Published in final edited form as:

Eur J Pharmacol. 2008 March 31; 583(1): . doi:10.1016/j.ejphar.2008.01.022.

Differential regulation of serotonin-1A receptor stimulated [³⁵S]GTPγS binding in the dorsal raphe nucleus by citalopram and escitalopram

Dania V. Rossi, Teresa F. Burke, and Julie G. Hensler

Department of Pharmacology, University of Texas Health Science Center-San Antonio, San Antonio, Texas 78229-3900

Abstract

The effect of chronic citalopram or escitalopram administration on 5-HT_{1A} receptor function in the dorsal raphe nucleus was determined by measuring [³⁵S]GTP S binding stimulated by the 5-HT_{1A} receptor agonist (R)-(+)-8-OH-DPAT (1nM-10μM). Although chronic administration of citalopram or escitalopram has been shown to desensitize somatodendritic 5-HT_{1A} autoreceptors, we found that escitalopram treatment decreased the efficacy of 5-HT_{1A} receptors to activate G-proteins, whereas citalopram treatment did not. The binding of [³H]8-OH-DPAT to the coupled, high affinity agonist state of the receptor was not altered by either treatment. Interestingly, escitalopram administration resulted in greater occupancy of serotonin transporter sites as measured by the inhibition of [³H]cyanoimipramine binding. As the binding and action of escitalopram is limited by the inactive enantiomer R-citalopram present in racemic citalopram, we propose that the regulation of 5-HT_{1A} receptor function in the dorsal raphe nucleus at the level of receptor-G protein interaction may be a result of greater inhibition of the serotonin transporter by escitalopram.

Keywords

quantitative autoradiography; serotonin-1A; citalopram; escitalopram; [³⁵S]GTP S binding; [³H]8-OH-DPAT

1. Introduction

Although administration of antidepressants results in rapid increases in monoaminergic neurotransmission, it has been hypothesized that neuroadaptive changes underlie the delay in therapeutic effects of antidepressants. One example of these neuroadaptive changes is the desensitization of serotonin-1A (5-HT_{1A}) autoreceptors in the dorsal raphe nucleus following chronic administration of selective serotonin reuptake inhibitors (SSRIs) (Blier and de Montigny, 1994; Hjorth et al., 2000).

Previously, we and others have shown that chronic treatment of rats with the SSRI fluoxetine results in a decrease in 5-HT_{1A} receptor-stimulated [³⁵S]GTP S binding in the

Address for correspondence: Julie G. Hensler, Ph.D., Department of Pharmacology, MC 7764, University of Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, TX 78229-3900, Tel. (210) 567-4236, Fax: (210) 567-4303, Email: hensler@uthscsa.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

dorsal raphe nucleus (Castro et al., 2003; Hensler, 2002; Shen et al., 2002). This decrease is not accompanied by a decrease in the coupled, high affinity agonist state of the 5-HT_{1A} receptor as measured by binding of the agonist radioligand [³H]8-OH-DPAT (Castro et al., 2003; Le Poul et al., 1995). Thus, it appears that the mechanism by which somatodendritic 5-HT_{1A} autoreceptors are desensitized following chronic fluoxetine treatment (e.g., Kreiss and Lucki, 1995; Le Poul et al., 1995) is a decrease in the efficacy of 5-HT_{1A} receptors to activate G proteins.

In the present study, we treated animals chronically with the SSRI citalopram or its active isomer S-citalopram (escitalopram). Since citalopram is a 1:1 mixture of R- and S-enantiomers, doses of citalopram and escitalopram were selected to administer equivalent amounts of the active S-enantiomer. The treatments chosen for this study have also been shown to result in desensitization of somatodendritic 5-HT_{1A} autoreceptor function in the dorsal raphe nucleus (Ceglia et al., 2004; Cremers et al., 2000). We hypothesized that the mechanism by which this desensitization occurs is through a decrease in the efficacy of 5-HT_{1A} receptors to activate G proteins.

2. Materials and Methods

Male Sprague-Dawley rats (Harlan, Indianapolis, IN) were treated for 14 days with citalopram (18 mg/kg/day) or saline; a second group of animals was treated with escitalopram (10 mg/kg/day) or saline. Drugs were administered via a subcutaneously implanted osmotic minipump (ALZET osmotic pumps, model 2ML2; Durect, Cupertino, CA). These studies were carried out in strict accordance with the *Guide for the Care and Use of Laboratory Animals* as adopted and promulgated by the National Institutes of Health, and were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Texas Health Science Center at San Antonio. Every effort was made to prevent animal suffering and to minimize the number of animals used.

