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Chronic citalopram treatment elevates serotonin synthesis in Flinders Sensitive and Flinders Resistant Lines of rats, with no significant effect on Sprague-Dawley rats

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Abstract

The influence of citalopram on regional 5-hydroxytryptamine (serotonin, 5-HT) synthesis, one of the most important pre-synaptic parameters of serotonergic neurotransmission, was studied. Sprague-Dawley (SPD) rats were used as the controls, and Flinders Resistant Line (FRL) rats were used as auxiliary controls, to hopefully obtain a better understanding of the effects of citalopram on Flinders Sensitive Line (FSL; “depressed”) rats. Regional 5-HT synthesis was evaluated using a radiographic method with a labelled tryptophan analog tracer. In each strain of rats, the animals were treated with citalopram (10 mg/kg/day) or saline for fourteen days. The groups consisted of between fourteen and twenty rats. There were six groups of rats with citalopram (CIT) and saline (SAL) groups in each of the strains (SPD-SAL, SPD-CIT, FRLSAL, FRL-CIT, FSL-SAL and FSL-CIT). A two-factor analysis of variance was used to evaluate the effect of the treatment (*e.g.*, SPD-SAL relative to SPD-CIT) followed by planned comparisons to evaluate the effect in each brain region. In addition, the planned comparison with appropriate contrast was used to evaluate a relative effects in SPD relative to FSL and FRL, and FSL relative to FRL groups. A statistical analysis was first performed in the *a priori* selected regions, because we had learned, from previous work, that it was possible to select the brain regions in which neurochemical variables had been altered by the disorder and subsequent antidepressant treatments. The results clearly show that citalopram treatment does not have an overall effect on synthesis in the control SPD rats; there was no significant ($p > 0.05$) difference between the SPD-SAL and SPD-CIT rats. In “depressed” FSL rats, citalopram produced a significant ($p < 0.05$) elevation of synthesis in seventeen out of thirty four regions, with a significant ($p < 0.05$) reduction in the dorsal and median raphe. In the FRL rats, there was a significant ($p < 0.05$) elevation in the synthesis in twenty-two out of thirty-four brain regions, with a reduction in the dorsal raphe. In addition to these regions magnus raphe was different in the SPD and FSL groups, but it was on the statistical grounds identified as an outlier. There were significant changes produced in the FSL and FRL rats in thirteen out of seventeen *a priori* selected brain regions, while in the SPD rats, citalopram produced significant changes in only four out of seventeen *a priori* selected regions. The statistical

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evaluation also revealed that changes produced by citalopram in the FSL and FRL rats were significantly greater than those in the SPD rats and that there was no significant difference between the effect produced in the FSL and FRL rats. The presented results suggest that in “depressed” FSL rats, the antidepressant citalopram elevates 5-HT synthesis, which probably in part relates to the reported improvement in behaviour with citalopram.

Keywords

depression model; autoradiography; tracer study; antidepressant treatment

Introduction

The evaluation of biological and behavioural parameters in animal models of depression is very important in obtaining a better understanding of depression (Nestler et al. 2002; Cryan et al. 2002; Cryan and Mombereau, 2004). A large volume of research data has been collected, both in animal models and humans on depression, but there is still no clear understanding of the pathophysiology of major depression, causes of it, or the way antidepressants work in alleviating the symptoms of major depression. Part of the problem is the fact that depression is a rather heterogeneous disorder and there is no consensus on which pathophysiological parameters are the most important in its description (O'Neil and Moore, 2003). The monoamine hypothesis of major depression is mainly based on the fact that drugs acting primarily through serotonergic and noradrenergic systems do alleviate symptoms of major depression in certain groups of patients. However, more recently, there have been several new hypotheses of neurotransmission deficiencies/alterations in depression proposed (Kiss, 2008; Vizi et al. 1995). These newer approaches could, in part, explain the creation of non-physiological circuitry which may underlie the depressive state. There is more and more data available suggesting the relationship between stressful effects of early life and vulnerability to the development of mood disorders (Liu et al. 2000; Sapolsky and Meaney, 1986). For example, maternal separation produces increased adult stress reactivity which results in an elevation of hormonal and corticosterone secretion (Heim and Nemeroff, 1999). Unfortunately, the majority of these studies have been performed in normal animals, which cannot take into account the role of genetics and/or environment-genetics interaction in mood disorders. Some studies, however, were also done in genetically altered animals: Flinders Sensitive Line (FSL) rats, while using Flinders Resistant Line (FRL) as controls (El Khoury et al. 2006). Unfortunately, these controls are not normal, as they have a higher resistance to cholinergic agonists than the rats from which they are derived, and comparisons of the neurochemical and/or behavioural variables may create confound in the interpretation of the results.

The baseline synthesis in FSL rats is lower than the synthesis in either the FRL or SPD rats (Hasegawa et al. 2006), with the baseline synthesis in FRL rats being rather similar to that in SPD rats. Both FSL and FRL rats were generated from the SPD rats by selective breeding. FSL rats have a behavioural supersensitivity and FRL rats have a sub-sensitivity to diisopropyl fluorophosphatase (Overstreet et al. 1979; Russel et al. 1982). This super-sensitivity accords with the cholinergic hypothesis of depression (Janowsky and Risch, 1984) and the hypothesis of serotonergic and cholinergic actions of antidepressants (Dilsaver, 1986; Janowsky and Risch, 1984; Plaznik et al. 1989). The measurements of regional 5-HT synthesis (Hasegawa et al. 2006), tissue levels of 5-HT and other neurochemical and behavioural variables (Overstreet et al. 2005; Yadid et al. 2000) suggest a very complicated interplay of different biochemical parameters in FSL rats. These studies have also documented a large spectrum of both neurochemical (*e.g.*, cholinergic, gabaergic, neurosteroids, dopaminergic, Hypothalamic-Pituitary-Adrenal axis; receptors; 5-HT_{2C}, 5-HT₃, GABA_A) and behavioural

(*e.g.*, immobility) deficiencies in FSL rats, relative to the control FRL or SPD rats (Yadid et al. 2000; Overstreet et al. 2005).

