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Acute treatment with fluvoxamine elevates rat brain serotonin synthesis in some terminal regions: An autoradiographic study

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Abstract

Introduction—A considerable body of evidence indicates the involvement of the neurotransmitter serotonin (5-HT) in the pathogenesis and treatment of depression.

Methods—The acute effect of fluvoxamine, on 5-HT synthesis rates was investigated in rat brain regions, using α-^{14}C-methyl-L-tryptophan as a tracer. Fluvoxamine (25 mg/kg) and saline (control) were injected intraperitoneally, one hour before the injection of the tracer (30 μCi).

Results—There was no significant effect of fluvoxamine on plasma free tryptophan. After Benjamini–Hochberg False Discovery Rate correction, a significant decrease in the 5-HT synthesis rate in the fluvoxamine treated rats, was found in the raphe magnus (−32%), but not in the median (−14%) and dorsal (−3%) raphe nuclei. In the regions with serotonergic axon terminals, significant increases in synthesis rates were observed in the dorsal (+41%) and ventral (+43%) hippocampus, visual (+38%), auditory (+65%) and parietal (+37%) cortex, and the substantia nigra pars compacta (+56%). There were no significant changes in the 5-HT synthesis rates in the median (+11%) and lateral (+24%) part of the caudate-putamen, nucleus accumbens (+5%), VTA (+16%) or frontal cortex (+ 6%).

Conclusions—The data show that the acute administration of fluvoxamine affects 5-HT synthesis rates in a regionally specific pattern, with a general elevation of the synthesis in the terminal regions and a reduction in some cell body structures. The reasons for the regional specific effect of fluvoxamine on 5-HT synthesis are unclear, but may be mediated by the presynaptic serotonergic autoreceptors.

Keywords

Autoradiography; Fluvoxamine; Serotonin synthesis; Selective serotonin reuptake inhibitor; Tracer method

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1. Introduction

Preclinical and clinical investigations suggest the involvement of the serotonergic system in the aetiology and treatment of depression [1,2]. Several neurochemical and behavioural studies [3–5] have found alterations in serotonergic neurotransmission after treatment with various classes of antidepressant drugs, including selective serotonin (5-hydroxytryptamine, 5-HT) reuptake inhibitors (SSRI), such as fluvoxamine [6].

In vitro fluvoxamine ((*E*)-5-methoxy-1-[4-(trifluoromethyl)phenyl]pentan-1-one *O*-2-aminoethyl oxim) inhibits 5-HT uptake in the monkey [7] and rat [8] brain synaptosomes with IC_{50} in a nanomolar range. Fluvoxamine is a weak inhibitor of noradrenaline or dopamine uptake [8], has little or no affinity for serotonergic [9], noradrenergic, dopaminergic and histaminergic receptors [8,10], and does not inhibit monoamine oxidase activity in the rat brain [8]. The major metabolite of fluvoxamine is fluvoxamine acid [11], which also has an antidepressant property [12].

Although fluvoxamine and other SSRIs were effective and widely used in the treatment of depression [12,13] and anxiety disorders [14], there are still some unanswered questions about their mechanism of action. The immediate effect of SSRIs on the inhibition of 5-HT uptake in vitro is in contrast to their delayed therapeutic effect [15]. Several lines of evidence suggest that the changes in sensitivity of somatodendritic $5\text{-}HT_{1A}$ and/or terminal $5\text{-}HT_{1B}$ autoreceptors [15,16] could have beneficial effect on the clinical response to acute antidepressant treatment. The controversy around the association between acute SSRI administration and suicidal behaviour in adults [17] and adolescents [18] may also be due to the unclear acute effect of antidepressants on the serotonergic system.

As the acute administration of SSRIs has important but unclear biochemical and behavioural effects, and taking into account that there are no data regarding the acute effect of fluvoxamine on 5-HT synthesis, the aim of the present study was to determine the 5-HT synthesis rate in a large number of rat brain regions using a specific autoradiographic method. Our hypothesis was that a single systemic administration of fluvoxamine affects 5-HT synthesis rates in a region-dependent manner.

