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Both acute and chronic buspirone treatments have different effects on regional 5-HT synthesis in Flinders Sensitive Line rats (a rat model of depression) than in control rats

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Abstract

The main objective of this investigation was to evaluate the effects of buspirone, a 5-HT_{1A} agonist with some partial agonist properties and also an antidepressant, on regional 5-HT synthesis in Flinders Sensitive Line (FSL) rats ("depressed"), and to compare the effects to the Flinders Resistant Line (FRL) control rats (not "depressed"). In addition results were compared to those previously reported in normal Sprague-Dawley (SPD) rats (normal control). Serotonin synthesis in both FSL and FRL rats was measured following acute and chronic treatments with buspirone. Both of these strains were derived from the SPD rats. No direct comparison was done between the FSL saline and FRL saline groups, or the FSL buspirone and FRL buspirone groups, because the objective of the studies was to evaluate effects of buspirone in these two strains. The results show that acute treatment with buspirone elevates 5-HT synthesis throughout the brain in the FRL rats. In the FSL rats, there were reductions in some brain regions (*e.g.*, dorsal and median raphe, amygdala, anterior olfactory nucleus, substantia nigra reticulata), while in other regions, there were increases in the synthesis observed (*e.g.*, frontal, parietal, visual and somatosensory cortices, ventral hippocampus). In twenty out of the thirty brain regions investigated in the FSL rats, there was no significant change in the synthesis following acute buspirone treatment. During the chronic treatment, buspirone produced a significant reduction of 5-HT synthesis in fifteen out of thirty brain regions in the FRL rats. In the FSL rats, buspirone produced a significant elevation of the synthesis in ten out of thirty brain regions. In both the FSL and FRL rats, buspirone produced rather different effects than those reported previously for SPD (normal) rats. The acute effect in the FSL rats was somewhat similar to the effect reported previously for the SPD rats, while in the FRL rats, the acute buspirone treatment produced an effect observed previously in treatments with 5-HT_{1A} antagonists suggesting an action of buspirone as partial agonist in FRL rats. The data suggest that with respect to 5-HT synthesis, FRL rats differ from SPD rats (a natural control; normal rats) and, as such, indicate that when the effects related to the serotonergic system

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(*e.g.*, influence of serotonergic drugs) are studied in the FSL rats and compared to those in the FRL rats, any conclusions drawn may not reflect differences relative to a normal rat.

Introduction

Serotonin is a brain neurotransmitter which acts through fifteen different receptors (Barnes and Sharp, 1999). One way in which 5-HT acts on the brain serotonergic neurotransmission and through which 5-HT modulates 5-HT synthesis are at the 5-HT_{1A} sites (Hutson et al., 1989; Blier and de Montigne, 1990; Blier et al., 1990; Okazawa et al., 1999; Tohyama et al. 2001 and 2007). It is now thought that 5-HT and/or the norepinephrine receptor adaptive processes in the brain are the basis for the efficacy of antidepressant treatments, including electroconvulsive therapy (Price et al, 1991; Deakin, 1991). Based on clinical studies, 5-HT_{1A} receptors seem to be involved in anxiety and depressive disorders because 5-HT_{1A} partial agonists, such as buspirone, have therapeutic values (Detke et al. 1995; Wieland and Lucki, 1990). Dysfunction of the brain serotonergic system has been postulated for quite some time as one of the biological parameters of depression (Judd, 1995). The alteration of serotonin synthesis has been found in two regularly used animal models of depression, the Flinders Sensitive Line (FSL) (Hasegawa et al. 2006) and olfactory bulbectomized (OBX) (Watanabe et al. 2003; Hasegawa et al. 2005) rats. Buspirone is a potent and selective agonist of 5-HT_{1A} receptors (De Vivo and Maayani, 1986; Hoyer and Boddeke, 1993) and it has antidepressant effects in animal models of depression (Kennett et al. 1987; Watanabe et al. 2006), as well as in humans (Goldberg and Finnerty, 1982; Schweizer et al. 1986; Lucki et al. 1994). In some situations, buspirone acts as a partial agonist at the 5-HT_{1A} receptors (De Vivo and Maayani, 1986, Hoyer and Boddeke, 1993). Buspirone also has region specific effects on the density of 5-HT_{1A} receptors, including normalizing open field activity in OBX rats (Sato et al. 2008), normalizing elevated 5-HT synthesis in OBX rats (Watanabe et al. 2006), and normalizing behavior abnormalities of Flinders Sensitive Line (FSL) rats (Overstreet et al. 1995 and 2005). It has been also shown that other 5-HT_{1A} agonist, flesinoxan, modulates 5-HT synthesis via 5-HT_{1A} receptors (Tohyama et al. 2007; Skelin et al. 2008). Taken together observations described above suggest that buspirone and other 5-HT_{1A} agonist modulate regional 5-HT synthesis which in part influences behavior.

5-HT_{1A} receptors are located both on the dendrites and soma of serotonergic neurons, and on the postsynaptic neurons with relatively high densities in the limbic and cortical regions (Verge et al. 1986). Pre- and post-synaptic 5-HT_{1A} receptors have similar biochemical and physiological characteristics (Radja et al. 1992) but their pharmacological properties are reasonably different (Meller et al., 1990).

Buspirone is a partial agonist on the 5-HT_{1A} receptor (De Vivo and Maayani, 1986). Buspirone was developed as an antipsychotic drug with D2 antagonistic properties, and anxiolytic and antidepressant properties (Kennett et al. 1987; Lucki et al. 1994). This means that with high extracellular availability of serotonin, buspirone would exert antagonistic action on 5HT_{1A} receptors, while with low serotonin availability it would act as an agonist (Lucki et al. 1994). One should keep in mind that buspirone has a pharmacologically active metabolite, α 2-adrenergic antagonist (Caccia et al. 1986), which could contribute to a chronic treatment.