On day 14 of treatment, animals were sacrificed. Trunk blood was collected to determine serum levels of citalopram or escitalopram (Clinical Psychopharmacology Laboratories, University of Texas Health Science Center at San Antonio). Brains were rapidly removed and frozen on powdered dry ice. Coronal sections of 20 µm thickness were cut at -17°C in a cryostat microtome and thaw-mounted onto gelatin-coated glass slides. Slide-mounted sections were stored at -80°C until used in quantitative autoradiographic experiments measuring (R)-(+)-8-hydroxy-2-(di-n-propylamino)tetralin hydrobromide (8-OH-DPAT)-stimulated [³⁵S]GTP S binding, 2-(N,N-di[2,3(n)-³H]propylamino)-8-hydroxy-1,2,3,4-tetrahydronaphthalene ([³H]-8-OH-DPAT) binding, and [³H]cyanoimipramine ([³H]CN-IMI) binding.

5-HT_{1A} receptor-stimulated [³⁵S]GTP S binding was performed as previously described (Rossi et al., 2006). Slide-mounted sections at the level of the dorsal raphe nucleus (plates 50-52) (Paxinos and Watson, 1998) were incubated in the absence or in the presence of (R)-(+)-8-OH-DPAT (1nM – 10µM). Basal [³⁵S]GTP S binding was defined in the absence of (R)-(+)-8-OH-DPAT. Nonspecific [³⁵S]GTP S binding was defined in the absence of (R)-(+)-8-OH-DPAT and in the presence of 10µM GTP S. Sections were exposed to Kodak Biomax MR film (Amersham) for 48 hours to generate autoradiograms.

The binding of [³H]8-OH-DPAT to 5-HT_{1A} receptors was performed as previously described (Rossi et al., 2006). Briefly, slide-mounted sections at the level of the dorsal raphe nucleus (plates 50-52) (Paxinos and Watson, 1998) were incubated in assay buffer containing 2 nM [³H]8-OH-DPAT. Nonspecific binding was defined by incubating adjacent sections in the presence of 10 µM N-[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl]-N-2-

pyridinylcyclohexane-carboxamide (WAY 100635). Sections were exposed to Kodak BioMax MR Film for a period of 9 weeks to generate autoradiograms.

The binding of [^3H]cyanoimipramine ([^3H]CN-IMI) to serotonin reuptake sites was performed as previously described (Gould et al., 2006; Kovachich et al., 1988). Briefly, slide-mounted sections at the level of the dorsal hippocampus (plates 32-34) (Paxinos and Watson, 1998) were incubated in assay buffer containing [^3H]CN-IMI (1 nM). Nonspecific binding was defined in the presence of 1 μM paroxetine. Sections were exposed to Kodak BioMax MR Film (Amersham) for a period of 4 weeks to generate autoradiograms.

Digitized autoradiograms were analyzed using NIH Image, version 1.47 (NIH, Bethesda, MD). Tissue sections were stained with thionin and brain areas were identified using the atlas of the rat brain (Paxinos and Watson, 1998). Autoradiograms of [^3H]8-OH-DPAT and [^3H]CN-IMI binding were quantified using simultaneously exposed [^3H] standards (ART-123, American Radiochemicals, St. Louis, MO), as previously described (Kovachich et al., 1988; Rossi et al., 2006). Specific binding was calculated by subtracting nonspecific binding from total binding on adjacent sections. Autoradiograms of (R)-(+)-8-OH-DPAT-stimulated [^{35}S]GTP S binding were quantified by the use of simultaneously exposed [^{14}C] standards (ARC-146, American Radiochemicals, St. Louis, MO), as previously described (Rossi et al., 2006). Nonspecific binding of [^{35}S]GTP S was subtracted from basal binding and from binding in the presence of R(+)-8-OH-DPAT. Specific (R)-(+)-8-OH-DPAT-stimulated binding was expressed as % above basal.

