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Both acute and subchronic treatments with pindolol, a 5-HT_{1A} and β_1 and β_2 adrenoceptor antagonist, elevate regional serotonin synthesis in the rat brain: An autoradiographic study

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ABSTRACT

Antidepressant treatments, including those that increase serotonin (5-HT) neurotransmission, require several weeks or months until the onset of the therapeutic effect in depressed patients. The negative feedback on 5-HT transmission exhibited by the 5-HT_{1A} and 5-HT_{1B} autoreceptors has been postulated as a possible delaying factor. The aim of the present study was to assess the effect of the acute and subchronic treatment with pindolol, a 5-$HT_{1A/1B}$, β_1 and β_2 adrenoceptor antagonist, on 5-HT synthesis, one of the key parameters of 5-HT neurotransmission. Male Sprague–Dawley (SPD) rats (180–220 g) were treated with pindolol or an adequate volume of saline, administered either acutely (15 mg/kg i.p.; SPD-AC-SAL, SPD-AC-TR) or subchronically (15 mg/kg day i.p. for 7 days; SPD-SUBCHR-SAL, SPD-SUBCHR-TR). Thirty minutes following the single i.p. injection (acute experiment) or at the 8th day following the commencement of the subchronic treatment (subchronic experiment), 5-HT synthesis was measured using α-[^{14}C]methyl-L-tryptophan autoradiography. The analysis of variance (ANOVA), followed by the Benjamini–Hochberg correction for multiple comparisons, revealed: (1) a significant increase of 5-HT synthesis in the SPD-AC-TR rats, relative to the SPD-AC-SAL rats in all brain regions examined except the substantia nigra – pars reticularis, dorsal subiculum, inferior olive, raphe magnus and raphe obscurus and (2) a significant increase of 5-HT synthesis in the SPD-SUBCHR-TR rats, relative to the SPD-SUBCHR-SAL rats in all brain regions except the median raphe, hypothalamus and raphe pontine. On the basis of these results, we hypothesized that the antagonism of the 5-$HT_{1A/1B}$ receptors prevents the negative feedback mediated by these receptors on 5-HT synthesis, resulting in a persistent increase of 5-HT synthesis. The results accord with clinical reports on the utility of pindolol in the augmentation of antidepressant treatment.

1. Introduction

Antidepressants acting on 5-HT neurotransmission, primarily selective serotonin reuptake inhibitors (SSRIs), have been used clinically for several decades, but the delayed onset of clinical improvement in treated patients, ranging from several weeks to months following the commencement of treatment, represents a significant problem (World Health Organization Mental Health Collaborating Centres, 1989). This therapeutic delay has been attributed to negative feedback mediated primarily by 5-HT_{1A} and 5-HT_{1B} autoreceptors (Stahl, 1998), found at the soma and dendrites of 5-HT neurons in the raphe nuclei and at the 5-HT terminals in the projection regions (reviewed by Nichols and Nichols, 2008). The stimulation of 5-HT_{1A} autoreceptors decreases the firing rates of 5-HT neurons (Scuvée-Moreau and Dresse, 1979; de Montigny et al., 1984), as well as 5-HT release (Rutter and Auerbach, 1993; McCall et al., 1994; Blier and Ward, 2003) and synthesis (Okazawa et al., 1999). Similarly, the stimulation of