Recently, we reported a rather different effect of acutely administered citalopram on regional serotonin synthesis in FSL rats compared to either SPD or FRL rats (Kanemaru et al. 2008). Acute citalopram treatment resulted in a reduction of the synthesis in FSL rats and an elevation of the synthesis in FRL rats, but without overall significant effect in the SPD rats. A relatively large reduction of regional 5-HT synthesis in FSL rats by acute citalopram is probably, at least in part, the result of the super-sensitivity of 5-HT_{1A} receptors, known to be involved in 5-HT synthesis control (Okazawa et al. 1999; Tohyama et al. 2001 and 2007) found in the FSL rats (Shayit et al. 2003), because there was no significant effect on the synthesis in normal SPD rats. An elevation of the synthesis observed in the FRL rats following acute citalopram (Kanemaru et al. 2008) suggests a rather different 5-HT synthesis control in this strain which probably relates to different intracellular signalling in SPD rats and/or a genetically created resistance to a cholinergic agonist.

Citalopram is a highly selective serotonin reuptake inhibitor (SSRI) with a high affinity and selectivity for serotonin (5-hydroxytryptamine, 5-HT) reuptake sites, with a low affinity for noradrenaline (NA) uptake sites (Hyttel J, 1977; Hiemke and Härtter 2000). It is also clinically effective as an antidepressant and currently prescribed for use (Milne and Goa, 1991). The blocking of the 5-HT transporter by SSRIs locally elevates the concentration of 5-HT, resulting in a greater availability of 5-HT to act on both pre- and post-synaptic 5-HT receptors (Bosker et al. 1995; Ceglia et al. 2004). Chronic treatments are required to alleviate depression, suggesting that some neuronal changes take place before the clinical effects are observed (Salomon et al. 1993; Garattini and Samanin, 1988). Similarly, in animal models of depression (*e.g.*, olfactory bulbectomized rats, FSL rats), only chronic treatments with antidepressants normalize behavioural as well as many neurochemical and endocrine abnormalities found in these models (Kelly et al. 1997; Overstreet et al. 2005). It has also been shown that chronic treatments with citalopram result in an elevation of extracellular concentration of 5-HT (Muraki et al. 2001; Raap and Van der Kar, 1999). This elevation in the extracellular concentration of 5-HT reduces the amount of 5-HT released, which subsequently, and over time, produces adaptive changes and the desensitization of 5-HT autoreceptors in normal rats (Goodwin, 1996; Blier and de Montigny, 1996; Li et al., 1997; Slaterry et al. 2004; Muraki et al. 2008). Following a chronic administration of SSRIs, the desensitization of the serotonergic 5-HT_{1A} autoreceptors on cell bodies and the 5-HT_{1B/D} on the terminals of serotonergic neurons has been documented (Blier et al. 1990; Inverzzini et al. 1992). A sub-chronic citalopram treatment in humans produces a region specific reduction of regional 5-HT synthesis, while a more chronic treatment produces an elevation of regional 5-HT synthesis, which correlates with the reduction of depression scores (Berney et al. 2008). It should be emphasized that these receptor sites and 5-HT synthesis represent only a small fraction of neurochemical parameters defining depression, a very complex state. Similarly, in animal models, only a fraction of behavioural and neurochemical parameters related to the depressed state in humans could be reproduced in any of the presently used animal models (Nestler et al. 2002; Cryan et al. 2002). Unfortunately, until now, a rather small amount of research in which neurochemical parameters were measured has been performed in good animal models of depression.

Because it is the case that only a chronic administration of citalopram produced behavioural changes (*e.g.*, making behaviour close to that in control rats) in the FSL rats (Overstreet et al. 2005; Yadid et al. 2000), it was important to investigate the effect of a chronic treatment of citalopram on regional 5-HT synthesis in FSL rats. The synthesis is one of the most important presynaptic processes related to serotonergic neurotransmission (Nelson, 1993). Further, in another rat model of depression, olfactory bulbectomized rats, citalopram normalized 5-HT synthesis and brought it to the level of sham operated rats (Hasegawa et al. 2005). Knowing

that citalopram has produced rather diverse effects in FSL, FRL and SPD rats following acute treatment (Kanemaru et al. 2008), it was important to study all three strains in a chronic treatment set-up to attempt to obtain a better understanding of citalopram action and possibly the reasons citalopram changes behaviour in FSL rats, but not in FRL rats. It was hypothesized that citalopram would elevate regional 5-HT synthesis in FSL rats as part of its antidepressant effect, because their baseline 5-HT synthesis is lower than in normal SPD rats, without a significant effect in normal SPD rats. This was based on the fact that citalopram does not change behaviour in normal rats and it has been known that there is an interaction between behaviour and brain 5-HT (Sato et al. 2008; Berney et al. 2008). The study in FRL rats was performed to see the effect on regional synthesis in the line of rats termed “resistant”, but very often used as controls in the study of FSL rats. This knowledge could be helpful in the understanding of citalopram action on the neurochemistry of the brain, because an acute treatment elevated regional 5-HT synthesis in the FRL rats (Kanemaru et al. 2008), suggesting a non-responsiveness of the autoreceptors to an elevation of the extracellular 5-HT. We hypothesized that a chronic treatment would have a similar effect on regional synthesis because of the inability of this indirect agonist to reduce the synthesis following acute treatment.