There have been several animal models of depression with different levels of similarity with human depression (Nestler et al., 2002; O'Neil and Moor, 2003; Willner and Mitchell, 2002). FSL rats are one of the better animal models of depression from the perspective of biological alteration (Yadid et al., 2000; Overstreet et al., 2005), and it has face, construct and predictive validity with a reasonably well-understood neurobiological basis of behavioural differences (Overstreet 2002; Overstreet et al., 2005). In a large majority of studies on the behaviour and

antidepressant actions in FSL rats, Flinders Resistant Line (FRL) rats were used as control animals. Both FSL and FRL rats were generated by selective breeding from Sprague-Dawley (SPD) rats (Overstreet et al., 1979; Russell et al., 1982). There are two lines which were selected as having a high (FSL) and low (FRL) sensitivity, to the anticholinesterase agent, diisopropyl fluorophosphatase. In comparison to the FRL and SPD rats, the FSL rats have reduced locomotion, increased anhedonia in response to chronic mild stress, increased rapid eye movement (REM) sleep, a reduction in REM sleep onset, and reduced body weight (Overstreet 2002; Overstreet et al., 2005). Similarly, as is the case with patients, chronic, but not acute, treatment of FSL rats with antidepressants, such as desipramine, imipramine, sertraline, or fluoxetine, improved their behavioural characteristics, relative to the FRL rats. In contrast to the FSL rats, the FRL rats do not express depressive-like behaviour or neurochemical abnormalities (Overstreet et al. 2005). It has been proposed that the FSL model may also model a predisposition to depression, as well as depression, *per se* (Willner and Mitchell, 2002).

The deficiency in regional 5-HT turnover/content (Zangen et al. 1997) and regional 5-HT synthesis (Hasegawa et al. 2006) in FSL rats suggests the possibility of creating a regional nonphysiological circuitry (Spont, 1992), similar to how it likely occurs in another rat model of depression, olfactory bulbectomized rats (Watanabe et al. 2003 and 2006; Hasegawa et al. 2006). However, in the FSL rats, this lower turnover is likely a result of lower release and/or lower 5-HT synthesis, in addition to changes in 5-HT induced dopamine release (Dremencov et al. 2005). This low release and synthesis probably does not provide enough 5-HT for the creation of normal circuitry, and at the same time increased levels of intracellular 5-HT (Zangen et al. 1997), which would then reduce 5-HT synthesis, as reported previously (Hasegawa et al. 2006), by inhibiting tryptophan hydroxylase (Macon et al. 1971; Hamon et al. 1972; Thierry et al. 1968). There is also a possibility that non-synaptic release of neurotransmitter (Vizi, 1980) and/or uptake of 5-HT into noradrenergic neurons (Vizi et al., 2004) contribute to different extend in these strains to the overall effects observed.

In this report, the effects of acute and chronic buspirone treatments are presented in the FSL and FRL rats. Here, as it has been case with a large majority of studies on FSL rats, the FRL rats were used as a control strain (group) in addition to a historical control group, SPD (Okazawa et al. 1999). The effects of buspirone observed in the both control groups (FRL reported here and SPD historical controls) were compared to those in the FSL rats. The main objective of these studies was the investigation of buspirone, known to act as an antidepressant in humans and animal models of depression, on regional 5-HT synthesis. The regional 5-HT synthesis was shown before to be one of the neurochemical parameters influenced by antidepressants, both in rats (Hasegawa et al. 2005, Mück-Šeler et al. 1996; Tsuiki et al. 1995) and depressed humans (Berney et al. 2008). Both acute and chronic studies were done in an attempt to obtain a better understanding of drug actions. The objective of the acute treatment was to obtain information on the initial response of the serotonergic system controlling 5-HT synthesis (e.g. 5-HT_{1A} sites; Okazawa et al. 1999; Tohyama et al. 2007; Skelin et al. 2008), while the chronic study was performed to evaluate the drug effect of a schedule generally used in antidepressant treatments. Both treatments followed the same schedule as those used previously for measurements in SPD rats, which gave us the opportunity to compare the data in normal SPD rats (Okazawa et al. 1999) to those obtained in the present study.

Materials and Methods

Animals and treatment delivery

All of the animals were produced at the Animal Facility of the Montreal Neurological Institute by congener breeding from two pairs provided by Dr. D.H. Overstreet (Center for Alcohol Studies, University of North Carolina, Chapel Hill, NC 27599-7178, U.S.A.). The rats were

housed in quarters with automatic light control (7 p.m. to 7 a.m. dark cycle) and they were sacrificed at approximately the same time of day. All animal procedures were in strict accordance with the Canadian Council on Animal Care guidelines, and were approved by the Animal Care Committee of McGill University. The night before the synthesis measurements, the animals were fasted overnight to stabilize the plasma glucose and amino acids. Water was given *ad libitum*. The rats were treated with a 5-HT_{1A} partial agonist, buspirone (Vander Maelen et al. 1986). In the acute experiments, the rats were injected with 10 mg/kg of buspirone 30 min before the tracer administration. In the chronic treatment, the buspirone was delivered continuously by mini-pump (model 2ML2; ALZA, Palo Alto, CA) at a dose of 10 mg/kg/day for fourteen days with the synthesis measurements done on the 15th day. The control rats were given the same volume of saline because saline was used as a solvent for buspirone. Fourteen days following the beginning of the treatment the rats' femoral artery and vein were cannulated under light halothane anesthesia (2-4% for the induction and 1% for the continuation). The doses of buspirone and the time of the tracer administration relative to the drug were selected on the basis of a previous study performed with SPD rats (Okazawa et al. 1999; normal rats).