Abbreviations: SPD, Sprague–Dawley; 5-HT, 5-hydroxytryptamine; α-[^{14}C]MTrp, α-[^{14}C]methyl-L-tryptophan; AO, anterior olfactory nucleus; AN, accumbens nucleus; Cincx, cingulate cortex; Fcx, frontal cortex; Scx, sensory cortex; Cl, claustrum; CPm, caudate putamen medial; CPl, caudate putamen lateral; MBF, medial forebrain bundle; GP, globus pallidus; CN, caudate putamen; Pcx, parietal cortex; Dhi, dorsal hippocampus; AMY, amygdala; DTh, dorsal thalamus; VTh, ventral thalamus; LG, lateral geniculate; Hyp, hypothalamus; DS, dorsal subiculum; Vhi, ventral hippocampus; CA3, Cornu Ammonis 3; Acx, auditory cortex; SC, superior colliculus; MG, medial geniculate; SNc, substantia nigra – pars compacta; SNr, substantial nigra – pars reticulata; VTA, ventral tegmental area; DR, dorsal raphe; MR, median raphe; Vcx, visual cortex; Encx, entorihinal cortex; IC, inferior colliculus; Rpo, raphe pontine; SO, superior olive; Rpa, raphe pallidus nucleus; IO, inferior olive; Ro, raphe obscurus nucleus; RM, raphe magnus; P, pineal body.

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5-HT_{1B} autoreceptors decreases the release (Engel et al., 1986) of 5-HT in the projection regions. One of the treatment strategies aimed to circumvent this feedback mechanism was the augmentation of SSRI treatment with pindolol, a non-selective antagonist of 5-$HT_{1A/1B}$, as well as β-adrenoceptors (Connolly and Thase, 2011). Several studies have shown that the augmentation of 5-HT reuptake inhibitors with pindolol results in a faster onset and/or higher rate of clinical improvement in patients diagnosed with major depression, (Artigas et al., 1994; Zanardi et al., 1997; Perez et al., 1997; Thome et al., 1997; Maes et al., 1999; Pérez et al., 2001; Portella et al., 2011), but others had mixed outcome (Martiny et al., 2012) or failed to show any benefit of this therapeutic regime over monotherapy with SSRIs (Berman et al., 1997; reviewed by Ballesteros and Callado, 2004). Pindolol augmentation has also been reported to produce a marked antidepressant effect in patients not responding to monotherapy with SSRIs or monoamine oxidase inhibitors (MAOI) (Blier and Bergeron, 1995; Artigas et al., 1994). This effect was selective for 5-HT-based antidepressants, as pindolol has shown no augmenting effect when combined with primarily norepinephrine (NE)-based antidepressants, such as desipramine or amitryptiline (Blier et al., 1997). Beside its augmentation effects on antidepressant treatments, (±)-pindolol has also augmented effects of selective 5-HT reuptake inhibitors in the treatment of obsessive–compulsive disorder (Fontenelle et al., 2007), as well as panic disorder (Sela et al., 2010).

Regarding the pharmacological profile of (±)-pindolol, it exhibits a comparable (in a nanomolar range) affinity for both the 5-HT_{1A} (Winter and Rabin, 1993) and 5-HT_{1B} receptors (Titeler et al., 1987), as well as a 2- to 4-fold higher affinity for β-adrenoceptors (Pazos et al., 1985). Pindolol appears to selectively bind to somatodendritic 5-HT_{1A} autoreceptors, without interacting with the postsynaptic 5-HT_{1A} receptors (Hirani et al., 2000; Saijo et al., 2012). Microdyalisis studies have shown that the co-application of (-)-pindolol with citalopram induces an additional increase of 5-HT extracellular levels, relative to citalopram alone (Hjorth and Auerbach, 1996; Romero et al., 1996). Similarly, the attenuation of 5-HT neuronal firing induced by acute treatment with another SSRI, paroxetine, was prevented by pre-treatment with 15 mg/kg of (−)-pindolol (delivered for 2 days via a subcutaneously implanted osmotic mini-pump (Romero et al., 1996)).

The effects of a combined treatment with (±)-pindolol and citalopram on 5-HT synthesis have been shown in the olfactory bulbectomized (OBX) rat model of depression (Nguyen et al., 2009). Due to the partial agonistic effects of pindolol on 5-HT_{1A} autoreceptors (Aulakh et al., 1988; Sánchez et al., 1996), the effects of pindolol on 5-HT synthesis may differ from increased extracellular concentrations of 5-HT, such as those induced by the selective 5-HT reuptake inhibitors (SSRIs), and a baseline condition.