2. Material and methods

2.1. Experimental animals

Sprague–Dawley rats weighing between 200 and 220 g were used in the study. The animals were kept under controlled temperature and a 12/12 h light/dark cycle (light on at 7 a.m.) for at least 3 days prior to the beginning of the experiments. All experiments were performed on animals deprived of food, but not water, 18 h beforehand.

Fluvoxamine maleate (Solvay Duphar) was dissolved in saline. The control rats were treated with the same amount of saline. All of the solutions were injected intraperitoneally (*i.p.*) at a volume of 1 ml/250 g.

2.2. Determination of 5-HT synthesis rate

Under light halothane (0.5%–1.0%) anaesthesia, plastic catheters were inserted in the femoral artery (for blood sampling) and vein (for the tracer injection). The rats were placed in loose-fitting plaster casts and allowed to awaken. A dose of 25 mg/kg of fluvoxamine (11 animals) and saline (8 control rats) was injected *i.p.* one hour prior to the tracer injection. The tracer, 30 μCi of α-^{14}C-methyl-L-tryptophan (α–[^{14}C] MTrp; specific activity of approximately 55 mCi/mmol; synthesized by us using the procedure described by Mzengeza et al. [19]) was injected intravenously in 1 ml of saline over 2 min, with an injection pump. With the beginning of the tracer injection, arterial blood samples were taken at progressively increased time intervals up to the time the rats were sacrificed. The blood samples were centrifuged for 3 min at 12,500 *g*. Twenty μl of plasma was deproteinized with 10 μl of 20% trichloroacetic acid. After mixing and spinning (2 min at 12,500 *g*), 20 μl of supernatant was taken for the radioactivity determination by a liquid scintillation counting to measure the plasma radioactivity (input function).

The animals were euthanized by guillotine one or two and half hours after the tracer injection. The brains were removed, frozen in freon and cut into 30 μm slices in a cryostat at approximately −20 °C. The brain sections were mounted on glass slides and exposed to X-ray film along with ^{14}C-polymer standards for 3–4 weeks to obtain the autoradiograms. The films were developed and radioactivity concentrations in different structures were determined using a microcomputer-based image analysing system (Image Calculator; Soquelec Ltd., Montreal) consisting of a video camera, a frame grabber, an IBM AT compatible computer, and appropriate software.

2.3. Calculation of 5-HT synthesis rate

The model for the estimation of the rate of 5-HT synthesis in the rat brain is based on three biological compartments: plasma, precursor and irreversible [20]. The movement of the tracer can be mathematically described by a set of differential equations with first-order rate constants [20,21].

The rate of 5-HT synthesis (R; nmol/g/min) can be calculated as $R=C_p\ K^*/LC$. C_p (nmol/ml) is the concentration of non-protein bound plasma tryptophan (free tryptophan). LC is the “lumped constant”, which is actually the ratio of the Michaelis–Menten constants for tryptophan and α-methyl-tryptophan (in relation to tryptophan hydroxylase) and the volume of distribution of tracer (methyl-tryptophan) and tracee (tryptophan). The LC was found to be uniform throughout the brain, having an average value of 0.42±0.07 [22,23]. K^* (nmol/g/min) is the constant for the unidirectional trapping of the tracer.

Total and free tryptophan concentrations were measured by the HPLC method [24] using a post-column o-phthalaldehyde derivatization as previously described [25].

2.4. Statistical analysis

The statistical analysis was performed by STATISTICA using a two-factor ANOVA analysis. The pineal body was not included in the ANOVA comparisons, because the pineal body is outside the blood brain barrier [26]. The post hoc evaluation was done by planned

comparison ANOVA. Planned comparison was selected because only a certain number of the total comparisons are of interest (*e.g.*, there is no interest in comparing synthesis in different brain regions in the same group of rats). In an attempt to remove false positive results, the Benjamini–Hochberg correction for the False Discovery Rate (FDR) was applied [27]. The $p<0.05$ was taken as significant.