Material and Methods

Citalopram used in the experiments reported here was a generous gift of H. Lundbeck A/S, of Copenhagen, Denmark. Citalopram-HBr was dissolved in saline (0.9% NaCl). The drug and saline in the control rats were delivered by osmotic minipump (Alzet osmotic minipump model 2ML2; ALZA, Palo Alto, CA, USA) implanted subcutaneously on the back of the rats. A dose of 10 mg/kg/day of citalopram was delivered for 14 days by minipump. This was the same dosage we used in previous studies (Hasegawa et al. 2005; Kanemaru et al. 2008). The control rats had the same volume of saline filled in the mini-pump and implanted in the same way as the rats that were given citalopram. The group of rats used in the present studies are: SPD-SAL (saline treated SPD rats), SPD-CIT (SPD treated with citalopram), FRL-SAL (FRL rats treated with saline), FRL-CIT (FRL rats treated with citalopram), FSL-SAL (FSL rats treated with saline), and FSL-CIT (FSL rats treated with citalopram). At the end of the experiment, all of the minipumps were checked for effectiveness by measuring whether they were empty of their contents to the extent expected. The α -[^{14}C]MTrp (α -[^{14}C]methyl-L-tryptophan) used in the experiments was obtained from Amersham Biosciences and had a specific activity of 55 mCi/mmol. All animal procedures were performed in strict accordance with the Canadian Council on Animal Care guidelines, and were approved by the Animal Care Committee of McGill University.

The detailed experimental procedures have been described in several of our previous publications (*e.g.*, Nagahiro et al. 1990; Hasegawa et al. 2005; Watanabe et al. 2003). We therefore only provide a brief description here. Male FSL and FRL rats (weighing around 200 g) were obtained from breeding colonies maintained at the Montreal Neurological Institute from virus free breeds kindly supplied by Dr. D. Overstreet (Center for Alcohol Studies, University of North Carolina, Chapel Hill, NC 27599–7178, U.S.A.), while male Sprague-Dawley (SPD) rats weighing about 200 g were obtained commercially (Charles River Canada, St. Constant, Quebec, Canada). In the present study, twenty eight rats were used in the SPD groups, thirty-one in the FRL, and thirty-eight in the FSL groups (Table 1). The SPD rats were housed in the animal facility (room temperature 22°C and on a 12h day-night cycle) for at least 3 days before being used in the experiments. The night before the synthesis measurements, the animals were fasted overnight to stabilize the plasma glucose and amino acids. At the end of the fourteen day treatment, the rats' femoral artery and vein were cannulated under light isoflurane anesthesia (2–4% for the induction and 1% for the continuation), the rats were placed in a loose fitting plaster cast and allowed to awaken. The arterial PO₂, PCO₂, pH, and hematocrit were checked at least two times during each experiment. All of the rats were killed between

noon and 2 PM. In each experimental group, approximately one half of the rats were sacrificed 60 min following the tracer injection, and the other half 150 min following the injection. The rats were always sacrificed in pairs to reduce possible bias. Following the injection of 30 μ Ci of α -[14 C]MTrp in 1 ml of saline (at a rate of progressively increased periods of time until decapitation. After centrifugation for 5 min at 9300xg, 20 μ L of red into plasma was transferred into 10 ml scintillating was solvents, 1 ml distilled water was added, and the radioactivity was measured by a liquid scintillation counting to obtain the input function (time activity curve). Fifty μ L of plasma was placed into an Ultrafree-MC filter and centrifuged at 12000 rpm for 10 min and the ultrafiltrate was used for the measurement of *free* tryptophan in the plasma. *Total* tryptophan in the plasma was measured in 20 μ L of the plasma pre-treated with 20% of tri-chloroacetic acid (TCA) (50 μ L plasma +25 μ L 20% TCA). An HPLC method using a fluorescence detector was used for the quantification of the plasma free and total tryptophan (Kanemaru et al. 2008; Nagahiro et al. 1990). After decapitation by a guillotine, the brains lices, were extracted, cut into 30 μ m slices, and exposed, to an X-ray film along with standards for approximately three weeks. The films, following exposure, were quantified using an image analysis system (MCID-4; Imaging Research Inc. St. Catharines, Ont. Canada), as previously detailed (Kanemaru et al. 2008). The brain regions were identified using a rat atlas (Paxinos and Watson, 1997) and were quantified bilaterally in four consecutive slices, but there was no left to right difference permitting averaging. Calculations of the uptake constant (K^a ; μ L/g/min) were performed as previously 2008). The rates of 5-HT synthesis (R; pmol/g/min) were calculated as: $R = C_p \times K^a / LC$, where C_p (pmol/mL) is the plasma concentration of free tryptophan. The LC (lumped constant) of 0.42 was used (Vanier et al. 1995).

Regions *a priori* selected

The brain limbic structures (cingulate (Cincx) and enthorinal (Encx) cortices, caudate, nucleus accumbens (AN), thalamus, hippocampus, hypothalamus (Hyp), and amygdala (Amy)), the cortical areas, and dorsal (DR) and median (MR) raphe were *a priori* selected brain regions for our evaluation. This selection of *a priori* regions was based on the current literature on the functional brain correlates of mood, cognitive, and autonomic dysregulations that characterize major depression (Baxter et al. 1989; Soares and Mann, 1997; Bremner et al. 1997; Mayberg, 2003). The PET studies of cerebral blood flow or glucose metabolism in patients with primary depression identified a pattern of corticolimbic, cingulate cortex, amygdala, basal ganglia and thalamus regional dysfunctions believed to underlie the pathogenesis or expression of major depressive disorder (Mayberg, 2003).

Statistical evaluation

The physiological data were analysed with Analysis of Variance (ANOVA) for each variable followed by Newman-Keuls post-hoc correction. The rates of 5-HT synthesis were first evaluated by two-factor ANOVA (factor-1 saline control or citalopram treatment, and factor-2 brain regions) to examine influence of treatment on the synthesis (e.g. SPD-SAL compared to SPD-CIT). The relative overall effects of citalopram were determined by using the user defined planned comparisons with appropriate contrasts following 2-way ANOVA on all six groups; the effect in SPD rats, relative to the FSL and FRL rats, and that in the FRL rats, relative to the FSL rats. The 2-way ANOVA on the groups in each strain of rats was followed by the planned comparisons (*a priori* selected number of comparisons), because only differences in any brain region between saline and citalopram groups were of interest. In general, the pineal body is considered to be outside of the brain and, as such, the synthesis rates were provided in the table but not used in the statistical evaluation. Statistical significance was assigned for $p < 0.05$. All statistical evaluations were done by Statistica 8.0 (StatSoft Inc., Tulsa, OK, USA).