Individual dose-response curves for (R)-(+)-8-OH-DPAT-stimulated [^{35}S]GTP S binding were fit by nonlinear regression to the model: $E = E_{\text{max}}/(1 + EC_{50}/[A])^n$, where E is the response at the (R)-(+)-8-OH-DPAT concentration [A], E_{max} is the maximal response, EC_{50} is the concentration of drug that yields a half-maximal response, and n is the slope factor (KaleidaGraph 4.0.1, Synergy Software, Reading, PA). Statistical comparisons for EC_{50} and E_{max} values were made using an unpaired t-test for two-group comparisons. Analysis of [^3H]CN-IMI binding in subregions of hippocampus was conducted using one-way ANOVA. F values reaching significance ($P < 0.05$) were evaluated further by post hoc analysis using Fisher's Protected Least Significant Difference test (GraphPad Prism 4, GraphPad Software Inc., San Diego, CA).

The materials used were obtained from the following sources: citalopram hydrobromide, escitalopram oxalate, and paroxetine hydrochloride (Shanco International Inc., Hazlet, NJ); [^{35}S]GTP S (1250 Ci/mmol, PerkinElmer Life Sciences Inc., Boston, MA); [^3H]8-OH-DPAT (210 Ci/mmol; GE Healthcare, Piscataway, NJ); [^3H]cyanoimipramine (60 Ci/mmol, American Radiochemicals, St. Louis, MO); (R)-(+)-8-hydroxy-DPAT hydrobromide (Tocris, Ellisville, MO); WAY 100635 maleate (Sigma/RBI, St. Louis, MO); GTP S (tetralithium salt) (Roche, Indianapolis, IN).

3. Results

In animals treated for 14 days with citalopram or escitalopram, steady-state serum levels were 189 ± 21.6 ng/ml ($n=6$) and 57.1 ± 9.05 ng/ml, ($n=7$), respectively. Since citalopram is a 1:1 mixture of R- and S-enantiomers, our data indicate that the citalopram and escitalopram treatments provided roughly equivalent amounts of the active S-enantiomer.

To determine whether treatment of rats with citalopram or escitalopram for 14 days results in a decrease in 5-HT_{1A} receptor sites in the dorsal raphe nucleus, we measured the binding of the agonist radioligand [^3H]8-OH-DPAT. There was no change in [^3H]8-OH-DPAT binding following chronic administration of citalopram [saline-treated: 445 ± 23.5 fmol/mg protein, ($n=6$); citalopram-treated: 435 ± 24.7 fmol/mg protein; ($n=6$)] or escitalopram

[saline-treated: 425 ± 14.4 fmol/mg protein, (n=5); escitalopram-treated: 397 ± 11.9 fmol/mg protein; (n=7)]. These data suggest that administration of citalopram or escitalopram for 14 days by osmotic minipump does not alter 5-HT_{1A} receptors in the coupled, high-affinity agonist state of the receptor in the dorsal raphe nucleus.

The effect of chronic citalopram administration on 5-HT_{1A} receptor function in the dorsal raphe nucleus was determined by measuring [³⁵S]GTP S binding stimulated by the 5-HT_{1A} receptor agonist (R)-(+)-8-OH-DPAT (1nM-10μM). Treatment of rats with citalopram for 14 days did not alter the potency (EC₅₀) [saline-treated: 57.7 ± 10.9 nM, (n=6); citalopram-treated: 64.1 ± 21.7 nM, (n=6)] or efficacy (E_{max}) [saline-treated: 63.5 ± 6.0 % above basal, (n=6); citalopram-treated: 63.8 ± 9.8 % above basal] of (R)-(+)-8-OH-DPAT to stimulate [³⁵S]GTP S binding (Fig. 1A). 5-HT_{1A} receptor-stimulated [³⁵S]GTP S binding in the dorsal raphe nucleus was also not altered by longer treatment with citalopram (i.e. 21 days) (data not shown).

By contrast, treatment of rats with escitalopram for 14 days decreased the efficacy (E_{max}) [saline-treated: 60.4 ± 3.4 % above basal, (n=6); escitalopram-treated: 45 ± 3.9 % above basal, (n=6); $P < 0.05$] of (R)-(+)-8-OH-DPAT to stimulate [³⁵S]GTP S binding in the dorsal raphe nucleus (Fig. 1B). The potency of (R)-(+)-8-OH-DPAT to stimulate [³⁵S]GTP S binding in the dorsal raphe was not altered [EC₅₀, saline-treated: 50.0 ± 6.0 nM, (n=6); escitalopram-treated: 62.9 ± 5.5 nM, (n=7)]. Our data suggest that chronic treatment with escitalopram, but not citalopram, alters the capacity of 5-HT_{1A} receptors to activate G proteins in the dorsal raphe nucleus.