Autoradiographic measurements

A detailed description of the experimental procedure is provided in our previous publications (e.g., Nagahiro et al. 1990; Okazawa et al. 1999; Nishi et al. 2006), and therefore only a short summary will be presented here. Following cannulation under halothane (1-2%) anesthesia, the rats were placed in a loose fitting plaster cast and allowed to awaken. In the acute experiments, tracer was injected 2 h following surgery. The arterial PO₂, PCO₂, pH, and hematocrit were checked at least two times during each experiment. In the acute experiments the blood gases were measured just before injection of buspirone or saline and fifty minutes after tracer injection (80 min after buspirone/saline injection). In the chronic experiments the blood gases were measured just before tracer injection and fifty minutes after tracer injection. To avoid the possible effect of circadian rhythm on the results, the tracer was always injected between noon and 2 PM. In each experimental group, 12-14 rats were used; one half was sacrificed 60 min following the tracer injection, and the other half 150 min following the injection. These two decapitation points were selected on the bases of the tissue time activity curves of this tracer and they represent a compromise between the tracer uptake and duration of experiment (Diksic et al 1990; Nagahiro et al. 1990). The rats were always sacrificed in pairs to avoid bias. Following the injection of 30 μ Ci of α -[¹⁴C]MTrp in 1 ml of saline (at a rate of 0.5 ml/min), 14 to 15 arterial blood samples (50 μ L each) were taken at progressively increased periods of time until decapitation. After centrifugation for 5 min at 9500 $\times$ g, 20 μ L of plasma was transferred into 10 ml scintillating 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). The progressively increased time intervals were selected because the plasma time activity curve changed progressively less as time after injection became longer (Diksic et al. 1990). *Free* tryptophan in the plasma was measured in an ultrafiltrate of plasma (50 μ L plasma into an Ultrafree-MC filter and centrifuged at 12000 rpm for 10 min). *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). The plasma free and total tryptophan were measured by an HPLC method using a fluorescence detector (Nagahiro et al. 1990). After decapitation by a guillotine, the brains 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 outlined (Nagahiro et al. 1990). The brain regions were identified using a rat atlas (Paxinos and Watson, 1997) and were quantified bilaterally in four consecutive slices. Calculations of the uptake constant (K^a ; μ L/g/min) were performed as previously described (Nagahiro et al. 1990; Nishi et al. 2006). 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).

Statistical Evaluation

First, a General Linear Model (GLM) analysis of variance was run on the results of the acute and chronic experiments to determine if there are differences between the saline treated (controls) and buspirone treated groups of rats, as well as if there is a significant interaction between the different groups and the brain regions among these groups. It should be noted that post-hoc planned comparisons were always done between the treatment group and its respective control group (*e.g.*, FSL saline relative to FSL buspirone). No direct comparisons were done between FSL-saline and FRL-saline, or FSL-buspirone and FRL-buspirone because of a possibility of creating a different level of a confound variable by injection (acute) or subcutaneous minipump (chronic) in these two strains. To find out which of the brain regions were different in any of the set (acute and chronic), one-way ANOVA planned comparisons were calculated for each comparison between the control and respective treatment group as part of the post-hoc evaluations. The $p < 0.05$ was taken to indicate significance. To obtain a quantitative overall estimate of the effect of treatment, a one sample two-tailed t-test was used on the mean ratio by comparing it to an ideal ratio of 1 ± 0 . When the ratio is significantly greater than one, the treatment produced a reduction in the synthesis and when it is smaller than one, the treatment produced an elevation in synthesis. All statistical evaluations were performed with Statistica 7.1 (StatSoft Inc., USA).

Results

There were no differences between the blood PaCO_2 , pH and hematocrit when the control and buspirone groups after either the acute or chronic treatments were compared (Table 1). Blood oxygen tensions (PaO_2) were significantly increased following the acute treatments with buspirone ($F(3,50)=33.0$; $p < 0.001$), and the increases were significant in the FSL ($F(1,25)=11.7$; $p < 0.002$) and FRL ($F(1,25)=268$; $p < 0.001$) rats. There were differences in the weight (mean $\pm$ SEM (standard error of the mean)) between the rats treated with saline and those treated with buspirone, and the weight gains were as follows: FSL rats (saline) = 72.2 ± 3.6 g and FSL rats (buspirone) = 45.5 ± 4.9 g. This is a significantly different result ($F(1,23)=17.2$; $p < 0.001$). In the FRL rats, there was no difference in weight gain (FRL rats (saline) = 67.6 ± 4.6 g and FRL rats (buspirone) = 62.0 ± 4.4 g; $F(1,26)=0.77$; $p > 0.05$). At the beginning of the treatments, there was no significant difference in the weight of rats between different experimental groups (Table 1). Also there were no differences in the mean age of rats when included in the protocol (acute-time of experiment; chronic-implantation of the minipump) or the mean weight at this time (Table 1). From this also follows that there was no difference in the mean weight gain in these rats between birth and inclusion into experimental protocols (Table 1). Because the rats were randomly included into different groups the weight gain from the birth was also compared for all FSL and FRL rats and it was found not be different (4.19 ± 0.06 g/day for FSL rats ($N=52$) and 4.18 ± 0.05 g/day for FRL rats ($N=52$)). At the time of inclusion into experiments the overall mean age of the FSL rats was 50.7 ± 0.6 [days] and of the FRL rats was 50.6 ± 0.7 [days] without any difference in age between individual experimental groups (Table 1).

Autoradiographic images exemplifying the variability of regional 5-HT synthesis across the brain are provided in Figures 1 and 2 for the acute and chronic treatments, respectively. A visual evaluation clearly shows that the synthesis is rather heterogeneous across the brain. Further, there is even a heterogeneity within some anatomical structures, such as the cortex (layer VI has a greater synthesis) and caudate (median part has greater synthesis). Both of these observations are similar to those found in normal rats (Okazawa et al., 1999).

