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Research report

5-HT_{2A} receptor antagonist M100907 reduces serotonin synthesis: An autoradiographic study

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ABSTRACT

The effects of the administration of the serotonin (5-HT)$_{2A}$ antagonist, M100907, on 5-HT synthesis rates, were evaluated using the α-[^{14}C]methyl-L-tryptophan (α-MTrp) autoradiographic method. In the treatment study, M100907 (10 mg/kg) was injected intraperitoneally 30 min before the α-MTrp injection (30 μCi over 2 min). A single dose of M100907 caused a significant decrease in the synthesis in the anterior olfactory nucleus, accumbens nucleus, frontal cortex, sensory-motor cortex, cingulate cortex, medial caudate-putamen, dorsal thalamus, substantia nigra, inferior collicus, raphe magnus nucleus, superior olive, and raphe pallidus nucleus.

These data suggest that the terminal 5-HT_{2A} receptors are involved in the regulation of 5-HT synthesis in the entire brain. Further, 5-HT synthesis is likely regulated by the 5-HT_{2A} antagonistic property of M100907 in the cortices, anterior olfactory nucleus, caudate putamen, and nucleus accumbens.

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1. Introduction

Serotonin (5-HT) cell bodies contained in the raphe nuclei have both ascending and descending axonal projections, and there are serotonergic projections from these cell bodies throughout the brain. 5-HT influences brain functions through the activation of numerous 5-HT receptor subtypes [4,14,64]. The 5-HT_2 receptor family is comprised of the 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors, which readily associate with the Gq family of G-proteins and upon activation, stimulate phospholipase C to increase phosphoinositide metabolism and cause a transient rise in intracellular free calcium [44,65,60]. The 5-HT_2 receptors have been reported to play a role in a remarkable range of central nervous system disorders, including anxiety, depression, migraine, obsessive compulsive disorder, excess impulsivity, anorexia nervosa, schizophrenia [1,12,5,21]. They also influence sleep and eating patterns [1,12,5,21]. Further, it has been shown that the administration of drugs with an affinity for the 5-HT_{2A} receptors (e.g., 5-HT_{2A} antagonists) can act synergistically with selective serotonin reuptake inhibitors (SSRIs) [43], by offering potential therapeutic advances in the treatment of some neuropsychiatric disorders [45].

5-HT_2 type binding sites were initially defined by their low affinity for [^{3}H]5-HT and a high affinity for [^{3}H]spiperone [40,58]. The density of 5-HT_2 receptors in the rat brain is highest in the claustrum, olfactory tubercle and layer IV of the cerebral cortex [56,67]. The 5-HT_{2A} receptors were also present in the anterior olfactory nucleus, piriform cortex, layers I and V of the neocortex, caudate putamen, nucleus accumbens, dentate gyrus, mammillary bodies, thalamus, hippocampus, brainstem, medulla, cerebellum and spinal cord [56,67]. 5-HT_{2A} receptor density was elevated in the olfactory bulbectomized (OBX) rats, a rat model of depression, and, antidepressant treatment down-regulated the density of the 5-HT_{2A} receptors in the OBX rats [67]. Given that the 5-HT_{2A} receptor shows distinct regional distributions, it is possible that it modulates specific physiological functions (e.g., "Executive Function"; [27]), as well as 5-HT synthesis.

The putative antipsychotic drug, *R*(+)-alpha-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)]-4-piperidine-methanol (M-100907), is a highly selective 5-HT_{2A} receptor antagonist with a significantly greater affinity (>500-fold) for the 5-HT_{2A} receptor over the D2 receptor [53,22], which may underlie its ability to achieve a high level of receptor occupancy in the absence of extrapyramidal-like symptoms in humans [3]. M100907 has been shown to elicit a positive response in a large number of preclinical paradigms designed to detect antipsychotic activity

Abbreviations: 5-HT, 5-hydroxytryptamine; α-MTrp, α-methyl-L-tryptophan; SSRI, selective serotonin reuptake inhibitor; M100907, R(+)-alpha-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)]-4-piperidine-methanol; OBX, olfactory bulbectomized rats; DV*, volume of distribution; LC, lumped constant; HPLC, high performance liquid chromatography; ANOVA, analysis of variance; TPH, tryptophan hydroxylase.