Given that citalopram and escitalopram administration provided roughly equivalent amounts of active S-enantiomer, we were surprised by the difference in the regulation of 5-HT_{1A} receptor function observed following these two drug treatments. To examine the occupancy of serotonin transporters following chronic citalopram or escitalopram administration, we measured the binding of [³H]CN-IMI. It is important to note that for these experiments the animals were sacrificed with the drug present. In sections taken at the level of the dorsal hippocampus from the same animals in which 5-HT_{1A} receptor binding and 5-HT_{1A} receptor-stimulated [³⁵S]GTP S binding was determined in the dorsal raphe nucleus, we found a marked decrease in the binding of [³H]CN-IMI to serotonin reuptake sites throughout the forebrain. Interestingly, there was a significantly greater reduction in [³H]CN-IMI binding in many brain regions from escitalopram-treated animals (Fig. 2), indicating greater occupancy of the serotonin transporter as a result of chronic escitalopram administration.

4. Discussion

In the present study, citalopram and escitalopram treatments provided roughly equivalent amounts of the active S-enantiomer. Chronic treatment with escitalopram decreased the efficacy of 5-HT_{1A} receptors to activate G-proteins, whereas chronic treatment with citalopram did not. Interestingly, chronic escitalopram administration resulted in greater occupancy of serotonin reuptake sites as measured by the inhibition of [³H]CN-IMI binding in forebrain regions.

Chronic administration of citalopram or escitalopram did not alter the binding of the agonist radioligand [³H]8-OH-DPAT in the dorsal raphe nucleus. These results are in agreement with previous findings that chronic treatment with the SSRIs fluoxetine or paroxetine does not alter [³H]8-OH-DPAT binding in the dorsal raphe nucleus (Castro et al., 2003; Le Poul et al., 1995). Thus, desensitization of somatodendritic 5-HT_{1A} autoreceptor function in the dorsal raphe nucleus following chronic SSRI administration is not due to a decrease in the coupled, high affinity agonist state of the 5-HT_{1A} receptor.

Chronic treatment with escitalopram decreased 5-HT_{1A} receptor-stimulated [³⁵S]GTP S binding in the dorsal raphe nucleus. By contrast, this measure of 5-HT_{1A} receptor function is not altered as a result of chronic administration of citalopram by osmotic minipump (present study), or intraperitoneal administration (Rossi et al, 2005), an indication that the route of administration is not a factor. Our data suggest that the desensitization of somatodendritic 5HT_{1A} autoreceptors observed in the electrophysiological and *in vivo* microdialysis experiments following chronic escitalopram treatment (Ceglia et al., 2004; El Mansari et al., 2005) is due to a decrease in the efficacy of 5-HT_{1A} receptors to activate G proteins in the dorsal raphe nucleus, whereas the desensitization of somatodendritic 5HT_{1A} autoreceptors observed following chronic citalopram treatment (Ceglia et al., 2004; Chaput et al., 1986; Cremers et al., 2000; El Mansari et al., 2005) may be due to changes distal to 5-HT_{1A} receptor-G protein interaction.

Citalopram and escitalopram have high affinity for serotonin transporters (Millan et al., 2001; Owens et al., 2001). It is not surprising therefore that the binding of [³H]CN-IMI to serotonin reuptake sites was reduced, as these binding experiments were conducted in the presence of escitalopram or citalopram. The decrease in serotonin transporter binding is likely due to occupancy of serotonin transporters by the drugs and not due to down-regulation of serotonin transporters (Gould et al., 2006).

The effect of citalopram to block 5-HT reuptake, as well as its antidepressant activity, resides in the active isomer escitalopram. Although inactive in terms of inhibiting 5-HT transport, the R(-) enantiomer of citalopram (R-citalopram) appears to limit or counteract the effects of escitalopram in inhibiting 5-HT reuptake (Sanchez, 2006). It has been proposed that R-citalopram may modulate the binding of escitalopram to its primary, high-affinity binding site on the serotonin transporter through an allosteric mechanism. Conversely, the binding of escitalopram to both the primary and the allosteric sites is hypothesized to be responsible for longer binding to and therefore greater inhibition of the serotonin transporter by escitalopram (Sanchez, 2006). The greater reduction in [³H]CN-IMI binding in forebrain regions of escitalopram animals (present study) is consistent with the positive allosteric binding of escitalopram to serotonin transporter sites.