3. Results

The plasma concentration of free tryptophan (10.2±4.2 nmol/ml) in the fluvoxamine treated group of rats was not significantly ($F(1,17)=0.08$; $p>0.7$; ANOVA) different from the plasma free tryptophan (8.7±3.1 nmol/ml) in the saline-treated (control) rats.

We have published numerous papers that included the set of representative autoradiograms [4,21,22,28–30]. Given this, and the fact that there is little information of value provided by this set, we did not include them in the current paper.

The two-factor ANOVA indicated a significant interaction in 5-HT synthesis rates between the brain regions and different groups of rats ($F(25,425)=36.7$; $p<0.0001$). A post hoc planned comparison revealed a significant reduction of the synthesis in the raphe magnus (−32%), median raphe (−14%), and medial forebrain bundle (−20%), and a non-significant decrease (−3%) in the dorsal raphe nuclei in fluvoxamine treated rats compared to the control (saline treated) rats (Table 1). However, after the Benjamini–Hochberg FDR correction, the decrease in the 5-HT synthesis rate remained significant only in the raphe magnus (Table 1).

In the majority of the other rat brain structures, the rate of 5-HT synthesis was increased in the fluvoxamine treated rats when compared to the controls. After the Benjamini–Hochberg FDR correction (Table 1), the significant increase in the 5-HT synthesis rate was found in some parts of the cortex such as visual (+38%) and auditory (+65%) cortex, whereas the increase in 5-HT synthesis rates in the sensory-motor (36%), and parietal (+37%) cortex lost significance (Table 1). The pronounced increase in the 5-HT synthesis rates was also observed in the nerve terminal areas, such as the dorsal (+41%) and ventral (+43%) hippocampus, dorsal (+23%) and ventral (+31%) thalamus, substantia nigra pars compacta (+56%), the medial geniculate body (+48%) and superior colliculus (+38%). The significant difference in 5-HT synthesis rates between the fluvoxamine treated and control rats in the substantia nigra pars reticulata (+23%), globus pallidus (+25%), lateral caudate (+23%), hypothalamus (18%) and inferior colliculus (+29%) was lost following correction (Table 1). Non-significant changes (from 4% to 29%) in 5-HT synthesis rates were observed before the Benjamini–Hochberg FDR correction in the frontal cortex, medial part of the caudate-putamen, nucleus accumbens, ventral tegmental area (VTA), superior olive and lateral geniculate body.

4. Discussion

The main finding of the present work is that the single systemic administration of the SSRI, fluvoxamine, affects 5-HT synthesis rates in a regionally specific pattern with an opposite effect on the synthesis rates in the areas of the serotonergic cell bodies (nuclei raphe) and

nerve terminals. An increase in 5-HT synthesis rates was found in the majority of regions with serotonergic axon terminals (hippocampus, thalamus, substantia nigra pars compacta, medial geniculate body), while in the nuclei raphe regions, a decrease in the rate was observed, particularly in the raphe magnus nucleus.

Previous studies have shown that the acute application of citalopram [4,31], fluoxetine [28], paroxetine [32] and fenfluramine [33] affects 5-HT synthesis rates similar to fluvoxamine in a different regionally specific manner. Further, as was the case with these other drugs, fluvoxamine decreased the 5-HT synthesis rate in the nuclei raphe, although its effect was less expressed and related mostly to 5-HT synthesis in the raphe magnus nucleus. The increase in the 5-HT synthesis rate in the areas of nerve terminals that was found after fluvoxamine was also observed in the above mentioned studies [4,28,31,33], but with slight differences in specific regions between the studies. The only exception was the study with paroxetine [32], in which decreased or unchanged (cortices) 5-HT synthesis rates were found in the axon terminals. Because all of the mentioned drugs, apart from fenfluramine, are part of the SSRI family and 5-HT synthesis rates were determined by the same autoradiographic method, the reasons for their dissimilar effect on 5-HT synthesis are unclear, but suggest that mechanisms other than the inhibition of 5-HT reuptake could be involved in the regulation of 5-HT synthesis rates.