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[38]. Several of these rodent paradigms indicate that M100907 can reverse the behavioral effects of an acute hyperdopaminergic state that has been induced by amphetamines [70], potentially through modification of its stimulated release of dopamine in the medial prefrontal cortex [57] and nucleus accumbens shell [42]. This stimulated release of dopamine has likely influence on serotonergic neurotransmission through neurotransmitter interaction [28]. As it has been stated by Nelson [50]: "the most important steps in the neurosecretion cycle are the synthesis of specific neurotransmitters in the presynaptic cell, accumulation of the transmitter into synaptic vesicles or secretory granules, measured secretion of the transmitter into synaptic cleft and reuptake of the neurotransmitter or its derivative". This clearly emphasizes that synthesis is a very important factor in neurotransmission and its control and its modulation needs to be investigated in more detail, as well as its relation to changes in behavior, before a good understanding of any antidepressant action can be obtained.

The autoradiographic method using labeled α-[^{14}C]methyl-L-tryptophan (α-[^{14}C] MTrp) for the measurement of regional 5-HT synthesis rates in the rat brain [49,37] relies on the unidirectional uptake (trapping) of α-MTrp, which is transported into the brain and has been shown to be converted, in part, to α-methyl-5-HT [23]. The trapping constant can be converted into 5-HT synthesis rates by division with a lumped constant (LC), which represents a conversion constant [23], followed by multiplication with the plasma free tryptophan (Trp; [49]).

Because data has implicated the involvement of 5-HT receptors in several brain disorders and given that an alteration in the functionality of 5-HT$_{2A}$ receptors has been reported in several affective disorders, it was felt to be important to evaluate a drug that acts on that receptor. It is also important to investigate whether 5-HT$_{2A}$ receptors play a role in the modulation of 5-HT synthesis, as the modulation of regional 5-HT synthesis appears to be part of antidepressant action [37,23,10,30,46]. This question was studied by investigating the influence of a selective 5-HT$_{2A}$ antagonist, M100907. The effects of M100907 on 5-HT synthesis in the rat brain using the autoradiographic α-[^{14}C]MTrp method were investigated.

2. Materials and methods

2.1. Animals

Sprague–Dawley male rats (Charles River) weighing (mean ± SEM) 213 ± 6 g (control rats) and 229 ± 5 g (treatment group) were housed in the animal facility (room temperature of 22 ± 2 °C with a 12-h day-night cycle) for at least 3 days before the administration of the drugs. The animals were fasted overnight to stabilize the plasma concentration of tryptophan (Trp), as well as the other amino acids, but water was given *ad libitum* before the α-[^{14}C]MTrp autoradiographic experiments. The stabilization of other amino acids is needed because several essential amino acids share the same transport system with Trp at the blood brain barrier [13,66,71]. To avoid any possible influence of the circadian rhythm on the measurements, the tracer was injected between 11:00 AM and 1:00 PM, and all of the rats were sacrificed between 1:00 PM and 3:00 PM. The body weight of each rat was recorded before the initial treatment of the drug. All surgical procedures and experiments were performed with the approval of the Animal Care Committee of the Montreal Neurological Institute of McGill University, and were done according to the procedures of the Canadian Council on Animal Care.

2.2. Drug

M100907 was synthesized according to the published procedure [76] and identification was performed using the melting point determination, ^{1}H NMR, and MS. The compound was dissolved in saline (0.9% NaCl). The control rats received saline injections. A dose of 10 mg/kg of M100907 or saline at a volume of 2 mL/kg was administered (with 12 rats in each group). Both the drug and saline were administered intraperitoneally 30 min before the injection of α-[^{14}C]MTrp. α-[^{14}C]MTrp was synthesized using the previously described procedure [48] and had a specific activity of 55 mCi/mmol.

2.3. Experimental procedure

The femoral artery and vein were cannulated with plastic catheters under light halothane (1.0–2.0%) anesthesia. The posterior limbs of the rats were fixed using a loose-fitting plaster cast, and the rats were allowed to awaken. The body temperatures of the rats were kept at approximately 37 °C with a heated lamp. In the acute treatment study, a dose of 10 mg/kg M100907 in 2 mL/kg of saline was injected intraperitoneally 2 h following the surgical treatment. The same volume of saline was injected into the control rats in the same manner. Thirty minutes after the drug injection, 30 μCi of α-[^{14}C]MTrp in 1 mL of saline was injected through a catheter into the femoral vein over 2 min by an injection pump (Harvard Apparatus, Model 55-2226).