Results

Autoradiograms qualitatively exemplifying regional variability of 5-HT synthesis are provided in Fig. 1 for all three sets of experiments. It is easy to notice that 5-HT synthesis is not homogeneous in many brain regions (*e.g.*, median part of caudate (CPm) has greater synthesis than the lateral (CPl) part; layer VI of the cortex has greater synthesis than other parts; DR is the structure with the greatest synthesis in the brain). The greatest synthesis was found to be in the pineal body (PB), however this structure is generally considered to be outside the brain and, as such, it was excluded from the statistical evaluations. A closer examination of the DR could reveal that the synthesis within this structure is not homogeneous; the lateral wings have somewhat lower synthesis than the centrally located part, but for the purpose of a direct comparison with previous results the DR was not separated into different parts.

The statistical evaluation of the physiological parameters revealed no significant differences in those parameters between the controls (saline treated) and citalopram treated rats in any of these strains in pH, pO₂, pCO₂, hematocrit, the weight at the beginning of experiment (200–220 g), and age (44–47 days). There was no difference in additional variables given in Table 1, except the weight gain ($F(5,89)=19.9$; $p<0.001$) and the weight at the beginning of experiment ($F(5,89)=3.5$; $p<0.01$). The post-hoc test revealed no difference in the weight gain between saline and citalopram groups within any of the strains, but there the weight gains in the SPD groups were greater than those in either FRL or FSL groups (Table 1). The post-hoc test for the weight at the beginning of experiments revealed only significant difference ($p<0.05$) between FRL-SAL and FRL-CIT groups (Table 1).

The mean weights (mean $\pm$ standard error of the mean (SEM)) of rats at the beginning of the experiments were very similar (Table 1), however despite a random assignments to the groups there was a small but significant difference between FRL-SAL and FRL-CIT groups (see above). The difference is rather small it is unlikely that it would create any confound in data interpretation because there was no difference in the age of rats in different groups (Table 1). At the beginning of the experiments, as stated above, there was no significant weight difference between the rats in any of the groups, but at the end of the experiments, the rats in the different groups had significant differences in their weights ($F(5,89)=18.9$; $p<0.001$). Because 5-HT synthesis is related to the plasma concentration free tryptophan (Trp) (Nishizawa et al. 1997), it is important to note that the plasma free or total tryptophan was not significantly different between the groups ($p>0.2$).

The regional 5-HT synthesis rates for both treatment groups and for all three strains are presented in Table 2. The Grubbs' test for outliers (Grubbs, 1969) revealed that the raphe magnus is an outlier (4.85; $N=953$; $p<0.001$) and, as such, this structure was removed from the final overall statistical comparisons. There is no obvious reason why this is the case.

The 2-way ANOVA on SPD groups, after excluding raphe magnus, revealed a significant difference between groups [$F(1,910)=5.85$; $p<0.02$] with a significant group-brain region interaction [$F(34,910)=4.06$; $p<0.001$]. The difference between groups loses significance after correction for multiple tests (three sets of groups were compared). The planned comparisons revealed significant differences in five out of thirty four regions tested (Table 2). In addition the values in the raphe magnus would be different between SPD-SAL and SPD-CIT groups. When an equivalent procedure was done on the *a priori* selected brain regions, the same conclusion was obtained (actual F-values not given).

Citalopram produced overall significant increase in 5-HT synthesis in the FSL rats (FSL-CIT) as compared to the saline treated rats (FSL-SAL) ($F(1,1260)=151.2$; $p<0.001$) with significant group-brain region interaction ($F(34,1260)=10.1$; $p<0.001$). The planned comparisons revealed significant differences in eighteen out of thirty-five regions evaluated with a

significant reduction of synthesis in the median and dorsal raphe nuclei in FSL-CIT group relative to FSL-SAL (Table 2). A statistical evaluation of the *apriori* selected brain regions revealed a significant effect of citalopram in thirteen out of seventeen *apriori* selected brain regions.

In the FRL rats, as mentioned in the Introduction section, the rats very often used as controls in FSL rat studies, the 2-factor ANOVA revealed a significant ($p<0.05$) increase in 5-HT synthesis in the citalopram treated group ($F(1,945)=225.5$; $p<0.001$) (FRL-CIT rats relative to the FRL-SAL group). The planned comparisons revealed significant differences in twenty-three out of thirty-five regions tested, and there was a reduction (-12.8%) of synthesis in the dorsal raphe (Table 2). The synthesis was significantly increased in several limbic structures (e.g., Amy, AN, Encx, thalamus, hippocampus). The largest increase was in the Scn (62.7%) and significant increases varied between few percent and 62.7% . Similarly, as in the FSL rats, citalopram produced a significant effect in thirteen out of seventeen *apriori* selected brain regions.

Overall relative effects between different strains of rats (e.g. effect in SPD compared to the effect in FSL; SPD-SAL minus SPD-CIT compared to FSL-SAL minus FSL-CIT) were tested with 2-factor ANOVA, using all six groups, followed by planned comparisons and appropriate contrasts. This showed that citalopram produced a significantly greater effect on the synthesis in the FSL and FRL rats than in the SPD rats ($F(1,3026)=50.1$; $p<0.0001$ and $F(1,3026)=163.9$; $p<0.001$ for FSL and FRL rats, respectively). When the effect produced in the FSL and FRL rats was compared by the planned comparison, it was found that the effect in the FRL rats was significantly greater ($F(1,3026)=43.6$; $p<0.001$). At the end of the treatment, the synthesis in SPD rats (SPD-CIT) was significantly greater than in the FSL-CIT ($F(1,3026)=3344.8$; $p<0.001$) or FRL-CIT ($F(1,3026)=3040$; $p<0.001$) groups. At the end of the treatment the synthesis in the FSL and FRL groups was marginally different (FSL-CIT compared to FRL-CIT; $F(1,3026)=3.8$; $p=0.05$), with the synthesis in the FSL-CIT group being greater.