Among many factors that may regulate 5-HT synthesis rates, the most important are blood tryptophan availability, the activity of tryptophan hydroxylase and the sensitivity of the pre- and/or postsynaptic mostly serotonergic receptors. As tryptophan is an essential amino acid and a crucial component for 5-HT synthesis, one possible explanation for the altered 5-HT synthesis rate after a single fluvoxamine application could be its influence on plasma tryptophan levels. However, this presumption is unlikely given that no difference in plasma free tryptophan levels was observed between the fluvoxamine and control group of rats in the present study. This is also in line with no effect of fluoxetine [28], citalopram [4,31] and paroxetine [32] treatments on plasma tryptophan levels in rats.

Another possibility is that fluvoxamine influences tryptophan hydroxylase activity as a rate-limiting enzyme in 5-HT biosynthesis [34]. Given that raphe nuclei are the serotonergic cell body regions that are very rich in tryptophan hydroxylase [35], the increase or decrease in 5-HT synthesis rate in the raphe nuclei observed after antidepressant administration may be related to an increase or decrease in tryptophan hydroxylase activity, respectively. To our knowledge, there is only one study [36] that showed no effect of fluvoxamine on tryptophan hydroxylase activity in rats. The effects of other SSRIs on tryptophan hydroxylase activity or its gene expression are inconsistent. The decreased [35], increased [37] and unaltered [36] tryptophan hydroxylase activity and gene expression were found after different antidepressant treatments. It has been shown that two SSRIs, fluoxetine and sertraline, up-regulate mRNA and protein levels of tryptophan hydroxylase in vitro [37]. A similar increase of tryptophan hydroxylase expression was found in vivo, after a 2 week injection of sertraline in rats [37]. However, a 1 to 32 day treatment with antidepressants of several classes such as SSRIs (citalopram, fluvoxamine), tricyclic (imipramine) and atypical (mianserin, amoxapine) antidepressants, did not change the levels of mRNA encoding several proteins of the serotonergic system, including tryptophan hydroxylase [36]. In

contrast, it has been recently reported [35] that citalopram and fluoxetine reduced the number of tryptophan hydroxylase positive cells in three out of four anatomically and functionally different raphe nuclei. Twenty-four hours after the citalopram injection, a 41%, 26% and 45% reduction in the number of tryptophan hydroxylase positive cells was observed in the dorsal raphe, median raphe and raphe magnus, respectively. Their results are in line with the decreased 5-HT synthesis rate observed in the raphe nuclei following the acute administration of paroxetine [32], fluoxetine [28] and fluvoxamine (present study).

In recent years, the role of the somatodendritic 5-HT_{1A} auto-receptors in the regulation of the neuronal firing rate and 5-HT synthesis has received major attention [38]. This acute effect was explained by the negative feedback exerted by 5-HT on the firing activity of 5-HT neurons via the 5-HT_{1A} autoreceptors. Although fluvoxamine in vitro has a low affinity for serotonergic and noradrenergic receptors [8–10,39], it is an in vivo indirect 5-HT_{1A} agonist, through the increase of extracellular 5-HT due to the inhibition of 5-HT reuptake. Our results of a regional specific effect of acute fluvoxamine application on 5-HT synthesis rates, with a decrease in the raphe area and an increase in the nerve terminals is in line with the opposite effect *i.e.* increased 5-HT synthesis rate in the raphe and decreased rate in the nerve terminals, after acute administration of the pure 5-HT_{1A} antagonist, WAY100635, on regional 5-HT synthesis [29].