It is also interesting to note that acute buspirone treatment in the FRL rats produced an overall elevation in 5-HT synthesis (the mean brain ratio ($\pm$ SEM) of the synthesis in the treated rats compared to the control rats was 1.48 ± 0.03 ; $N=29$, $t=13.9$; $p<0.001$), while in the FSL rats, the ratio was not different from one (± 0) (the mean ratio $=1.02 \pm 0.03$; $N=30$, $t=0.73$; $p>0.1$). It is important to note that in the FSL rats (Table 2), there were increases in the synthesis in some regions (*e.g.*, cortices, ventral hippocampus), while in other regions (*e.g.*, amygdala, anterior olfactory nucleus, dorsal and median raphe), there were reductions in the synthesis (Table 2). This contributed to the mean ratio not being different from one. In the chronic buspirone treatment (Table 3), the evaluation of the ratios showed a small but significant overall increase in 5-HT synthesis in the FSL rats treated with buspirone relative to those treated with saline (the ratio was 1.07 ± 0.02 ; $N=30$; $t=3.07$; $p<0.005$), while the chronic treatment in the FRL rats produced an overall reduction in the FRL rats (ratio was 0.89 ± 0.01 ; $N=29$; $t=-8.08$; $p<0.001$).

The GLM analysis of the acute data (Table 2) showed a highly significant difference between the four groups ($F(3, 47)=11.3$; $p<0.001$) and an interaction of 5-HT synthesis between the acute groups and the brain regions (Region*group interaction had $F(84,1316)=138.3$; $p<0.001$), suggesting a significant effect of this acute buspirone treatment on the synthesis in these groups, as well as an interaction between the synthesis and brain regions. A planned comparison as a post-hoc evaluation revealed differences in nine out of twenty-nine brain regions (Table 2; note that the pineal body is not part of the brain) when comparisons between the saline and buspirone treated rats were performed in the FSL rats. In the FRL rats there were differences in all brain regions except the dorsal thalamus, substantia nigra reticulata, and raphe magnus (Table 2). In the acutely treated FSL rats, the largest reduction in 5-HT synthesis was found in the substantia nigra reticulata (32%), followed by a reduction in the anterior olfactory nucleus (17%) (Table 2). There were significant increases in all cortical regions except the entorhinal cortex, with the largest increase in the visual cortex (49%), followed by a 32% increase in the parietal cortex. In the comparisons between the saline (control) and buspirone treated FRL rats, the largest increases in synthesis were observed in the ventral hippocampus (74%), followed by the frontal cortex (73%). The smallest, yet still significant, increase was found in the dorsal raphe (17%), followed by the raphe magnus (23%) (Table 2).

The GLM analysis of data from the chronic buspirone treatment indicated a highly significant group-region interaction ($F(87,1421)=13.3$; $p<0.001$), and was on the border of significance in the group effect; $F(3,49)=2.8$; $p=0.05$. A planned comparison, as part of the post-hoc analysis, revealed a difference in thirteen out of twenty-nine brain regions in the comparisons of the FSL groups (saline and respective buspirone group), and fifteen out of twenty-nine regions in the comparisons of the FRL groups (Table 3).

When saline and buspirone chronically treated FSL rats were compared, there were significant elevations in the synthesis in all brain regions showing differences, except the median (-21.9%) and pontine (-8.9%) raphe nuclei, where there were significant reductions of synthesis (Table 3). The largest increase was observed in the parietal cortex (32.8%) and the somatosensory cortex (32.2%). Intermediate significant increases were observed in the lateral part of the caudate (14.3%), globus pallidus (13.5%), VTA (13.7%), entorhinal cortex (10.6%), and amygdala (12.9%) (Table 3).

In the comparison of the FRL saline and buspirone chronically treated groups, the greatest reduction in synthesis was found in the locus ceruleus (-28.6%), the substantia nigra compacta (-23.0%), and the hypothalamus (-22.5%) (Table 3). It is also important to note that in many limbic structures, chronic treatment with buspirone produced a significant reduction in synthesis (*e.g.*, anterior olfactory nucleus, accumbens, cingulate and entorhinal cortices). There were significant reductions of synthesis in several brain regions in the FRL rats, while in some of the limbic and cortical regions in the FSL rats, chronic buspirone produced a

significant elevation in synthesis (enthorinal, frontal, somatosensory, and visual cortices, amygdala, dorsal hippocampus).

Discussion

The most important finding reported here is the observation that buspirone, a 5-HT_{1A} agonist with some partial agonistic properties (De Vivo and Maayani, 1986; Hoyer and Boddeke, 1993), elevated synthesis in the FRL rats (non “depressed” rats; used as control rats) following acute treatment (Table 2), and reduced synthesis in several limbic structures following chronic treatment (Table 3). With respect to the FSL rats, there was a mixed effect: there were reductions in some regions and increases in others (Table 2). Following chronic treatment with 10 mg/kg/day (constant delivery) for fourteen days, there were significant increases of brain 5-HT synthesis in many brain regions in the FSL rats, but a reduction in the median and pontine raphe (Table 3). There was also a significant reduction of the synthesis in many brain regions in the FRL rats as well as an overall reduction (Table 3). These results (Tables 2 and 3) are in contrast to those reported in SPD rats (the strain from which the FSL and FRL rats were derived) (Okazawa et al. 1999). In SPD rats, following an acute treatment, there was a reduction in synthesis throughout the brain, while after chronic treatment, the synthesis was only reduced in a few brain regions (Okazawa et al. 1999). From these data (presented here and in Okazawa et al. 1999), it is clear that following acute treatment in the FRL rats, buspirone acts as a partial agonist, while in the FSL rats, buspirone acts more like an indirect agonist (e.g. selective serotonin reuptake inhibitor). The actions in both strains are in contrast to the clear agonistic properties of buspirone previously reported in SPD rats (Okazawa et al. 1999).