A graphical presentation of the relative changes of regional 5-HT synthesis produced by citalopram on a subset of selected brain regions is provided in Fig. 2. From this, it is easy to see that citalopram produced substantial changes throughout the brain in FSL and FRL rats, while the changes in SPD rats are rather minor and occurred only in a few brain regions. Also the changes in the FRL rats produced by citalopram treatment seem to be the greatest.

Discussion

The main findings of the present studies are: 1) Antidepressant and SSRI citalopram produces a significant elevation of 5-HT synthesis in “depressed” FSL rats (differences between FSL-SAL and FSL-CIT groups), while in the control SPD rats, there was no significant effect of citalopram when an analysis was performed on the *apriori* selected brain regions or after removing the raphe magnus, identified as an outlier (planned comparison of SPD-SAL and SPD-CIT groups); 2) There was a significant elevation of the synthesis in the FRL rats (FRL-SAL compared to FRL-CIT); 3) The elevation of 5-HT synthesis was greater in the FRL rats (difference between the FRL-SAL and FRL-CIT groups) than the FSL (FSL-SAL relative to FSL-CIT) rats; 3) An elevation of synthesis was found in eighteen regions of the FSL rats, and in twenty-three regions of the FRL rats, out of thirty-five evaluated. A synthesis reduction was found in the dorsal raphe; 4) In the SPD rats, an effect was observed, using the planned comparison, in only six regions – a reduction in three and an elevation in three; 5) The elevation of 5-HT synthesis coincides with reported normalization of behavioural parameters in FSL rats produced with citalopram (Overstreet et al. 2004).

Because citalopram, when administered at 10 mg/kg/day, also normalized the behavioural variable in this model of depression (Overstreet et al. 2004), one could hypothesize that there is a relationship between regional 5-HT synthesis and the normalization of “depressive” symptoms in FSL rats. This suggests that the alleviation of behavioural “depressive” symptoms (*e.g.*, time of immobility) in the FSL rats correlates with an increase of brain 5-HT synthesis in this rat model of depression. The treatment also brought the regional synthesis in the FSL rats close to those found in the normal SPD rats. Similarly, buspirone treatment produced a normalization of the open field activity and a normalization of regional 5-HT synthesis in the OBX rats (Watanabe et al. 2006; Sato et al. 2008). Further, citalopram produced a normalization of regional 5-HT synthesis in OBX rats (Hasegawa et al. 2005). In depressed humans, regional 5-HT synthesis correlated with Hamilton depression scores before treatment and the treatment produced regionally specific changes (some increases and some decreases) in 5-HT synthesis (Berney et al. 2007), suggesting a region specific correlation between 5-HT synthesis and behaviour.

However, this is not as simple as it may appear, as citalopram did not produce significant changes in the immobility of FRL rats (Overstreet et al. 2004), while in the present study produced the largest increase in 5-HT synthesis among studied strain (Table 2). This would suggest that the response in FRL rats could be related to the cholinergic resistance present in this strain and interactions between cholinergic and serotonergic systems (Jeltsch-David et al. 2008; Rutz et al. 2006). Taken together, the results, as well as previous reports, suggest that the regional imbalance in the synthesis and/or 5-HT regional concentration may be the most important factors in the manifestation and subsequent alleviation of depressive symptoms. It has been suggested previously that probably the most important factor in depression and the action of antidepressants is the relative imbalance in synthesis between different brain regions, rather than the absolute values of the synthesis as such (Rosa-Neto et al. 2004; Berney et al. 2008). Mayberg et al. (2000) showed regional specific changes produced in glucose utilization by antidepressant treatment. Sensitivity to cholinergic agonists has been related to depression in humans (Janowsky et al. 1994), suggesting that the sensitivity is related to the FSL rats as a model of depression, and that the resistance of FRL rats may be something different than in normal rats. Chronic treatment with escitalopram given in food pellets in FSL rats stressed by maternal separation produced a reduction in the number of BrdU positive cells (reduction of neurogenesis) (Petersen et al. 2008). The authors suggested that this reduction could be related to a decrease of glia. Because of the fact that a large fraction of 5-HT metabolised extraneuronally is done in glia cells, this reduction in the glia could influence the homeostasis of 5-HT and, with this, regional synthesis. After acute citalopram treatment, synthesis in FSL rats is reduced (Kanemaru et al. 2008) probably through the activation of autoreceptors. A subsequent elevation of extracellular 5-HT by SSRIs would desensitize the receptors (Blier et al. 1990), reduce the number of glia (Petersen et al. 2008) and create low levels of 5-HT in the neurotransmitter realisable pool, which would result in the need for elevated 5-HT synthesis, as reported here. It is not surprising that citalopram did not produce a large effect in the SPD rats because it has been reported by many investigators that antidepressants do not have much effect in normal humans or normal animals.

The reduction of synthesis in the cell body regions in all three strains of rats (Table 2) after chronic treatment is in line with the reduction reported previously in the sham operated SPD rats treated with the same treatment protocol (Hasegawa et al. 2005), as well as with fluoxetine results, another SSRI (Mück-Seler et al. 1996). Increases in the SPD-CIT rats, relative to the SPD-SAL rats, were found in the AONuc, Cincx and ventral hippocampus (HiV). These observations suggest that in the SPD rats, there are effects on synthesis in some regions important in behaviour (*e.g.*, Amy, Cincx), but it is likely that these changes are not sufficient to have a significant effect on behaviour, as citalopram does not effect immobility in SPD rats (Rygula et al. 2006; Breuer et al. 2007).