Arterial blood samples (40 μL each) were taken at progressively larger intervals, starting from the beginning of the tracer injection to the decapitation of the rats (taken at: 0.5, 1, 1.5, 2, 3, 5, 10, 20, 30, 45, 50, 55, 60 min for the 60 min experiments, and at: 0.5, 1, 1.5, 2, 3, 5, 10, 30, 60, 90, 120, 140, 145, 150 min for the 150 min experiments). The total volume of the blood taken was about 0.56 mL, and the blood was always replaced by saline. The blood samples were centrifuged for 3 min at 9,300 g, and 20 μL of plasma was taken for liquid scintillation counting to measure the plasma radioactivity needed for an arterial input function. Physiological parameters of arterial samples (PO_2, PCO_2, pH, and hematocrit) were measured at least twice in each experiment. Five additional blood samples were taken to measure the plasma concentrations of total and free Trp, using the method described below.

The rats were guillotined 60 or 150 min following the tracer injection, as required by the experimental protocol [49]. The brains were removed, frozen in cold 2-methylbutane, and sliced into 30 μm thickness in a cryostat at −20 °C. The brain slices were mounted on glass slides and exposed to X-ray films along with ^{14}C-polymer standards (American Radiolabel Co., St. Louis, MO, USA; calibrated to 30 μm thickness of the brain tissue) for 3 weeks to obtain the autoradiograms.

2.4. The measurement of plasma Trp concentration

Five plasma samples (total of about 0.4 mL; always replaced by saline) were taken at different times to determine the total and free (non-albumin-bound) Trp concentrations in the plasma. Forty μL of plasma was deproteinized with 20 μL of 20% trichloroacetic acid and used to measure the total Trp concentration in the plasma. After the sample was mixed with a vortex-mixer and centrifuged, 40 μL of supernatant was stored in a freezer (−84 °C) until it was analyzed for total Trp. An additional 40 μL of plasma was filtered through a Biomax-10 filter (10,000 MW cut-off, Millipore Co., Bedford, MA, USA) spinning at 9300 × *g* for 10 min, and was also stored in a freezer (−84 °C) until it was analyzed for free Trp. The total and free Trp concentrations were measured using HPLC (high performance liquid chromatography) with fluorescence detection [71].

2.5. Measurement of the α-MTrp trapping and calculation of 5-HT synthesis

A set of representative autoradiograms illustrating 5-HT synthesis in the rat brain as determined by the α-MTrp method have been presented in many previous publications [49,37,24,52,75,30,31] and their addition would not provide any new information, because the logarithmic scale between the tracer concentration (represented by 5-HT synthesis) and optical density. All that can be seen from these images is the brain non-homogenous distribution. The resultant images on the X-ray film were analyzed using a microcomputer-based image analyzing system (MCID/M4-Image Analysis System, Imaging Research Inc., Canada) and tissue equivalent calibrated standards as detailed in previous publications [49,37,23,30]. The optical densities were converted into tissue radioactivity concentration (*n*Ci/g). The tissue concentrations of the tracer were measured separately in 39 brain structures. Averages of six readings (bilaterally) in each brain structure in three consecutive sections was obtained. In this investigation the dorsal raphe was not separated into the dorsal, ventral or lateral parts. Instead, the tracer concentration of the entire structure was measured in all of the slices and the average value was used in the calculation of 5-HT synthesis. The tissue radioactivity concentrations were converted into volume of distribution (DV*; mL/g) by dividing tissue concentrations [$C^*_t(T)$; *n*Ci/g] by the plasma tracer concentration [$C^*_P(T)$; *n*Ci/mL] at the end of the experiment. There was a significant linear relationship between DV and Θ (min) [$\Theta = \int_0^T C^*(t)dt/C^*(T)$] with a slope K (mL/g/min; trapping constant for α-[^{14}C]MTrp as previously described [49,24] in all of the structures investigated. Note that the rats killed at different clock times would have different exposure time Θ [min] making a better definition of the straight line. The rate of 5-HT synthesis R; pmol/g/min) is calculated by converting K (slopes obtained from fits of DV as function of Θ) to K^T ($K^T = K/LC$) and then multiplying the calculated K^T by the plasma free (non-protein-bound) Trp (Cp; pmol/mL); $R = Cp\,K/LC = Cp\,K^T$. The use of plasma free Trp in this calculation is based upon reports indicating that this plasma fraction is in dynamic equilibrium with brain Trp [13,66,71]. The LC was measured in vivo and found to be 0.42 ± 0.07 [78], uniform throughout the rat brain. In the present experiments, all o the linear relationships between Θ [min] and DV* [mL/g] had a significant ($p < 0.05$; F-statistics) and positive slope [49].

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