In addition, the presynaptic autoreceptors on the nerve terminals, presumably 5-HT_{1B} receptors, are also activated by the transiently increased concentration of 5-HT and react by reducing the release of the neurotransmitter [40]. Our results of increased 5-HT synthesis rates in the nerve terminal areas suggest an independent receptor-mediated regulation of the 5-HT synthesis rates in the nerve terminals to compensate the decreased intracellular pool of 5-HT. The increases observed in the present study could be a transient elevation of the synthesis to replenish the depleted releasable 5-HT pool in the terminal regions.

The mechanism(s) by which fluvoxamine affects the regional 5-HT synthesis rates after a single application is not fully characterized. In vivo, the processes of release and reuptake are the main mechanisms that control the extracellular (*i.e.,* functional pool) 5-HT. As fluvoxamine is a specific and selective inhibitor of the 5-HT transporter, an increase in extracellular 5-HT could be expected after its administration. It could be argued that following acute administration, synaptic fluvoxamine levels were too low for considerable inhibition of the 5-HT reuptake process with a consequence of insufficient increase in synaptic 5-HT levels. It seems that this presumption is unlikely, given that 2 h after its oral administration in rats, the plasma and brain peak values of fluvoxamine were 126 nM and 2.7 μM, respectively [41].

Microdialysis studies [41,42] have found that acute fluvoxamine application does not affect the extracellular 5-HT levels uniformly throughout the brain with a larger increase in extracellular 5-HT concentration in the cell body area than in the nerve terminal areas. In rats, a systemic [42] fluvoxamine administration induced a several fold larger increase in the extracellular 5-HT concentration compared to the basal levels (before the drug administration) in the raphe nuclei than in the frontal cortex. In addition, the time dependent peak in the extracellular 5-HT concentration, found in the dorsal raphe, was not observed in

the frontal cortex [42]. In another study [41], a single oral administration of fluvoxamine induced a 270% and 191% augmentation of baseline extracellular 5-HT levels in the median raphe and dorsal hippocampus, respectively. This process also involves regional mechanisms, as the efflux of 5-HT is increased by local infusion of fluvoxamine via the dialysis probe.

In contrast to other SSRIs, fluvoxamine is a potent agonist of sigma-1 receptors [43] with Ki ten and a one hundred times higher than the Ki for fluoxetine and paroxetine [44], respectively. The significance of sigma-1 receptors for the antidepressant effect of fluvoxamine is still unclear, as under physiological conditions, a fluvoxamine induced increase in prefrontal cortex extracellular levels of 5-HT, dopamine and noradrenaline is independent on sigma-1 receptors [45].

Our results of the unchanged rate of 5-HT synthesis in some areas of the nerve terminals like the VTA, frontal cortex, striatum (*i.e.*, globus pallidus, lateral and medial caudate, nucleus accumbens) after fluvoxamine treatment could be explained by the interaction between the 5-HT and dopaminergic systems. The presence of the neuronal pool of dopamine within the dorsal raphe and the significant increase of 5-HT and dopamine levels were described in this brain region following the local administration of amphetamine [46]. Parsons and Justice [47] observed that 5-HT increases extracellular dopamine concentrations in the nucleus accumbens. On the other hand, the dopaminergic neurons have 5-HT receptors that permit the tonic control of dopamine release in the midbrain, striatum and nucleus accumbens [48]. It has been shown that systemic fluvoxamine administration induced a moderate increase in extracellular dopamine levels in the prefrontal cortex of rats [49] and mice [45], and the dorsal striatum of rats [50], but did not influence dopamine levels in the thalamus [50] of rats. The unchanged 5-HT synthesis rate in the nerve terminals following acute fluvoxamine administration could be explained by a fluvoxamine effect on extracellular dopamine levels at least in the area of dopaminergic nerve terminals like the striatum. Extracellular dopamine could be transported in the 5-HT neurons [51] and could inhibit tryptophan hydroxylase activity [52] in the striatal serotonergic axonal terminals.

