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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.

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-HT_{1A} and/or terminal 5-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

Abbreviations: α-[^{14}C]MTrp, α-^{14}C-methyl-L-tryptophan; VTA, ventral tegmental area.

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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