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Chronic citalopram treatment elevates serotonin synthesis in flinders sensitive and flinders resistant lines of rats, with no significant effect on Sprague–Dawley rats

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ABSTRACT

The influence of citalopram on regional 5-hydroxytryptamine (serotonin, 5-HT) synthesis, one of the most important presynaptic parameters of serotonergic neurotransmission, was studied. Sprague–Dawley (SPD) rats were used as the controls, and Flinders Resistant Line (FRL) rats were used as auxiliary controls, to hopefully obtain a better understanding of the effects of citalopram on Flinders Sensitive Line (FSL; "depressed") rats. Regional 5-HT synthesis was evaluated using a radiographic method with a labelled tryptophan analog tracer. In each strain of rats, the animals were treated with citalopram (10 mg/(kg day)) or saline for 14 days. The groups consisted of between fourteen and twenty rats. There were six groups of rats with citalopram (CIT) and saline (SAL) groups in each of the strains (SPD–SAL, SPD–CIT, FRL–SAL, FRL–CIT, FSL–SAL and FSL–CIT). A two-factor analysis of variance was used to evaluate the effect of the treatment c., SPD-SAL relative to SPD-CIT) followed by planned comparisons to evaluate the effect in each brain region. In addition, the planned comparison with appropriate contrast was used to evaluate a relative effects in SPD relative to FSL and FRL, and FSL relative to FRL groups. A statistical analysis was first performed in the *a priori* selected regions, because we had learned, from previous work, that it was possible to select the brain regions in which neurochemical variables had been altered by the disorder and subsequent antidepressant treatments. The results clearly show that citalopram treatment does not have an overall effect on synthesis in the control SPD rats; there was no significant ($p > 0.05$) difference between the SPD–SAL and SPD–CIT rats. In "depressed" FSL rats, citalopram produced a significant ($p < 0.05$) elevation of synthesis in seventeen out of thirty-four regions, with a significant ($p < 0.05$) reduction in the dorsal and median raphe. In the FRL rats, there was a significant ($p < 0.05$) elevation in the synthesis in twenty-two out of thirty-four brain regions, with a reduction in the dorsal raphe. In addition to these regions magnus raphe was different in the SPD and FSL groups, but it was on the statistical grounds identified as an outlier. There were significant changes produced in the FSL and FRL rats in thirteen out of seventeen *a priori* selected brain regions, while in the SPD rats, citalopram produced significant changes in only four out of seventeen *a priori* selected regions. The statistical evaluation also revealed that changes produced by citalopram in the FSL and FRL rats were significantly greater than those in the SPD rats and that there was no significant difference between the effect produced in the FSL and FRL rats. The presented results suggest that in "depressed" FSL rats, the antidepressant citalopram elevates 5-HT synthesis, which probably in part relates to the reported improved in behaviour with citalopram.

1. Introduction

The evaluation of biological and behavioural parameters in animal models of depression is very important in obtaining a better understanding of depression (Nestler et al., 2002; Cryan et al., 2002; Cryan and Mombereau, 2004). A large volume of research data has been collected, both in animal models and humans on depression, but there is still no clear understanding of the pathophysiology of major depression, causes of it, or the way

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antidepressants work in alleviating the symptoms of major depression. Part of the problem is the fact that depression is a rather heterogeneous disorder and there is no consensus on which pathophysiological parameters are the most important in its description (O'Neil and Moore, 2003). The monoamine hypothesis of major depression is mainly based on the fact that drugs acting primarily through serotonergic and noradrenergic systems do alleviate symptoms of major depression in certain groups of patients. However, more recently, there have been several new hypotheses of neurotransmission deficiencies/alterations in depression proposed (Kiss, 2008; Vizi et al., 1995). These newer approaches could, in part, explain the creation of non-physiological circuitry, which may underlie the depressive state. There is more and more data available suggesting the relationship between stressful effects of early life and vulnerability to the development of mood disorders (Liu et al., 2000; Sapolsky and Meaney, 1986). For example, maternal separation produces increased adult stress reactivity, which results in an elevation of hormonal, and corticosterone secretion (Heim and Nemeroff, 1999). Unfortunately, the majority of these studies have been performed in normal animals, which cannot take into account the role of genetics and/or environment–genetics interaction in mood disorders. Some studies, however, were also done in genetically altered animals: Flinders Sensitive Line (FSL) rats, while using Flinders Resistant Line (FRL) as controls (El Khoury et al., 2006). Unfortunately, these controls are not normal, as they have a higher resistance to cholinergic agonists than the rats from which they are derived, and comparisons of the neurochemical and/or behavioural variables may create confound in the interpretation of the results.

The baseline synthesis in FSL rats is lower than the synthesis in either the FRL or SPD rats (Hasegawa et al., 2006), with the baseline synthesis in FRL rats being rather similar to that in SPD rats. Both FSL and FRL rats were generated from the SPD rats by selective breeding. FSL rats have a behavioural super-sensitivity and FRL rats have a sub-sensitivity to diisopropyl fluorophosphatase (Overstreet et al., 1979; Russell et al., 1982). This super-sensitivity accords with the cholinergic hypothesis of depression (Janowsky and Risch, 1984) and the hypothesis of serotonergic and cholinergic actions of antidepressants (Dilsaver, 1986; Janowsky and Risch, 1984; Plaznik et al., 1989). The measurements of regional 5-HT synthesis (Hasegawa et al., 2006), tissue levels of 5-HT and other neurochemical and behavioural variables (Overstreet et al., 2005; Yadid et al., 2000) suggest a very complicated interplay of different biochemical parameters in FSL rats. These studies have also documented a large spectrum of both neurochemical (*e.g.*, cholinergic, gabanergic, neurosteroids, dopaminergic, hypothalamic–pituitary–adrenal axis; receptors; 5-HT_{2C}, 5-HT_3, $GABA_A$) and behavioural (*e.g.*, immobility) deficiencies in FSL rats, relative to the control FRL or SPD rats (Yadid et al., 2000; Overstreet et al., 2005; Nishi et al., 2008).