4.1. Conclusion

In conclusion, we found, using an autoradiographic method, that the single administration of the 5-HT uptake blocker, fluvoxamine, affected 5-HT synthesis rates throughout the brain. A significant (raphe magnus) and a trend (dorsal and median raphe) of decrease in 5-HT synthesis rates in serotoninergic cell body areas, and increased (hippocampus, substantia nigra, hypothalamus) or unchanged (caudate-putamen, nucleus accumbens) 5-HT synthesis rates were found in the nerve terminal regions. The reasons for the regional specific effect of fluvoxamine on 5-HT synthesis are unclear, but could presumably be mediated by the presynaptic serotonergic autoreceptors.

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Abbreviations

α–[^{14}C]MTrp	α-^{14}C-methyl-L-tryptophan
VTA	ventral tegmental area

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Table 1

Effect of fluvoxamine (25 mg/kg *i.p.*) or saline (control) on 5-HT synthesis rates in the rat brain regions and pineal body.

Structures	5-HT synthesis rate (pmol/g/min)[a]		ANOVA	
	Controls (*N*=8)	Fluvoxamine (*N*=11)	Percent difference[b]	F(1,17); p
Raphe nuclei				
-Dorsal	144±18	140±18	−3%	0.22 ; <0.60
-Median	134±21	115±12	−14%	6.4; <0.02[d]
-Magnus	62±9	43±10	−32%	18.1 ; <0.0006[c]
Cortices				
-Visual	30±5	41±7	38%	14.3; <0.001[c]
-Auditory	26±6	43±9	65%	21.4; <0.003[c]
-Parietal	29±7	39±9	37%	6.8; <0.02[d]
-Sensory-motor	32±7	43±12	36%	5.4; <0.04[d]
-Frontal	33±7	35±9	7%	0.27; >0.60
Basal ganglia				
-Globus pallidus	39±10	48±7	25%	5.36; <0.04[d]
-Caudate-lateral	38±8	47±10	23%	4.4 ; <0.05[d]
-Caudate-medial	54±9	60±10	12%	1.81; >0.10
-Nucleus accumbens	65±9	68±6	4%	0.76; >0.30
Thalamus				
-Ventral	32±5	42±9	31%	7.99; <0.010[c]
-Dorsal	38±6	46±9	23%	4.74; <0.050[c]
Hippocampus				
-Ventral	42±5	59±9	43%	23.0; <0.0002[c]
-Dorsal	38±8	54±4	41%	33.0; <0.0003[c]
Substantia nigra				
-Reticulata	28±4	34±7	23%	4.7; <0.05[d]
-Compacta	30±4	44±9	56%	16.7; <0.0008[c]
Geniculate body				
-Medial	30±6	45±10	48%	14.1; <0.002[c]
-Lateral	43±7	47±9	8%	1.09; >0.3
VTA	43±6	50±9	15%	3.6; >0.07
MFB	48±6	39±9	−20%	6.00; <0.03[d]
Hypothalamus	34±5	40±6	18%	5.30; <0.04[d]
Superior colliculus	28±6	39±10	38%	7.60; <0.02[c]
Inferior colliculus	24±7	32±7	29%	6.0; <0.03[d]
Superior olive	34±17	34±11	2%	0.08; 1.000

Structures	5-HT synthesis rate (pmol/g/min)[a]		ANOVA	
	Controls (*N*=8)	Fluvoxamine (*N*=11)	Percent difference[b]	F(1,17); p
Pineal body	705±262	1178±298	Not tested	

[a] 5-HT synthesis rates are expressed as mean±SD. The number of animals is given in brackets.

[b] Calculated as a percent (%) of the synthesis rates in the control group.

[c] Significant difference in 5-HT synthesis rates between the fluvoxamine and control group following the Benjamini–Hochberg correction.

[d] Identifies the brain regions with $p<0.05$ which lost significance following Benjamini–Hochberg correction.