In the present studies the FRL and FSL rats were of the same age, had the same weight gain between birth and inclusion into protocols, and their weights were not different (Table 1). This is in disagreement with previous reports in which difference in weight was reported (Russel et al. 1982; Wörtwein et al. 2006), but in those studies rats were 90 days old, the age almost double to rats used in the present studies. This suggests that at an older age FSL and FRL rats do not gain weight equally. Another difference is the fact that in our experiments weights of rats were taken after being deprived food for twelve hours; the fasting is required by the protocol.

Effect of acute buspirone

An elevation in arterial blood oxygen in the FRL rats and the FSL rats could also contribute to the observed effects of buspirone on synthesis. Elevations of blood oxygen tensions are in line with the effects reported for buspirone (Okazawa et al. 1999) and other 5-HT_{1A} agonists (Tohyama et al. 2002 and 2007). The possibility that elevation in blood oxygen could elevate brain 5-HT synthesis in FRL rats (Table 2) accords with observations in humans (Nishikawa et al. 2005).

The greatest contrast in the buspirone treatment was observed between the FSL and FRL rats administered 10 mg/kg of buspirone (Table 2). Significant increases were observed in almost all of the brain regions investigated in the FRL rats, and the increases were quite large (Table 2). In the FSL rats, acute buspirone administration, as compared to that of saline, produced some elevation and some reduction of synthesis. The increases observed in the FRL rats suggest that the action of buspirone in this strain of rats is more like an antagonist, because such regional increases in 5-HT synthesis were previously observed following treatment with the 5-HT_{1A} antagonist, WAY-100635 (Tohyama et al. 2001). The reduction of synthesis in the FSL rats in the raphe nuclei and cell body regions are probably the result of action through the 5-HT_{1A} autoreceptors that are present on the dendrites (this has been proposed as an agonistic effect; Blier et al. 1993). However, increases in some terminal regions would suggest different actions in the cell bodies and terminal regions.

There is also the possibility that buspirone, and/or extra 5-HT present in the brain, interacted with another neurotransmitter system (*e.g.*, cholinergic, dopaminergic), after occupying the 5-HT_{1A} sites, which resulted in a reduction in the 5-HT inhibitory effects and, as a result, stimulated the synthesis in the FRL rats. This would be in line with the action of the antidepressant, desipramine, which reduced extracellular dopamine levels in FSL rats (no similar study was done in the FRL rats) (Zangen et al. 1999), relative to SPD rats. In addition, there is also the possibility of an interaction between the serotonergic and noradrenergic systems (Harkin et al. 1999). This interaction could be the result of a metabolite of buspirone, 1-(2-pyrimidinyl)-piperazine, which is a α 2-adrenoreceptor and D2 antagonist and can release dopamine (Gobert et al. 1999).

It has been shown that both 5-HT_{1A} agonists (Okazawa et al. 1999; Tohyama et al. 2007; Skelin et al. 2008) and antagonists (Tohyama et al. 2001) can modulate 5-HT synthesis in normal rats. This suggests the importance of this site in synthesis control and likely also for antidepressant action (Blier and Ward, 2003). An elevation of 5-HT synthesis observed in some terminal regions in the FSL rats following acute treatment is similar to an elevation observed in the SPD rats following treatments with fluoxetine (Mück-Seler et al. 1996; Tsuiki et al. 1995). This could indicate antidepressant action of buspirone in FSL rats. This elevation could be a result of the observation that in some of the terminal regions, the FSL rats have lower amounts of 5-HT in the neuronal releasable pool (Zangen et al. 1997; Hasegawa et al. 2006), which serotonergic neurons would need to replenish through synthesis stimulated by intracellular signaling (which has not yet been found). It has been reported that FSL rats have rather different biochemical and behavioral responses than FRL rats, to a 5-HT_{1A} agonist and also to acute stress (Overstreet et al. 1995; Overstreet, 2002; Zambello et al. 2008). This suggests possible different responses of the brain serotonergic system in these two strains of rats to the activation of 5-HT_{1A} receptors. This also suggests that both FSL and FRL rats have different intracellular handling of the activation of 5-HT_{1A} receptors with buspirone than SPD rats. The possibility of different intracellular signaling was also demonstrated through the different activation of peptide mRNA by electroconvulsive stimuli (Jimenez-Vasquez et al. 2000 and 2007), maternal separation (Wörtwein et al. 2006), stress and apomorphine induced hypothermia (Pucilowski and Overstreet, 1993), 5-HT_{1A} agonist induced hypothermia (Overstreet et al. 2005), 5-HT receptor mRNA expression (Österlund et al. 1999), striatal density of high-affinity nicotinic acetylcholine receptors (Auta et al. 2000), and swim test immobility (Friedman et al. 2006).

The acute effect could also be related to different levels of receptor internalization in FSL and FRL rats, as receptor internalization in normal rats has been reported, following acute treatment with another 5-HT_{1A} agonist, 8-OH-DPAT (Zimmer et al. 2004). It is important to note that following chronic treatment (see below), there were significant reductions of synthesis in the substantia nigra reticulata (the region containing a large density of dopaminergic cell bodies) in the FSL rats, without a significant effect in the FRL rats (Table 2). This reduction could be a result of a 5-HT-dopamine interaction in the FSL rats that didn't occur in the FRL rats, through serotonergic receptors found on dopaminergic neurons (Freed and Yamamoto, 1985).