Although citalopram and escitalopram have been shown to desensitize somatodendritic 5-HT_{1A} autoreceptors, our data suggest that the mechanism(s) of desensitization may differ. Perhaps the differences between the effects of chronic administration of citalopram or escitalopram on the regulation of 5-HT_{1A} receptor function in the dorsal raphe nucleus may be accounted for by allosteric modulation of the action of escitalopram by R-citalopram. It is not unreasonable to propose that longer binding to and therefore greater inhibition of the serotonin transporter by escitalopram may result in greater activation of 5-HT_{1A} receptors and regulation of 5-HT_{1A} receptor function at the level of receptor-G protein interaction, as opposed to regulation of 5-HT_{1A} receptor function distal to receptor-G protein interaction. It would be of interest to determine whether differences in the mechanism of somatodendritic 5-HT_{1A} autoreceptor desensitization are related to antidepressant drug efficacy, therapeutic response or clinical outcome.

Acknowledgments

This work was supported by US PHS grants MH 52369 (JGH). We thank Ms. Melissa McCasland for excellent technical assistance, and Dr. Wouter Koek for helpful discussions and critical reading of the manuscript.

References

- Blier P, de Montigny C. Current advances and trends in the treatment of depression. Trends Pharmacol Sci. 1994; 15:220–226. [PubMed: 7940983]

- Castro EM, Diaz A, del Olmo E, Pazos A. Chronic fluoxetine induces opposite changes in G protein coupling at pre and postsynaptic 5-HT_{1A} receptors in rat brain. *Neuropharmacology*. 2003; 44:93–101. [PubMed: 12559126]
- Ceglia I, Acconcia S, Fracasso C, Colovic M, Caccia S, Invernizzi RW. Effects of chronic treatment with escitalopram or citalopram on extracellular 5-HT in the prefrontal cortex of rats: role of 5-HT_{1A} receptors. *Br J Pharmacol*. 2004; 142:469–478. [PubMed: 15148253]
- Chaput Y, de Montigny C, Blier P. Effects of a selective 5-HT reuptake blocker, citalopram, on the sensitivity of 5-HT autoreceptors: electrophysiological studies in the rat brain. *Naunyn Schmiedebergs Arch Pharmacol*. 1986; 333:342–348. [PubMed: 3022157]
- Cremers TI, Spoelstra EN, de Boer P, Bosker FJ, Mork A, den Boer JA, Westerink BH, Wikstrom HV. Desensitisation of 5-HT autoreceptors upon pharmacokinetically monitored chronic treatment with citalopram. *Eur J Pharmacol*. 2000; 397:351–357. [PubMed: 10844134]
- Czachura JF, Rasmussen K. Effects of acute and chronic administration of fluoxetine on the activity of serotonergic neurons in the dorsal raphe nucleus of the rat. *Naunyn Schmiedebergs Arch Pharmacol*. 2000; 362:266–275. [PubMed: 10997729]
- El Mansari M, Sanchez C, Chouvet G, Renaud B, Haddjeri N. Effects of acute and long-term administration of escitalopram and citalopram on serotonin neurotransmission: an in vivo electrophysiological study in rat brain. *Neuropsychopharmacology*. 2005; 30:1269–1277. [PubMed: 15702136]
- Gould GG, Altamirano AV, Javors MA, Frazer A. A comparison of the chronic treatment effects of venlafaxine and other antidepressants on serotonin and norepinephrine transporters. *Biol Psychiatry*. 2006; 59:408–414. [PubMed: 16140280]
- Hensler J, Durgam H. Regulation of 5-HT_{1A} receptor-stimulated [35S]-GtpgammaS binding as measured by quantitative autoradiography following chronic agonist administration. *Br J Pharmacol*. 2001; 132:605–611. [PubMed: 11159711]
- Hensler JG. Differential regulation of 5-HT_{1A} receptor-G protein interactions in brain following chronic antidepressant administration. *Neuropsychopharmacology*. 2002; 26:565–573. [PubMed: 11927181]
- Hensler JG, Kovachich GB, Frazer A. A quantitative autoradiographic study of serotonin_{1A} receptor regulation. *Neuropsychopharmacology*. 1991; 4:131–144. [PubMed: 2025379]
- Hjorth S, Bengtsson HJ, Kullberg A, Carlzon D, Peilott H, Auerbach SB. Serotonin autoreceptor function and antidepressant drug action. *J Psychopharmacol*. 2000; 14:177–185. [PubMed: 10890313]
- Kovachich GB, Aronson CE, Brunswick DJ, Frazer A. Quantitative autoradiography of serotonin uptake sites in rat brain using [3H]-cyanoimipramine. *Brain Res*. 1988; 454:78–88. [PubMed: 2970277]
- Kreiss DS, Lucki I. Effects of acute and repeated administration of antidepressant drugs on extracellular levels of 5-hydroxytryptamine measured in vivo. *J Pharmacol Exp Ther*. 1995; 274:866–876. [PubMed: 7636750]
- Le Poul E, Laaris N, Doucet E, Laporte AM, Hamon M, Lanfumey L. Early desensitization of somatodendritic 5-HT_{1A} autoreceptors in rats treated with fluoxetine or paroxetine. *Naunyn Schmiedebergs Arch Pharmacol*. 1995; 352:141–148. [PubMed: 7477436]
- Millan MJ, Gobert A, Lejeune F, Newman-Tancredi A, Rivet JM, Auclair A, Peglioni JL. S33005, a novel ligand at both serotonin and norepinephrine transporters: I. Receptor binding, electrophysiological, and neurochemical profile in comparison with venlafaxine, reboxetine, citalopram, and clomipramine. *J Pharmacol Exp Ther*. 2001; 298:565–580. [PubMed: 11454918]
- Mork A, Kreilgaard M, Sanchez C. The R-enantiomer of citalopram counteracts escitalopram-induced increase in extracellular 5-HT in the frontal cortex of freely moving rats. *Neuropharmacology*. 2003; 45:167–173. [PubMed: 12842122]
- Newman ME, Shalom G, Ran A, Gur E, Van de Kar LD. Chronic fluoxetine-induced desensitization of 5-HT_{1A} and 5-HT_{1B} autoreceptors: regional differences and effects of WAY-100635. *Eur J Pharmacol*. 2004; 486:25–30. [PubMed: 14751404]