The effects of pindolol on 5-HT synthesis have not yet been studied in isolation. The aim of the present study is to quantify the effects of acute (15 mg/kg i.p.) or subchronic (15 mg/kg day i.p., for 7 days) treatment with (±)-pindolol on 5-HT synthesis rates in Sprague–Dawley (SPD) rats, using α-[^{14}C]methyl-L-tryptophan (α-[^{14}C]MTrp) autoradiography. The subchronic experiments were needed to answer the question whether the receptors would become desensitized and, as such, stop the modulation of synthesis. The 5-HT synthesis was chosen as a biological parameter because it is one of the most important factors of serotonergic neurotransmission (Nelson, 1993). Studies in rats have shown the utility of α-[^{14}C]MTrp autoradiography in measuring 5-HT synthesis rates. Briefly, α-[^{14}C]MTrp is a radioactively labeled analog of Tryptophan (Trp), an amino-acid precursor in 5-HT synthesis. The intravenous injection of α-[^{14}C]MTrp and the subsequent measurement of the tracer levels in the plasma and brain tissue allow the calculation of the α-[^{14}C]MTrp net uptake. This parameter was shown to be independent of the protein synthesis and it presumably reflects the rates of brain 5-HT synthesis (for a review, see Diksic and Young, 2001). Positron emission tomography (PET) studies in human clinical and healthy control populations, using α-methyl-L-tryptophan labeled with the short living isotope ^{11}C, have shown that the regional levels of brain 5-HT synthesis in humans differ between currently depressed patients and healthy subjects (Rosa-Neto et al., 2004) and show the correlation between gender and history of depression (Frey et al., 2010). In addition, it was shown that there is a more rapid and greater increase of 5-HT synthesis in the prefrontal cortex (BA 9) when citalopram is combined with pindolol, compared to citalopram alone (Berney et al., 2008).

2. Materials and methods

2.1. Animals

Following arrival at the animal facility of the Montreal Neurological Institute, the male SPD rats (Charles River, St. Constant, Quebec, Canada) were allowed to acclimate to the new environment for at least 3 days before the start of the experiment, to prevent the influence of novelty-induced stress on the experimental results. The rats were housed two per cage, with the room temperature of 22 ± 2 °C and a 12-h day–night cycle. At the beginning of the experiment, the rats weighed 180–220 g. Before the α-[^{14}C]MTrp autoradiographic experiments, 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*. 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 in the subchronic experiment was recorded before the initial treatment of the drug, as well as on the day of surgical procedure. 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 treatment

(±)-Pindolol hydrochloride was obtained from a commercial supplier (Tocris Bioscience, Business Park, Ellisville, Missouri, USA). The compound was dissolved in saline (0.9% NaCl) with the small addition of 0.1 N HCl to adjust the pH to 5. The control rats were injected with the same solvent. In the acute experiments, a dose of 15 mg/kg of (±)pindolol or solvent at a volume of 2 mL/kg was administered approximately 60 min before the infusion of α-[^{14}C]MTrp. Both the drug and solvent were administered intraperitoneally (i.p.). In the subchronic treatments, the rats received i.p. either a drug or solvent injections (7.5 mg/kg) twice a day (9 am and 9 pm) for 7 days, and were used in the tracer experiments on the 8th day (e.g., approximately 12–14 h after the last drug or solvent injection).

2.3. Experimental procedure

The femoral artery and vein were cannulated with plastic catheters under light halothane (1.0–2.0%) anaesthesia. The posterior limbs of the rats were fixed using a loose-fitting plaster cast, and the rats were allowed to awaken. The rats' body temperatures were kept at approximately 37 °C with a heating lamp. Sixty minutes after the drug injection (acute experiment) or at least 2 h following the termination of anaesthesia (subchronic experiment), 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