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CHRONIC FLUOXETINE RESCUES CHANGES IN PLASMA MEMBRANE DENSITY OF 5-HT1A AUTORECEPTORS AND SEROTONIN TRANSPORTERS IN THE OLFACTORY BULBECTOMY RODENT MODEL OF DEPRESSION

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Abstract—Reduced serotonin (5-HT) neurotransmission is postulated to underlie the pathogenesis of depression. The serotonin transporter (SERT) and 5-HT1A auto-receptors act in concert to ensure homeostasis of serotonin (5-HT) neurotransmission and regulation of their cell surface expression represent efficient mechanisms to maintain this homeostasis. Thus, we investigated the changes in the subcellular distribution of SERT and 5-HT1A receptors (5-HT1AR) in the rat olfactory bulbectomy model of depression using immuno-gold labeling and electron microscopy, and examined the effect of chronic treatment with the antidepressant, fluoxetine, a serotonin reuptake inhibitor, on the subcellular distribution of SERT and 5-HT1AR. The density of plasma membrane labeling of 5-HT1A auto-receptors on dendrites of dorsal raphe neurons was increased after bulbectomy, but the 5-HT1A hetero-receptor membrane labeling on dendrites of CA3 hippocampal neurons was not. The density of membrane labeling of SERTs was increased both in dendrites of dorsal raphe neuron and axon terminals in the hippocampus after bulbectomy. However, the proportion of 5-HT1AR and SERT membrane labeling relative to total labeling was unchanged, suggesting an increase in protein levels. The increases in 5-HT1AR and SERTs membrane labeling induced by bulbectomy were reversed by chronic fluoxetine treatment, and these changes were associated with a reduction in the relative proportion of membrane versus total labeling, consistent with a protein shift between subcellular compartments. Our findings support the hypothesis that changes in efficacy of serotonergic neurotransmission in this model of depression depends on both activity and density of cell surface-expressed SERT and 5-HT1A auto-receptors.

Key words: depression, antidepressant, animal model, olfactory bulbectomy, 5-HT1A receptor, serotonin transporter, 5-HT1A auto-receptor, 5-HT1A hetero-receptor, hippocampus, dorsal raphe nucleus.

INTRODUCTION

A reduced activity of serotonergic neurotransmission is postulated in the pathogenesis of depression (Meltzer, 1989; Ressler and Nemeroff, 2000) and medication enhancing central serotonergic activity is widely used as treatment (Nemeroff, 1998). Impairment of serotonin (5-HT) neurotransmission by depletion of 5-HT or inhibition of 5-HT synthesis precipitates symptoms of depression (Young et al., 1985; Delgado et al., 1990; Meltzer, 1990; Maes and Meltzer, 1995). Conversely, facilitation of 5-HT neurotransmission by precursor loading, nonselective serotonin agonists, monoamine oxidase inhibitors and selective serotonin reuptake inhibitors, elevates mood (Meltzer, 1990; Maes and Meltzer, 1995).

5-HT neurons are scattered along the midline throughout the rostral-caudal extent of the brainstem, with the largest populations in the median and dorsal raphe nuclei. 5-HT cells send projections throughout the forebrain to limbic, striatal, and cortical regions (Adell et al., 2002). 5-HT1A receptors (5-HT1ARs) and serotonin transporters (SERTs) play a key role in maintaining 5-HT homeostasis due to their location on presynaptic 5-HT neurons (Barnes and Sharp, 1999) and 5-HT projection targets (Varnäs et al., 2004; Kish et al., 2005). 5-HT1ARs act as somatodendritic auto-receptors on 5-HT neurons and as postsynaptic hetero-receptors in 5-HT projection areas (Kia et al., 1996; Riad et al., 2000). SERTs are present on 5-HT neuron soma, dendrites and axon terminals (Zhou et al., 1996, 1998a; Tao-Cheng and Zhou, 1999) and limit 5-HT neurotransmission via 5-HT reuptake (Barnes and Sharp, 1999; Hoyer et al., 2002).

SERTs are molecular targets for clinically effective antidepressants (Blakely et al., 1994; Schloss and Williams, 1998). Selective serotonin reuptake inhibitors bind to SERT, thereby blocking 5-HT uptake

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Abbreviations: 5-HT, serotonin (5-hydroxytryptamine); 5-HT1AR, serotonin-1A receptor; SERT, serotonin transporter; OBX, olfactory bulbectomy; DRN, dorsal raphe nucleus.

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and enhancing synaptic 5-HT levels. Although inhibition of uptake is achieved rapidly and efficiently by antidepressant medication, mood improvement only occurs after several days/weeks of treatment (Coyle and Duman, 2003; Schloss and Henn, 2004; Millan, 2006). This delay suggests that serotonin uptake inhibition alone cannot account for the therapeutic effect and that other regulatory mechanisms must come into play. A candidate mechanism is the regulation of 5-HT clearance by modulation of SERT protein expression on the cell surface. Indeed, long-term treatment with selective serotonin reuptake inhibitors leads to a significant reduction of SERT density in hippocampus and dorsal raphe nucleus (Piñeyro et al., 1994; Benmansour et al., 1999; Kittler et al., 2010; Descarries and Riad, 2012). Moreover, antidepressants were found to reduce serotonin clearance in vivo to a greater extent by down-regulation of SERT than by its acute blockade (Benmansour et al., 1999, 2002).