Recently, we reported a rather different effect of acutely administered citalopram on regional serotonin synthesis in FSL rats compared to either SPD or FRL rats (Kanemaru et al., 2008). Acute citalopram treatment resulted in a reduction of the synthesis in FSL rats and an elevation of the synthesis in FRL rats, but without overall significant effect in the SPD rats. A relatively large reduction of regional 5-HT synthesis in FSL rats by acute citalopram is probably, at least in part, the result of the super-sensitivity of 5-HT_{1A} receptors, known to be involved in 5-HT synthesis control (Okazawa et al., 1999; Tohyama et al., 2001, 2007) found in the FSL rats (Shayit et al., 2003), because there was no significant effect on the synthesis in normal SPD rats. An elevation of the synthesis observed in the FRL rats following acute citalopram (Kanemaru et al., 2008) suggests a rather different 5-HT synthesis control in this strain which probably relates to different intracellular signalling than in SPD rats and/or a genetically created resistance to a cholinergic agonist.

Citalopram is a highly selective serotonin reuptake inhibitor (SSRI) with a high affinity and selectivity for serotonin (5-hydroxytryptamine, 5-HT) reuptake sites, with a low affinity for noradrenaline (NA) uptake sites (Hyttel, 1977; Hiemke and Härtter, 2000). It is also clinically effective as an antidepressant and currently prescribed for use (Milne and Goa, 1991). The blocking of the 5-HT transporter by SSRIs locally elevates the concentration of 5-HT, resulting in a greater availability of 5-HT to act on both pre- and post-synaptic 5-HT receptors (Bosker et al., 1995; Ceglia et al., 2004). Chronic treatments are required to alleviate depression, suggesting that some neuronal changes take place before the clinical effects are observed (Salomon et al., 1993; Garattini and Samanin, 1988). Similarly, in animal models of depression (*e.g.*, olfactory bulbectomized rats, FSL rats), only chronic treatments with antidepressants normalize behavioural as well as many neurochemical and endocrine abnormalities found in these models (Kelly et al., 1997; Overstreet et al., 2005; Hasegawa et al., 2005). It has also been shown that chronic treatments with citalopram result in an elevation of extracellular concentration of 5-HT (Muraki et al., 2001; Raap and Van de Kar, 1999). This elevation in the extracellular concentration of 5-HT reduces the amount of 5-HT released, which subsequently, and over time, produces adoptive changes and the desensitization of 5-HT autoreceptors in normal rats (Goodwin, 1996; Blier and de Montigny, 1996; Li et al., 1997; Slattery et al., 2004; Muraki et al., 2008). Following a chronic administration of SSRIs, the desensitization of the serotonergic 5-HT_{1A} autoreceptors on cell bodies and the 5-$HT_{1B/D}$ on the terminals of serotonergic neurons has been documented (Blier et al., 1990; Invernizzi et al., 1992; Dobson et al., 2004; Tohyama et al., 2002). A sub-chronic citalopram treatment in humans produces a region specific reduction of regional 5-HT synthesis, while a more chronic treatment produces an elevation of regional 5-HT synthesis, which correlates with the reduction of depression scores (Berney et al., 2008). Is should be emphasized that these receptor sites and 5-HT synthesis represent only a small fraction of neurochemical parameters defining depression, a very complex state. Similarly, in animal models, only a fraction of behavioural and neurochemical parameters related to the depressed state in humans could be reproduced in any of the presently used animal models (Nestler et al., 2002; Cryan et al., 2002). Unfortunately, until now, a rather small amount of research in which neurochemical parameters were measured has been performed in good animal models of depression.

Because it is the case that only a chronic administration of citalopram produced behavioural changes (*e.g.*, making behaviour close to that in control rats) in the FSL rats (Overstreet et al., 2005; Yadid et al., 2000), it was important to investigate the effect of a chronic treatment of citalopram on regional 5-HT synthesis in FSL rats. The synthesis is one of the most important presynaptic processes related to serotonergic neurotransmission (Nelson, 1993). Further, in another rat model of depression, olfactory bulbectomized rats, citalopram normalized 5-HT synthesis and brought it to the level of sham-operated rats (Hasegawa et al., 2005). Knowing that citalopram has produced rather diverse effects in FSL, FRL and SPD rats following acute treatment (Kanemaru et al., 2008), it was important to study all three strains in a chronic treatment set-up to attempt to obtain a better understanding of citalopram action and possibly the reasons citalopram changes behaviour in FSL rats, but not in FRL rats. It was hypothesized that citalopram would elevate regional 5-HT synthesis in FSL rats as part of its antidepressant effect, because their baseline 5-HT synthesis is lower than in normal SPD rats, without a significant effect in normal SPD rats. This was based on the fact that citalopram does not change behaviour in normal rats and it has been known that there is an interaction between