ABSTRACT

Major depression (MDD) is one of the leading global causes of all non-fatal burden of disease. Involving monoaminergic imbalances, but also hormonal, structural and inflammatory alterations, the underlying pathogenesis remains incompletely understood. The antidepressant drug fluoxetine, which may be considered the “prototype” of all selective serotonin reuptake inhibitors (SSRI), appears to affect all of these processes. Interestingly, this is also the case for retinoic acid (RA), the highly potent active metabolite of vitamin A. In this review, we discuss RA signaling as a central mechanism of action – and missing link – for the multiple, pleiotropic effects of fluoxetine in the CNS, suggesting that direct inhibition of CYP-450-mediated RA catabolism by fluoxetine results in increased local concentration, and enhanced paracrine RA signaling in the CNS.

Enhancement of Local Retionic Acid Signaling: A Pivotal Mechanism in Fluoxetine’s Pleiotropic Actions

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Introduction

Mental and substance use disorders are the leading global cause of all non-fatal burden of disease with major depressive disorder (MDD) having the biggest impact in this group1. While the neurobiological mechanisms underlying MDD are highly complex and not yet fully understood, treatment with antidepressant substances, first serendipity discovered, started over 50 years ago. Revelation of a common monoaminergic mode of action has significantly contributed to the neurobiological understanding of MDD pathogenesis and led to the “monoamine hypothesis”2. Fluoxetine, which was the first selective serotonin reuptake inhibitor (SSRI) that was approved by the Food and Drug Administration (FDA) in 1987, may be considered the “prototype” of all modern, selective monoaminergic antidepressants. Although numerous substances with a selective monoaminergic mechanism of action have followed and been approved since, fluoxetine is still effectively used in clinical practice. Fluoxetine, but also other selective monoaminergic antidepressants, do not exhibit any immediate antidepressant actions. In fact, antidepressant action is known to occur no earlier than 2-3 weeks after onset of treatment, a fact suggesting other processes, secondary to (altered) monoaminergic signaling, to be responsible for and involved in an antidepressant mechanism of action. Therefore, many additional theories about fluoxetine’s antidepressant mode of action are discussed. Several specific, but likely “serotonin-independent” effects of fluoxetine have been demonstrated, including enhancement of synaptic plasticity in hippocampal neurons3,3, anti-inflammatory mechanisms4, but also a serotonin-independent effect on the acid sphingomyelinase-ceramide system5,6,7.
Interestingly, one of the hallmark characteristics of fluoxetine is its rather long half-life and its ability to interact strongly with the cytochrome P450 (CYP450)-system. While this fact has mainly been of interest in the context of drug-drug interactions, it is noteworthy that CYP450 enzymes are rather ubiquitously expressed, also (or especially) in the brain, where they are believed to contribute to specific local small molecule levels.

The metabolism of retinoids

Retinoic acid (RA), the active metabolite of Vitamin A, is one of the most important, CNS-active small molecules regulated by local CYP450-catabolism. As mentioned above, fluoxetine is known to inhibit a number of likely RA-degrading CYP450-isozymes. Therefore, a local interaction in the brain may be of relevance for local RA concentrations as well. Most interestingly, we were able to demonstrate this relationship in a series of previous experiments, indicating a direct inhibitory effect of fluoxetine on local RA degradation in brain tissue, pointing towards altered RA-signaling as a putative missing link between the multiple, RA-like (and serotonin-independent) effects of fluoxetine. RA is a crucial CNS morphogen, involved in patterning and neuronal differentiation in embryonic brain development, but also an essential factor in neuronal plasticity and regeneration of the adult CNS. RA belongs to the group of endogenous retinoids, which are metabolites of retinol and other precursors termed “vitamin A” or beta-carotene. After oral uptake, vitamin A-derivatives are stored as retinyl esters, mainly in the liver. Transport to tissue targets takes place via the blood as retinol bound to retinol-binding protein (RBP). Uptake into the target cells has been demonstrated to involve binding to membrane receptor STRA6. Subsequently, retinol is oxidized by the retinol dehydrogenase (ROLDH) into retinal and in a second step via retinaldehyde dehydrogenase (RALDH) into RA, the final active metabolite which can diffuse to other, neighboring cells, generating “RA gradients”, which again depend not only on the local rate of synthesis, but more importantly on the local rates of degradation via the CYP450-system. Resembling a central, endogenous neurotrophic process, an involvement of altered RALDH function has been suggested by Gruenblatt and Riederer to play a role in the pathogenesis of neurodegenerative disorders such as Alzheimers’ disease at the genetic, protein expression level and at the level of enzymatic activity. With respect to RA’s molecular actions, after binding to cellular retinoic acid binding protein (CRABP) I and II, CRABP II assists RA to enter the nucleus and to reach its nuclear receptors. RA binds to retinoic acid receptors (RARα, RARβ and RARγ) and retinoic X receptors (RXRa, RXRβ and RXRγ) which in turn bind to retinoic acid response elements (RARE), inducing ligand-dependent transcription.