Chronic effects of buspirone

In the chronic buspirone treatment, the effects were somewhat opposite in the FSL and FRL rats; there was an elevation of synthesis in many brain regions in the FSL rats (Table 3) with a significant reduction of synthesis in many brain regions in the FRL rats (Table 3). An elevation of synthesis in the cortical regions of the FSL rats, as well as some limbic structure areas (*e.g.*, amygdala, lateral part of caudate-putamen, entorhinal cortex) could be an indication of increased 5-HT neurotransmission as a result of antidepressant action of buspirone (Kennett et al. 1987; Lucki et al. 1994; Watanabe et al. 2006). A significant reduction in the median and pontine raphe in the FSL rats treated chronically with buspirone (Table 3) was somewhat

unexpected, because desensitization can be expected following chronic treatment, as was the case in the SPD rats (Okazawa et al. 1999). The reduction of synthesis in many terminal regions of the FRL rats could indicate that the 5-HT_{1A} receptors have not been desensitized and/or intracellular signaling has not adapted to the new buspirone state, which is opposite to the effect in the SPD rats (Okazawa et al. 1999). This reduction of synthesis could also be an indication of increased sensitivity of 5-HT_{1A} receptors and other receptors (*e.g.*, 5-HT_{1B}) controlling the synthesis. As there were no differences in synthesis in the cell body regions between the saline and buspirone groups in the chronically treated FRL rats (Table 3), this would indicate that the 5-HT_{1A} autoreceptors were desensitized in the cell bodies. The reduction in the FRL rats was observed in many limbic regions (*e.g.*, accumbens, anterior olfactory nucleus, cingulate cortex, hypothalamus), the regions expected to be targeted by antidepressants. At the same time, these results indicate that 5-HT_{1A} autoreceptors in the cell bodies and/or the terminal 5-HT_{1A} receptors, which through postsynaptic loops could control serotonergic neurotransmission (Blier and de Montigny, 1990; Blier et al. 1990), were not desensitized by buspirone in FRL rats (Table 3) in contrast to more or less complete desensitization in the SPD rats (Okazawa et al. 1999). It was not expected that buspirone would change the synthesis in the brain limbic structures (present data), because it did not affect behavior in FRL rats, while the behaviour in the FSL rats was affected by it (Overstreet et al. 1995 and 2005; Overstreet, 2002).

It has been reported that naive FRL rats have similar 5-HT synthesis rates to SPD rats (Hasegawa et al. 2006). In SPD rats, buspirone produced a reduction following acute treatment, and very little effect following chronic treatment (Okazawa et al. 1999). Because of this, an elevation in 5-HT synthesis following an acute buspirone treatment (Table 2), and a significant reduction in many terminal regions following chronic treatment (Table 3) have not been expected. This suggests that the sensitivity of 5-HT_{1A} receptors to buspirone and/or intracellular signal transduction in FRL rats is different than that in either the FSL and SPD rats. This hypothesis of different receptor sensitivity and/or signaling could be related to the fact that the FRL rats were selected to be more resistant to a cholinergic drug, diisopropyl fluorophosphatase (Overstreet et al. 1979; Russell et al. 1982), and they show different thermal responses to 5-HT_{1A} agonists (Overstreet et al. 2005).

5-HT_{1A} autoreceptors play an inhibitory role, while the role of postsynaptic 5-HT_{1A} receptors in control of 5-HT synthesis and release is not fully understood (Blier and de Montigny, 1987). The activation of 5-HT_{1A} autoreceptors with an agonist produces an inhibitory effect, resulting in the reduction of the firing rate, decreased 5-HT release, both in the terminal regions and the cell bodies (Bosker et al. 1996). 5-HT_{1A} initiated post-synaptic loops have also been identified (Blier and de Montigny, 1987). These loops are probably controlling the actions of drugs after chronic treatment. The different responses of these loops are probably the result of different effects observed in the FRL rats, relative to the FSL rats. In addition to the cell body 5-HT_{1A} autoreceptors, serotonergic neurons have terminal 5-HT_{1B} autoreceptors which have also been shown to modulate, in a regional specific way, 5-HT synthesis (Tohyama et al. 2002; Dobson et al. 2004; Hasegawa et al. 2005). Further, 5-HT_{1B} receptors can control 5-HT release independently of 5-HT_{1A} sites (Barton and Huston, 1999). These terminal autoreceptors could be, in part, responsible for synthesis modulation following chronic treatment.

Interactions between different monoaminergic systems in the modulation of 5-HT release and synthesis could also be an important factor, especially in studies in which the drug investigated has an active metabolite, as is the case with buspirone. For example, 8-OH-DPAT, a 5-HT_{1A} agonist, reduces extracellular levels of 5-HT, while increasing extracellular levels of dopamine in the normal rat cortex (Gobert et al. 1998), which could then act on the serotonergic system. Similarly, flesinoxan, another 5-HT_{1A} agonist, produces a dose dependent increase in the firing rate of dopaminergic neurons in the ventral tegmental area (Lejeune and Millan, 1998). By this dopamine-5-HT neuronal interaction 5-HT synthesis could be modulated complicating

interpretation of the results in the FSL rats, the strain reported to have region specific abnormalities in 5-HT and dopamine release, as well as region specific abnormalities in the tissue concentrations of these two amines (Zangen et al. 1997; Yadid et al. 2000).

In conclusion, it can be stated that buspirone, a selective 5-HT_{1A} partial agonist, produced very different effects on regional 5-HT synthesis in the FSL and FRL rats, which were also different from those reported for SPD (normal) rats. Because the FRL rats have been used as a control strain in many behavioural and neurochemical studies done on FSL rats, the results presented point to a possible problem. The differences of these effects are obvious in both the chronic and acute treatments. Assuming that the elevation in regional 5-HT synthesis in the FSL rats following chronic buspirone treatment represents increased serotonergic neurotransmission, it could be proposed that this would correlate with buspirone antidepressant activity as shown previously in both animal (e.g. normalization of the immobility in FSL rats) and human studies. The data presented here suggest that the use of FRL rats as control animals in many previous studies could have created some difficulties in the interpretation of the results and their relation to the treatment of depressive symptoms in this rat model of depression.

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Figure 1.