The 5-HT1A auto-receptor is another key regulator of serotonergic neurotransmission. Activation of somatodendritic 5-HT1A auto-receptors by locally released 5-HT inhibits firing of 5-HT neurons, thereby reducing 5-HT actions throughout the brain (Blier and de Montigny, 1987; Sprouse and Aghajanian, 1987; Hutson et al., 1989; Sharp et al., 1989; Adell et al., 2002). In animal studies of antidepressant treatment with selective serotonin reuptake inhibitors, the firing rate of 5-HT neurons is reduced at initiation of treatment and recovers over weeks, corresponding with the time course of 5-HT1A auto-receptor desensitization and/or internalization (Li et al., 1996; Le Poul et al., 1997; Czachura and Rasmussen, 2000; Aznavour et al., 2006; Riad et al., 2008). Because 5-HT1A hetero-receptors on 5-HT neuron projection targets do not desensitize or internalize (Kennett et al., 1987; Beer et al., 1990; Haddjeri et al., 1998; Piñeyro and Blier, 1999; Le Poul et al., 2000; Riad et al., 2001, 2004, 2008), the net effect of 5-HT1A auto-receptor internalization is enhancement of serotonergic transmission. Selective serotonin reuptake inhibitors work by inhibiting 5-HT reuptake and thus their effect of signal amplification is dependent on 5-HT release after 5-HT neuron firing. So attenuated 5-HT neuron firing, due to 5-HT1A auto-receptor activation, will undermine the signal-enhancing effect of selective serotonin reuptake inhibitors because less 5-HT is being released in the first place. The 5-HT1A auto-receptors desensitization model of antidepressant action postulates that chronic antidepressant treatment reduces 5-HT1A auto-receptor-mediated inhibitory feedback in 5-HT neurons by desensitization/downregulation of 5-HT1A auto-receptors, resulting in increased 5-HT neuron firing and increased postsynaptic serotonergic signaling.

5-HT1AR and SERT are, thus, of central interest in psychopharmacology of depression. We demonstrated previously that acute treatment of normal rats with the selective serotonin reuptake inhibitor fluoxetine leads to internalization of plasma membrane 5-HT1A auto-receptors in dorsal raphe 5-HT neurons, but not of 5-HT1A hetero-receptors in hippocampus (Riad et al., 2001, 2004). In addition, SERT was internalized in both cell bodies and axon terminals of 5-HT neurons after chronic but not acute fluoxetine treatment (Descarries and Riad, 2012). However, whether fluoxetine treatment induces similar changes in SERT and 5-HT1AR localization in an animal model of depression remains to be determined.

Olfactory bulbectomy in rat is a model of depression with behavioral alterations that are reversed by chronic antidepressant treatment, similar to major depression in humans (Kelly et al., 1997; Bourin et al., 2001; Song and Leonard, 2005). Thus, in the present study, we investigated the changes in subcellular distribution of SERT and 5-HT1AR in the bulbectomized rat model of depression, and their reversal by chronic fluoxetine treatment. We used electron microscopy and immunogold labeling to quantify SERT labeling in the plasma membrane and cytoplasmic compartments of 5-HT neuron dendrites in dorsal raphe nucleus and axon terminals in CA3 hippocampus, as well as 5-HT1A auto-receptors on dorsal raphe neurons and 5-HT1A hetero-receptors on hippocampal neuron. We found that bulbectomy resulted in increased 5-HT1A auto-receptor and SERT surface expression and these changes were reversed by chronic fluoxetine treatment. In addition, analysis of the proportion of membrane relative to total labeling suggests that the changes in 5-HT1AR and SERT induced by bulbectomy may be due to increase in protein levels, whereas the decrease in membrane labeling induced by fluoxetine treatment may be due to proteins shifting between subcellular compartments.

EXPERIMENTAL PROCEDURES

Animals

Adult male Sprague–Dawley rats (250 ± 50 g body weight; Charles River, St-Constant, QC, Canada) were housed at a constant temperature (21 °C) and humidity (60%), under a fixed 12-h light/dark cycle and free access to food and water. All procedures involving animals and their care were conducted in strict accordance with the Guide to care and use of experimental animals (Ed. 2) of the Canadian Council on Animal. The experimental protocols were approved by the Comité de déontologie pour l'expérimentation sur des animaux (CDEA) of the Université de Montréal. Six groups of five animals were used: (1) a group of un-operated and saline-treated rats (the control group to which the results of all other groups were normalized to); (2) a group of un-operated and fluoxetine-treated rats; (3) a group of sham-operated and saline-treated rats; (4) a group of sham-operated and fluoxetine-treated rats; (5) a group of bulbectomized and saline-treated rats; and (6) a group of bulbectomized and fluoxetine-treated rats.

Olfactory bulbectomy

Olfactory bulbectomy was performed on rats anesthetized with a mixture of isoflurane (3–3.5%, O2 0.6 L/min) and mounted onto a stereotaxic apparatus. The surface of the skull was exposed and a rectangular hole was drilled on the portion of the skull overlying the olfactory bulbs. The caudal end of the bulbs was severed from

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