The elevation of regional synthesis in “depressed” FSL rats in the terminal regions of serotonergic neurons with a significant reduction in the dorsal and median raphe, and a significant elevation in a large number of brain regions, including the limbic structures (*e.g.*, Amy, Cincx, HiD, thalamus), are novel observations. However, there were several regions in which citalopram did not produce a significant effect (Table 2). These observations suggest that the action of antidepressants is not uniform throughout the brain. A similar observation has been proposed for humans suffering from depression in studies in which it was shown that differences in regional 5-HT synthesis between depressed and matched normal subjects are not uniform throughout the brain (Rosa-Neto et al. 2004), suggesting the importance of a regional imbalance in 5-HT synthesis. This could also relate to region specific changes produced by citalopram in patients (Berney et al. 2008). The fact that this chronic treatment with citalopram produced a relatively small overall reduction of synthesis in SPD rats, while producing an increase in “depressed” FSL rats is of special importance because naïve FSL rats have significantly lower 5-HT synthesis than control SPD rats (Hasegawa et al. 2006).

It has been reported previously that several antidepressants reduce immobility in the swim test in the FSL rats without a significant effect in FRL rats (FRL used as controls) (Overstreet, 2002). Citalopram (Overstreet et al. 2004) and escitalopram (an active stereoisomer of citalopram; El Khouri et al. 2006) have also shown to be effective in increasing swimming in the FSL rats as part of their antidepressant activity, without significant effect in FRL rats. Other antidepressants (setraline, desipramine, imipramine, paroxetine, fluoxetine) had a similar effect on immobility in FSL rats without an effect in FRL rats (Overstreet, 2002). Some of these same drugs showed antidepressant characteristics in other rat models of depression (*e.g.*, high and low sensitive DPAT rats, chronic mild stress model, olfactory bulbectomized rats) (Overstreet, 2002; Papp et al. 2002; Sanchez et al. 2003; Hasegawa et al. 2005; Sato et al. 2008; Watanabe et al. 2006). In accordance with those antidepressant actions in FSL rats, the present results show that while the behaviour of the FSL rats is improved, regional 5-HT synthesis is elevated in the fields of serotonergic terminals. This elevated synthesis accords with increased 5-HT turnover produced in FSL rats by the antidepressant, desipramine (Zangen et al. 1997). Because the FSL rats have the same extracellular levels of 5-HT as the SPD controls (Dremencov et al. 2005), the action of SSRIs would produce a lack of 5-HT in the ready realisable pool, as was reported for fluoxetine (Geyer et al. 1978), and the system, likely through a second messenger(s), would respond by an elevation of synthesis.

We recently reported the effects of acutely administered citalopram on regional synthesis in both the FSL and FRL rats and compared the effects with those in SPD rats (Kanemaru et al. 2008). In that study, acute treatment with citalopram compared to that with saline, has not produced a significant effect in the SPD rats, but it elevated synthesis in a few brain regions (*e.g.*, AONuc, Cincx) and reduced synthesis in the dorsal and median raphe nuclei. This acute treatment produced a significant reduction in the FSL rats and an elevation in the FRL rats. Because the synthesis is elevated in the FRL rats even following chronic treatment, this suggests that the receptor controlling the synthesis in the FRL rats does not respond to an extracellular elevation of 5-HT, as it does in the FSL or SPD rats. Here, it is assumed that chronic citalopram would elevate extracellular 5-HT as is the case in normal rats (Invernizzi et al. 1992). It might then appear that there is no relationship between 5-HT synthesis and behaviour in FRL rats. However, there is a possibility that cholinergic resistance present in the FRL rats through the interaction between the cholinergic and serotonergic systems, and possibly other monoaminergic systems, is masking the behavioural influence of 5-HT. This hypothesis is supported by the fact that regional 5-HT synthesis is negatively correlated with a number of suicidal attempts (Leyton et al. 2006), with the Hamilton depression scores (Berney et al. 2008), and having positive and negative correlations with joy and sadness, respectively (Perreau-Linck et al. 2007).

FSL rats have lower 5-HT in the realisable pool (derived from lower 5-HT turnover (Zangen et al. 1997) and lower 5-HT synthesis (Hasegawa et al. 2006) which, coupled with a supersensitivity to 5-HT autoreceptors (Overstreet et al. 1998 and 2005; Kanemaru et al. 2008), suggests that following chronic treatment with citalopram, the receptors and/or intracellular signalling modifies regional control, resulting in the elevation of synthesis (present report). The elevation of synthesis in the FRL rats, both after acute (Kanemaru et al. 2008) and chronic administration (present data), suggest that the receptors and/or intracellular signalling (second and third messengers) controlling 5-HT synthesis do not respond the same way as in the SPD control rats to an elevation of extracellular 5-HT. A synthesis elevation could also be related to a neuronal response to replenish the realisable pool after citalopram blocks the reuptake of 5-HT, because the realisable pool is the first one to be replenished with newly synthesized 5-HT (Geyer et al. 1978; Hamon et al. 1979). This hypothesis is supported by immunoassaying results showing a reduction of 5-HT stain in the intracellular compartment in SSRI treated rats (Geyer et al. 1978).

In the present studies, statistically significant differences were produced by citalopram in some brain regions in SPD rats (*e.g.*, amygdala, dorsal raphe), despite a lack of overall effect of citalopram after the removal of an outlier (in which synthesis was affected by citalopram in the sham operated rats (Hasegawa et al. 2006)). A very small effect of citalopram was produced in the SPD rats following acute citalopram treatment (Kanemaru et al. 2008), further suggesting that measurements of neurochemical effects of citalopram in normal rats have little relevance to action in the “depressed” brain.

The results obtained in the FRL rats may also be of interest because they suggest that citalopram, an indirect 5-HT agonist, after prolonged treatment, can produce a desensitization of receptors controlling 5-HT synthesis. This follows from the fact that after this chronic treatment, there were several brain regions (*e.g.*, cingulate, frontal cortex, median raphe) in which 5-HT synthesis did not differ between the citalopram and saline treated groups (Table 2). However, there were elevations in the synthesis in these brain regions following acute treatments (Kanemaru et al. 2008). It is interesting to note that while 5-HT synthesis, after an acute treatment, was elevated in the dorsal and median raphe (Kanemaru et al. 2008), it was significantly reduced in the dorsal raphe following chronic treatment, suggesting that after prolonged activation of autoreceptors and/or second messengers, the synthesis became influenced by elevated extracellular levels of 5-HT. This has also been reported for 5-HT_{1A} agonists (Okazawa et al. 1999; Tohyama et al. 2007) and another SSRI fluoxetine (Mück-Šeler et al. 1996). In normal rats, chronic paroxetine treatment resulted in a reduction of synthesis (Yamane et al. 2001).