A set of autoradiograms exemplifying inhomogeneities of the regional 5-HT synthesis in FSL and FRL rats treated acutely with buspirone (10 mg/kg) and control rats treated with saline. The meanings of the abbreviations are: Amy-amygdala; AN-accumbens; DR-dorsal raphe; MR-median raphe; CPm-caudate-putamen (median); CPI-caudate-putamen (lateral); ThD-thalamus (dorsal); ThV-thalamus (ventral); PB-pinael body; VI-layer VI of the cortex; SmCx-sensory-motor cortex; Encx-Entorhinal Cortex.

Figure 2.

A set of autoradiograms exemplifying inhomogeneities of the regional 5-HT synthesis in FSL and FRL rats treated chronically (14-day treatments with mini pump) with buspirone (10 mg/kg/day) and control rats treated with saline. The abbreviations are given in the caption of Fig. 1.

Table 1

Physiological parameters of FSL and FRL rats in both acute and chronic experiments. The values are provided as means± Standard Error of the Mean (SEM)

Variable	FSL				FRL			
	Acute		Chronic		Acute		Chronic	
	Saline (N=13)	Buspirone (N=13)	Saline (N=12)	Buspirone (N=14)	Saline (N=12)	Buspirone (N=13)	Saline (N=14)	Buspirone (N=13)
pH	7.42±0.01	7.43±0.01	7.44±0.0	7.42±0.01	7.42±0.01	7.40±0.01	7.41±0.01	7.42±0.01
pO ₂ (mmHg)	95.0±2.6*	111.0±3.9	97.0±3.2	99.4±2.0	92.0±1.7*	124.7±1.1	84.0±2.7	92.5±2.0
pCO ₂ (mmHg)	34.4±0.7	33.8±2.2	34.7±1.2	34.3±1.0	37.0±0.9	36.5±0.8	39.7±1.7	38.5±0.6
Hematocrit (%)	44.9±0.7	45.5±2.0	47.0±1.2	44.1±2.3	46.0±0.8	45.4±0.9	47.8±1.7	45.9±0.6
Free Trp (nmol/mL)	8.0±0.7	8.4±0.7	6.8±0.7	6.8±0.6	7.2±0.7	8.4±0.7	7.6±0.6	9.4±0.8
Total Trp (nmol/mL)	48±3.6	58.0±2.8	48.0±4.3	52.0±2.9	51.0±4.3	54.0±4.2	50.0±3.7	45.0±4.7
Weight gain (g)	NA	NA	72.2±3.3	45.5±4.6*	NA	NA	67.6±4.6	62.0±4.4
Entry weights (g)	205.8±1.7	213.0±2.3	215.1±2.5	210.9±2.2	207.8±2.7	203.5±3.0	214.5±1.9	212.3±1.9
Weight at tracer injection (g)	As above	As above	287.3±5.2	256.4±4.7	As above	As above	282.1±5.1	274.3±5.4
Entry age (days)	49.9±1.6	51.2±1.1	50.9±1.0	50.8±1.0	50.7±0.5	52.6±1.3	49.4±1.4	49.7±2.0
Weight gain from birth (g)	4.20±0.13	4.20±0.10	4.24±0.07	4.16±0.09	4.06±0.09	3.90±0.10	4.39±0.12	4.35±0.16

* Significantly (p<0.05; ANOVA with Newman-Keuls post-hoc evaluation) different from the respective control group.

Table 2

Regional 5-HT synthesis rates (pmol/g/min) in saline or buspirone (10 mg/kg; i.p.) acutely treated FSL and FRL rats. The values are provided as mean±SEM for the number of rats provided in Table 1.

REGIONS ¹	FSL Rats (Acute)			FRL Rats (Acute)		
	Saline	Buspirone	%Change ²	Saline	Buspirone	%Change ²
AN	50.5±2.4	52.3±2.4	NS	46.8±2.0	72.6±2.1	55*
AMY	37.6±1.4	33.0±1.4	-12*	28.9±1.3	44.9±1.9	55*
AONu	41.2±1.6	34.1±1.7	-17*	29.7±1.8	39.8±1.6	34*
Acx	22.9±2.4	27.3±1.3	NS	23.7±1.3	37.4±1.6	57*
Cincx	27.5±2.4	26.2±1.7	NS	19.9±1.3	29.6±1.7	49*
Encx	32.0±2.7	33.7±1.2	NS	29.1±1.3	44.1±1.6	52*
Fex	25.0±1.9	30.1±1.8	20*	21.4±1.4	36.9±1.6	73*
Pcx	18.7±2.4	24.6±1.6	32*	20.3±1.5	33.0±1.5	63*
Smcx	22.1±2.1	27.8±1.7	26*	20.0±1.5	33.7±1.6	69*
Vcx	19.6±3.1	29.1±1.4	49*	22.6±2.0	39.1±1.6	73*
CPm	37.6±2.4	36.3±1.6	NS	38.0±1.5	56.4±2.0	48*
CPI	29.0±2.1	28.3±1.5	NS	29.6±1.4	43.5±1.6	47*
GP	27.7±1.4	25.0±1.5	NS	20.3±1.1	30.5±1.8	50*
MBF	23.1±2.4	23.5±2.2	NS	16.8±1.7	30.5±2.3	82*
HiD	28.6±2.4	28.2±1.3	NS	27.9±1.4	45.3±2.0	62*
HiV	34.2±3.0	41.1±1.1	20*	31.3±1.5	54.5±1.9	74*
Hyp	34.6±1.9	29.8±2.7	NS	27.2±1.2	39.4±3.1	45*
ThD	22.9±2.2	22.2±1.3	NS	21.0±1.1	25.6±1.9	NS
ThV	21.6±2.9	23.3±1.7	NS	15.9±1.1	26.8±2.4	69*
LG	24.7±2.1	22.7±1.5	NS	22.5±1.0	33.5±1.8	49*
MG	16.3±3.1	17.8±1.2	NS	18.6±1.6	28.7±2.0	54*
SNc	23.2±2.3	21.3±1.0	NS	20.8±1.5	30.9±2.0	49*
SNr	24.9±1.4	16.9±1.4	-32*	17.1±1.2	19.5±1.5	NS
VTA	25.3±2.3	25.2±1.2	NS	28.6±1.9	34.6±2.4	21*
DR	101±4	84.6±1.2	-16*	141±5	164±6	17*
MR	74.6±3.4	63.3±1.6	-15*	87.9±4.1	121±4	38*

