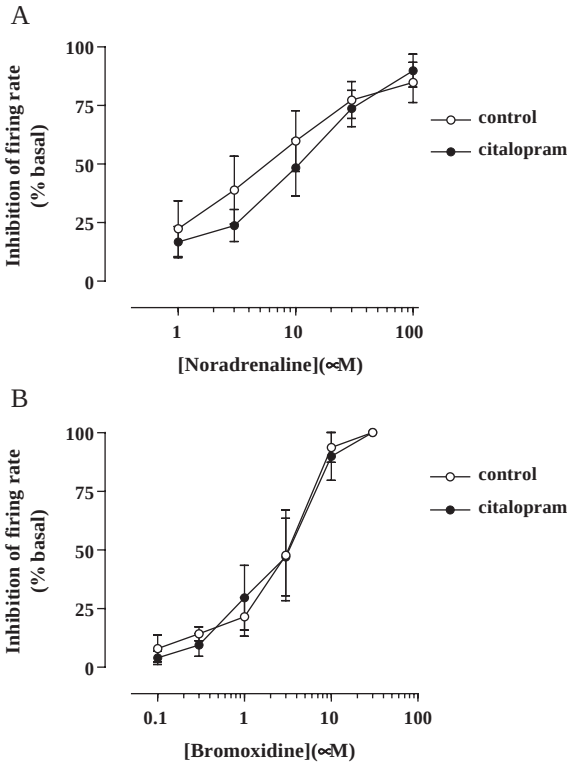

Fig. 5. Concentration–effect curves for (A) noradrenaline (control group, $n=5$; citalopram-treated group, $n=5$) and (B) bromoxidine (control group, $n=4$; citalopram-treated group, $n=5$). Symbols represent means±S.E.M. of the percentage inhibition of the firing rate with respect to the basal firing rate of n rats. The horizontal axes represent the cumulative concentrations of bromoxidine applied at 10 min intervals or concentrations of noradrenaline applied during 1 min intervals. The lines through the data are theoretical curves constructed from the means of the concentration–effect curve parameters.

4. Discussion

In the present study, we have shown that bath application of the SSRI citalopram inhibited the basal firing rate of locus coeruleus neurons and enhanced the inhibitory effect of noradrenaline on these neurons. This inhibitory effect of citalopram was not blocked by 5-HT synthesis inhibition nor reversed by application of different serotoninergic and opioid antagonists. In contrast, idazoxan produced a partial reversion of the inhibitory effect of this drug. However, the endogenous ligand 5-HT, as well as the 5-HT_{1A} receptor agonist 8-OH-DPAT and the 5-HT_2 receptor agonist DOI, inhibited locus coeruleus neurons. Curiously, 5-HT did not modify the effects of noradrenaline. These results indicate

that citalopram may act on locus coeruleus neurons through various mechanisms: it may indirectly produce an increase in the levels of noradrenaline in the locus coeruleus; this noradrenaline would also inhibit the firing rate of locus coeruleus neurons by means of activation of α_2-adrenoceptors. However, the fact that the citalopram effect was reversed only partially by the α_2-adrenoceptor antagonist and mimicked by 5-HT and 5-HT_{1A} and 5-HT_2 receptor agonists suggests that a 5-HT-mediated mechanism may also underlie the citalopram effect.

Our results are consistent with in vivo studies showing that citalopram induces a decrease in the firing rate of the locus coeruleus and a parallel increase in the extracellular levels of noradrenaline in this nucleus (Mateo et al., 2000), together with an enhancement of noradrenaline levels in projecting areas (David et al., 2003). Thus, our results further contribute to demonstrating that at least part of the effect of citalopram is due to increased activation of inhibitory α_2-adrenoceptors, likely in response to an increased availability of noradrenaline, since we show that locus coeruleus inhibition due to citalopram was not blocked by 5-HT synthesis inhibition with PCPA, nor reversed by 5-HT receptor antagonists, but was partially reversed by idazoxan (an α_2-adrenoceptor antagonist). It is important to point out that in in vitro recording assays, α_2-adrenoceptor antagonists alone do not elicit any effect on locus coeruleus neurons, but they do block the inhibitory effect due to α_2-adrenoceptor agonists (Williams et al., 1985; Illes and Norenberg, 1990; Pineda and Aghajanian, 1997; Ugedo et al., 1998). In addition, we observed that citalopram potentiated the effect of exogenous noradrenaline which could be interpreted as an increase in the availability of noradrenaline in the synaptic cleft by inhibiting not only 5-HT uptake but also noradrenaline uptake. So although radioligand binding studies have shown that citalopram is the most selective SSRI, being about 3000-fold weaker in blocking the noradrenaline transporter than the 5-HT transporter (Popik, 1999; Sánchez and Hyttel, 1999), citalopram has been shown to inhibit not only the 5-HT transporter but also the noradrenaline transporter in vivo with a comparable potency (David et al., 2003). Furthermore, citalopram has been reported to inhibit [^{3}H] noradrenaline uptake into synaptosomes, almost at similar concentrations (25–50 μM) (Hughes and Stanford, 1998). Therefore, citalopram appears to alter noradrenergic neurons through a mechanism, which is dependent on increased levels of noradrenaline in the synaptic cleft. In that line, a recent study has shown that the effect of citalopram on 5-HT levels is attenuated on mice unable to synthesize noradrenaline (Cryan et al., 2004). In addition, other mechanisms appear to be involved for example it could be that citalopram cause a small hyperpolarizating effects on the cell which do not affect cell firing but add up to the effects of NA.