A significant elevation of the synthesis in many terminal brain regions following chronic treatment in the FRL rats suggest, as was concluded following acute treatment (Kanemaru et al. 2008), that the synthesis in FRL rats is differently controlled in this strain than the control in either the SPD or FSL rats. When comparing the synthesis in the FSL and FRL rats after citalopram treatment, one can notice that the regional synthesis is very similar, suggesting that there is some kind of relationship between regional synthesis and behavioural tests, because after citalopram treatment, the behaviour of the FSL and FRL rats are about the same in the FRL and FSL rats. However, the synthesis in neither of these rats is at the level found in the normal rat (SPD), suggesting that maybe the absolute levels of the regional synthesis are less important than the relative differences between brain regions. In the OBX rats, another rat model of depression which has elevated serotonin synthesis (Watanabe et al. 2003; Hasegawa et al. 2005), citalopram treatment produced a normalization of regional synthesis; it reduced it to the levels found in the sham operated rats (Hasegawa et al. 2005). It should be noted that OBX and FSL rats also represent two different models of depression; the former one is a model of agitated depression (Kelly et al. 1997) while the latter one is a model of depression with

psychomotor retardation (Overstreet et al. 2005). The data presented clearly show that when the baseline synthesis is pathologically low (*e.g.*, FSL rats), citalopram can elevate it, and *vice versa*.

In conclusion, the present data suggest that citalopram, a SSRI and antidepressant, produces region specific increases of 5-HT synthesis in many limbic and cortical regions in FSL (“depressed”) rats without any significant effect on the synthesis in normal SPD rats. This elevation in the synthesis coincides with an improvement of behaviour in the citalopram treated FSL rats. Because citalopram also produced an elevation of synthesis in the FRL rats, both following acute (Kanemaru et al. 2008) and chronic (present data) treatments, but does not change behaviour in these rats (Overstreet et al. 2005), it is suggested that a cholinergic resistance produced a mutation which is incompatible with the normal rat. As a result of this and previously reported findings in the FSL and FRL rats, the use of FRL rats in the study of FSL rats could produce a confound in data interpretation.

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Figure 1.

A set of autoradiographic images exemplifying regional variability in serotonin synthesis in SPD, FRL and FSL groups of rats treated with saline (-SAL; subcutaneous minipump; 14 days) and citalopram (-CIT; 10 mg/kg/day by subcutaneous minipump for 14 days). Several brain regions are approximately identified in these autoradiograms and they are: AN-accumbens; Cinex-cingulate cortex; CPI-caudate lateral part; CPm-caudate median part; DR-dorsal raphe; Layer VI-sixth layer of cortex; HiD-dorsal hippocampus; HiV-ventral hippocampus; Hyp-hypothalamus; MR-median raphe; Pcx-parietal cortex; Scxsomatosensory cortex; ThD-dorsal thalamus; ThV-ventral thalamus; Vcx-visual cortex.

Figure 2.

A graphical representation of the citalopram effect on the regional serotonin synthesis calculated as percent of the synthesis in respective control. The error bars are SEM calculated as the square root of the sum of square of the relative errors in individual values.

Table 1

Physiological parameters of the rats in different treatment groups[/]

Parameter	SPD-SAL N=14	SPD-CIT N=14	FRL-SAL N=15	FRL-CIT N=14	FSL-SAL N=18	FSL-CIT N=20
Gain Weight (g)	95.2±4.4 ^a	93.8±3.2 ^b	64.2±3.1 ^c	69.6±2.8	70.1±2.2 ^d	74.9±1.6
Free-Trp (nmol/ml)	9.46±0.61	9.17±0.81	8.48±0.68	8.7±1.0	8.74±0.81	8.97±0.71
Total-Trp (nmol/ml)	66.6±4.9	62.5±5.7	53.8±3.7	55.8±3.5	61.2±3.3	56.9±2.8

[/] The values are provided as mean± standard error of the mean (SEM). The number of animals in each group is given as an “N” under the group identification.

^a Significantly different from the gain in either the FRL-SAL or FSL-SAL groups

^b Significantly different from the gain in either the FRL-CIT or FSL-CIT groups

^c Significantly different from the gain in the FRL-CIT group

^d Significantly different from the gain in the FSL-CIT group

Table 2
Regional 5-HT synthesis in FSL, FRL and SPD rats treated with saline or citalopram (10 mg/kg/day for 14 days; s.e. minipump)¹