- Owens MJ, Knight DL, Nemeroff CB. Second-generation SSRIs: human monoamine transporter binding profile of escitalopram and R-fluoxetine. *Biol Psychiatry*. 2001; 50:345–350. [PubMed: 11543737]
- Owens MJ, Morgan WN, Plott SJ, Nemeroff CB. Neurotransmitter receptor and transporter binding profile of antidepressants and their metabolites. *J Pharmacol Exp Ther*. 1997; 283:1305–1322. [PubMed: 9400006]
- Pejchal T, Foley MA, Kosofsky BE, Waeber C. Chronic fluoxetine treatment selectively uncouples raphe 5-HT(1A) receptors as measured by [(35)S]-GTP gamma S autoradiography. *Br J Pharmacol*. 2002; 135:1115–1122. [PubMed: 11877317]
- Rossi DV, Valdez M, Gould GG, Hensler JG. Chronic administration of venlafaxine fails to attenuate 5-HT1A receptor function at the level of receptor-G protein interaction. *Int J Neuropsychopharmacol*. 2006; 9:393–406. [PubMed: 16035959]
- Shen C, Li H, Meller E. Repeated treatment with antidepressants differentially alters 5-HT1A agonist-stimulated [³⁵S]GTP gamma S binding in rat brain regions. *Neuropharmacology*. 2002; 42:1031–1038. [PubMed: 12128004]

Fig. 1. Effect of chronic treatment with **A)** citalopram or **B)** escitalopram on 5-HT_{1A} receptor stimulated [³⁵S]GTP S binding in the dorsal raphe nucleus. Specific [³⁵S]GTP S binding is expressed as % above basal. Shown are the mean $\pm$ S.E.M. **A)** saline (n = 6), citalopram (n=6); **B)** saline (n=6), escitalopram (n=7).

Fig. 2.

Chronic treatment with escitalopram results in a greater reduction in the specific binding of [³H]CN-IMI (1 nM) to serotonin reuptake sites in forebrain. Specific [³H]CN-IMI binding in regions of brain from escitalopram- or citalopram- treated animals is expressed as % Decrease from respective saline controls. * P < 0.05 post hoc Fisher's Protected Least Significant Difference test.