The application of 5-HT mimicked the inhibitory effect of citalopram, suggesting that a 5-HT mechanism may contribute citalopram inhibition. However, 5-HT did not modify the effects due to noradrenaline, suggesting an absence of interaction with noradrenaline uptake. The fact that 5-HT terminals do not synapse with all noradrenaline neurons (Van Bockstaele, 2000) may be the reason for citalopram and 5-HT only affect a subpopulation of locus coeruleus neurons. Our study indicates that locus coeruleus neuron activity may be modulated not only indirectly by 5-HT from the raphe nucleus (Haddjeri et al., 1997), but also directly via locally produced 5-HT.

The effect of citalopram was similar to that of 5-HT, and the 5-HT_{1A} and 5-HT_2 receptor agonists, in that all of these agents decreased the firing rate in a similar percentage of tested cells. The locus coeruleus is known to express both 5-HT_{1A} (Weissmann-Nanopoulos et al., 1985 and Pazos and Palacios, 1985) and 5-HT_2 receptors (Lopez-Gimenez et al., 1997 and Cornea-Hébert et al., 1999); both receptors have been shown to be able to modulate locus coeruleus neuron activity (Haddjeri et al., 1997; Szabo and Blier, 2002). However, the 5-HT_{1A} receptor agonist 8-OH-DPAT enhances the activity of locus coeruleus neurons by a mechanism which is entirely dependent on intact 5-HT neurons (Haddjeri et al., 1997). In contrast, we report that this drug inhibited locus coeruleus activity; a similar result was found by Bobker and Williams (Bobker and Williams, 1989) who observed that 8-OH-DPAT inhibited the depolarizing synaptic potentials of locus coeruleus neurons in brain slices in vitro. It is also conceivable that citalopram activates 5-HT receptors. In this context, it has been shown that citalopram interacts directly with 5-HT_2 receptors (Pälvimäki et al., 1996). Thus, other subtypes of 5-HT receptors such as 5-HT_3 and 5-HT_7 receptors are also present in the brain (Vanhoenacker et al., 2000; Van Hooft and Yakel, 2003). However, we did not detect any effect when 5-HT_3 and 5-HT_7 receptor agonists were applied to the locus coeruleus. Moreover, we did not observe any reversion of the citalopram effect with 5-HT_3 and 5-HT_7 receptor antagonists. The possibility that several mechanisms may underlie the effects of citalopram would explain why it was not possible to block these effects with 5-HT receptor antagonists. In addition, interactions between antidepressants and the opioid system have been reported (Berrocoso et al., 2004) although in the present study naloxone did not altered the citalopram effect.

It has been hypothesized that chronic treatment with citalopram may alter the function of noradrenergic receptors (Petersen and Mork, 1996). Indeed, 14 days treatment with citalopram (Szabo et al., 2000) or fluoxetine (Grant and Weiss, 2001) has been reported to decrease the spontaneous firing activity of locus coeruleus neurons. However, in the present study, prolonged administration of citalopram with minipumps did not alter the basal firing activity or the sensitivity of α_2-adrenoceptors in the locus coeruleus. These discrepancies could be due to the presence of the antidepressant in blood, since the minipumps were in place during the experiments and an inhibitory effect of citalopram, when we applied the drug directly in the bath,

was observed (see above). However, we carried out experiments 24 h after removing the minipumps, i.e. when the concentration of citalopram in the blood was undetectable (Hyttel et al., 1984). These discrepancies may also be due to the fact that in the mentioned in vivo studies, neurons were under the control of various mechanisms located both within and outside the locus coeruleus, while in our in vitro preparation, effects on the locus coeruleus were directly assessed, since the complex influence of the afferents which the locus coeruleus receives were avoided. No adaptive changes were detected after 14 days of prolonged treatment with minipumps either, when citalopram was applied intraperitoneally every 12 h (data not shown). This lack of change may be due to complex compensatory mechanisms affecting 5-HT and noradrenaline levels. In fact, we did not observe any alterations in α_2-adrenoceptor sensitivity after prolonged treatment with duloxetine, a dual 5-HT and noradrenaline reuptake inhibitor. Corroborating our results Gobbi et al. (1997) did not find changes in 5-HT uptake binding sites after 14-days of citalopram treatment.

In summary, the results of the present study demonstrate that the selective 5-HT uptake inhibitor citalopram inhibits locus coeruleus neuron activity through an α_2 adrenoceptor-related mechanism. Although 5-HT receptor-related mechanisms might be involved they were not detected. No adaptive changes were observed in neuron activity or α_2-adrenoceptor sensitivity after prolonged citalopram administration. Thus, our results point to the involvement of regulation of the noradrenergic system, which is known to play an important role in major depression (Svensson, 2000), in the therapeutic antidepressant effects of SSRIs. These findings are relevant for the optimization of the pharmacotherapy of depression.

5. Uncited reference

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Acknowledgements

This work was supported by the Basque Government (PI 99/0026) and the UPV-EHU (00026.327-13590/2001). The authors wish to thank the agency ACTS (http://www.euskalnet.net/acts) for improving the English of this manuscript.

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