Regions ³	SPD-SAL ² R±SEM (N=14)	SPD-CIT ² R±SEM (N=14)	FSL-SAL ² R±SEM (N=18)	FSL-CIT ² R±SEM (N=20)	FRL-SAL ² R±SEM (N=15)	FRL-CIT ² R±SEM (N=14)
Acx	57.6±1.7	59.0±1.9	28.62±0.92	35.6±1.0 ^b	23.19±0.37	27.8±1.5
AMY	63.5±1.8 ^a	51.6±2.2	31.77±0.92	41.9±1.0 ^b	31.74±0.73	40.2±1.3 ^c
AN	72.2±2.3	74.5±3.2	50.5±1.3	50.7±1.5	40.28±0.87	59.6±1.0 ^c
AONucl	53.9±1.7 ^a	61.7±2.0	33.1±1.1	37.4±1.1	31.63±0.80	37.4±1.8 ^c
Cinex	46.8±2.0 ^a	53.7±1.8	31.5±1.1	44.4±1.8 ^b	24.81±0.51	28.8±1.1
Cl	67.2±2.1	68.3±2.3	40.3±1.0	42.1±1.3	31.12±0.50	40.1±1.4 ^c
CN	60.8±1.9	61.0±2.0	35.9±1.0	41.9±1.2 ^b	27.64±0.55	41.3±1.2 ^c
CPI	55.6±1.7	54.0±2.0	33.79±0.94	37.7±1.1	24.83±0.50	36.0±1.4 ^c
CPm	74.2±1.9	69.6±2.3	43.4±1.0	46.8±1.3	32.59±0.71	44.6±1.1 ^c
DR	190.4±3.7 ^a	174.8±7.4	135.1±3.6	118.7±3.5 ^b	152.4±2.8	132.9±4.8 ^c
DS	47.0±1.7	47.5±1.7	23.22±0.92	32.0±1.0 ^b	19.97±0.55	22.9±1.3
Encx	70.4±1.9	68.2±2.4	40.78±0.83	43.0±1.0	28.72±0.65	38.7±1.5 ^c
Fcx	46.9±1.7	49.7±1.7	26.0±1.0	32.8±1.1 ^b	21.93±0.47	26.3±1.4
GP	51.9±1.7	54.8±1.9	29.2±1.1	35.1±1.2 ^b	23.63±0.50	35.0±1.4 ^c
HiD	67.3±1.9	68.1±2.3	37.4±1.0	42.3±1.1 ^b	29.03±0.61	41.5±1.1 ^c
HiV	78.2±2.0 ^a	88.3±2.5	45.5±1.1	49.4±1.2	33.88±0.75	42.3±1.5 ^c
Hyp	66.9±1.9	67.1±2.6	37.3±1.3	43.4±1.2 ^b	36.6±1.0	46.8±1.2 ^c
LC	63.4±2.4	60.5±2.5	29.12±0.93	32.2±1.1	25.7±1.0	32.7±1.5 ^c
LG	53.0±1.8	53.8±1.8	27.51±0.83	35.8±1.2 ^b	26.60±0.51	30.0±1.0
MBF	61.4±1.8	58.8±2.1	30.8±1.0	34.3±1.1	29.06±0.85	41.9±2.2 ^c
MG	51.7±2.0	53.4±2.1	26.6±1.1	33.6±1.2 ^b	22.81±0.44	26.5±1.3
MR	133.6±3.5	132.4±7.0	93.7±2.9	75.8±3.3 ^b	93.3±2.4	97.8±3.2
PB	680.±12.	710.±16.	610.±10.	630.±10.	708.±10.	692.±18.
Pcx	52.0±1.4	47.7±1.8	22.9±1.1	32.15±0.91 ^b	20.63±0.60	27.4±1.5 ^c

Regions ³	SPD-SAL ² R±SEM (N=14)	SPD-CIT ² R±SEM (N=14)	FSL-SAL ² R±SEM (N=18)	FSL-CIT ² R±SEM (N=20)	FRL-SAL ² R±SEM (N=15)	FRL-CIT ² R±SEM (N=14)
R_mag	91.3±4.4 ^a	60.0±3.9	43.7±1.5	54.3±1.6 ^b	54.5±1.9	59.1±7.5
Rpo	75.0±2.8	76.5±3.2	47.3±1.4	46.2±1.6	35.6±1.2	55.8±1.4 ^c
SC	53.0±1.8	50.6±2.1	24.1±1.0	31.0±1.1 ^b	21.69±0.40	17.9±1.7
Sen	74.9±2.4	74.9±2.5	45.5±1.6	48.0±1.6	39.22±0.84	63.8±2.2 ^c
Sex	53.2±1.5	50.9±1.8	27.6±1.1	31.5±1.0	19.91±0.45	27.3±1.4 ^c
Sep.n.	57.3±1.6	56.1±1.9	31.0±1.0	35.6±1.0 ^b	25.14±0.51	33.0±1.2 ^c
SNc	52.1±1.9	53.6±2.2	28.19±0.82	28.2±1.2	23.33±0.65	27.0±1.3
SNr	43.1±1.5	43.4±1.9	21.7±1.0	25.0±1.1	21.35±0.66	26.1±1.5
ThD	48.6±1.6	46.0±2.1	22.4±1.0	31.1±1.0 ^b	20.88±0.59	26.8±1.1 ^c
ThV	47.5±1.8	49.0±1.9	26.1±1.0	32.5±1.1 ^b	19.00±0.65	29.1±1.0 ^c
Vcx	56.3±1.7	57.4±2.3	26.4±1.1	34.9±1.0 ^b	23.91±0.47	30.2±1.4 ^c
VTA	59.6±1.7	60.1±2.0	33.81±0.92	35.2±1.2	29.63±0.79	36.0±1.4 ^c

¹ Synthesis rates are provided in pmol/g/min as the mean ± standard error of the mean (SEM)

² The number below the group identification is the number of rats in each particular group.

³ Abbreviation for the regions are: Aex-auditory cortex; AMY-amigdala; AN-accumbens; AONuc-anterior olfactory nucleus; Cinex-cingulate cortex; Cl-claustrum; CN-caudate nucleus; CPI-caudate-putamen (lateral); CPm-caudate-putamen (median); DR-dorsal raphe; DS-dorsal subiculum; Enex-enthorinal cortex; Fcx-frontal cortex; GP-globus pallidus; HiD-hippocampus (dorsal); HiV-hippocampus (ventral);Hyp-hypothalamus; LC-locus coeruleus; LG-lateral geniculate; MBF-median forebrain bundle; MG-medina-geniculate; MR-median raphe; PB-pineal body; Pex-parietal cortex; R_mag-raphe magnus; Rpo-pontine raphe; SC-superio colliculus; Sen-supra-chiasmatic Nucleus; Sex-somatosensory cortex; Sep.n.-septal nucleus; Snc-substantia nigra compacta; Snr-substantia nigra reticulata; ThD-thalamus (dorsal); ThV-thalamus (ventral); Vcx-visual ortex; VTA-ventral tegmental area.

^aThe synthesis in the SPD-SAL group is significantly different from the SPD-CIT group.

^bThe synthesis in the FSL-SAL group is significantly different from the FSL-CIT group.

^cThe synthesis in the FRL-SAL group is significantly different from the FRL-CIT group.