REGIONS ¹	FSL Rats (Acute)		FRL Rats (Acute)	
	Saline	Buspirone	Saline	Buspirone
Rpo	42.3±3.8	37.8±2.1	38.8±2.2	55.8±1.7
RM	29.3±2.6	27.7±1.6	29.3±1.8	36.0±1.5
LC	24.2±2.7	28.6±2.1	18.8±1.5	20.8±2.3
PB	783±20	820±17	733±18	749±21
			%Change ²	%Change ²
			NS	44*
			NS	23
			NS	NS
			NS	NS

¹The meanings of the abbreviations are as follows: AONu-Medial Anterior Olfactory Area; AN-Accumbens Nucleus; Cinex-Anterior Cingulate Cortex; Fex-Frontal Cortex; Smcx-Somatosensory Cortex; CPn-Caudate-Putamen (Medial); CPI-Caudate-Putamen (Lateral); MBF-Medial Forbrain Bundle; GP-Globus Pallidus; Pcx-Parietal Cortex; HiD-Hippocampus (dorsal); HiV-Hippocampus (ventral); AMY-Amygdala; ThD-Thalamus (dorsal area); ThV-Thalamus (ventral areas); LG-Lateral Geniculate; Hyp-Hypothalamus; Acx-Auditory Cortex; MG-Medial Geniculate; SNC-Substantia Nigra pars compacta; SNr-Substantia Nigra pars reticulata; VTA-Ventral Tegmental Area; DR-Dorsal Raphe; RM-Median Raphe; Vex-Visual Cortex; Encx-Entorhinal Cortex; Rpo-Pontine Raphe; RM-Raphe Magnus; LC-Locus Ceruleus; PB-Pineal body

²NS stands for no significant difference between the buspirone and respective control groups;

* indicates significance found in post hoc planned comparisons with p<0.05.

Table 3

Regional 5-HT synthesis rates (pmol/g/min) in saline or buspirone (10 mg/kg/day, 14 days) chronically treated FSL and FRL rats. The values are provided as mean±SEM for the number of rats provided in Table 1 and the abbreviation under Table 2.

REGIONS ¹	FSL Rats (Chronic)			FRL Rats (Chronic)		
	Buspirone	%Change ²	Saline	Buspirone	%Change ²	
AN	57.4±2.3	NS	48.4±1.4	42.6±2.1	-12.0*	
AMY	34.0±1.6	13.9*	35.2±0.7	31.8±1.4	NS	
AONu	40.0±1.9	NS	33.7±1.0	30.0±1.2	-11.0*	
Acx	31.5±1.4	NS	29.6±0.8	28.1±1.1	NS	
Cincx	31.6±1.4	NS	28.7±0.8	22.7±1.1	-20.9*	
Enex	36.8±1.3	10.6*	37.5±0.9	32.5±1.2	-13.3*	
Fcx	24.7±1.4	24.7*	27.1±0.7	23.4±1.1	-13.7*	
Pex	23.6±1.4	33.1*	24.7±0.7	24.3±1.2	NS	
Sex	24.0±1.3	32.1*	26.0±0.8	22.6±1.1	-13.1*	
Vcx	28.1±1.2	22.4*	30.5±0.8	27.9±1.2	NS	
CPm	44.6±1.6	NS	43.2±1.1	37.8±1.8	-12.5*	
CPI	33.0±1.4	14.6*	34.7±0.8	30.3±1.4	-12.7*	
GP	27.5±1.4	13.5*	28.1±0.7	27.9±1.3	NS	
MBF	36.0±1.6	NS	31.7±0.8	25.2±1.3	-20.5*	
HiD	31.1±1.2	13.2*	35.1±0.9	32.5±1.5	NS	
HiV	45.3±1.7	NS	42.3±1.1	39.1±1.4	NS	
Hyp	39.3±2.2	NS	40.5±1.1	31.4±1.8	-22.5*	
ThD	27.6±1.4	NS	27.6±0.7	24.9±1.3	NS	
ThV	27.5±1.4	12.0*	28.0±0.7	25.4±1.2	NS	
LG	27.3±1.3	NS	31.9±0.8	27.2±1.2	-14.7*	
MG	30.0±1.5	NS	31.8±1.0	27.3±1.0	-14.2*	
LC	38.4±1.4	NS	35.2±0.9	25.1±1.0	-28.7*	
SNc	32.0±1.3	NS	31.6±1.0	24.3±1.1	-23.1*	
SNr	20.1±1.4	NS	21.8±1.0	19.6±1.4	NS	
VTA	33.4±1.4	NS	34.5±1.0	29.6±1.2	-14.2*	
DR	109.5±2.8	NS	103.0±3.1	108.±1.1	NS	

REGIONS ¹	FSL Rats (Chronic)		FRL Rats (Chronic)	
	Buspirone	%Change ²	Saline	%Change ²
MR	93.2±2.0	72.8±2.2	74.1±2.0	72.0±5.3
Rpo	49.5±1.4	45.1±1.1	45.8±1.0	44.2±1.6
RM	33.3±1.2	33.7±1.1	48.5±1.3	43.9±1.9
PB	949 ±38.	869 ±35.	795.±18.	820±21.

¹The meanings of the abbreviations are provided in Table 2.

²NS stands for no significant difference between the buspirone and respective control groups;

* indicates significance found in post hoc planned comparisons with p<0.05.