RA Signaling in MDD

RA is involved in several MDD related processes, which is most strikingly demonstrated in a knockout mouse, where, ablation of RXY was found to lead to depressive-like behavior in the mice.

Moreover, chronic treatment with isoretinoin, the 13-cis-isomer and prodrug of retinoic acid that is used in acne therapy, is well known to cause severe depressive symptoms including suicide. Given the tight regulation of RA homeostasis by local synthesis and differential local degradation via fine-tuned feedback mechanisms, it is not surprising that opposite effects can be observed for chronic, high-dose treatment with retinoids (inducing depressive-like behavior) and for acute, short-term treatment, which was reported to have antidepressive effects similar to those observed for fluoxetine.

During embryonic development, a negative feedback was shown for RA on the expression of ROLDHs and thereby its own synthesis. In almost all other developing or adult tissues, local degradation of RA via CYP450 enzymes is positively feedback-regulated by RA with RAR involvement. Several isozymes of CYP450, including retinoid specific CYP26 isozymes but also less well characterized ones, have been identified to be involved in RA-catabolism. While in adults CYP26A1 is mainly expressed in the liver, CYP26B1 has been shown to have the highest levels in the CNS. Highlighting the clinical impact of the CYP450-system in the context of MDD, an association has been found between functionally relevant polymorphisms of RA-catabolizing CYP450-isozymes, such as CYP2C19, on the one hand, and response to antidepressant treatment as well as prevalence of depressive symptoms and risk for depression and suicide itself on the other hand. Consequently, transgenic mice expressing the mutant human CYP2C19 gene were found to exhibit hippocampal atrophy and depressive-like symptoms. Against the background of RA’s involvement in MDD pathogenesis and its catabolism via CYP450-isozymes, RA signaling might represent a missing link between pathologically increased catabolism (e.g. via aberrant CYP2C19 isoforms) and altered state of mood and behavior.

Common targets of fluoxetine and RA in MDD

Fluoxetine and RA exhibit several effects in common on MDD related processes, supporting the hypothesis of enhanced RA signaling as an underlying mechanism for fluoxetine’s serotonin-independent pleiotropic effects. Synaptic plasticity including structural and functional processes in neurons due to environmental influences such as injury, stress and inflammation, is well known to be affected during MDD pathogenesis. Interestingly, RA signaling can directly affect homeostatic synaptic plasticity via RARα and the Calcium-dependent protein...
phosphatase calcineurin (CaN)\textsuperscript{33}. Altered CaN function has also been linked to psychiatric disorders\textsuperscript{34}, and CaN activity has been shown to be upregulated in murine hippocampus after chronic fluoxetine treatment. Moreover, upregulation of CaN leads to a sensibilisation of mice to the behavioral effects of fluoxetine\textsuperscript{35}, overexpression of human CaN upstream regulator of calcineurin 1 (RCAN1) results in increased anxiety and RCAN1 knock-out mice show a lack of early fluoxetine response\textsuperscript{36}. Taken together, these results point towards an involvement of CaN (and

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**Figure 1:** Retinoid homeostasis and involvement in MDD-related processes. Retinol, which is stored mainly in the liver, is released to the bloodstream bound to retinoid-binding proteins (RBPs). After uptake into target cells, retinol is oxidized via retinol dehydrogenases (ROLDH) and retinaldehyde dehydrogenases (RALDH) to retinoic acid (RA). RA then enters the nucleus of the same cell (autocrine actions) or diffuses to the nuclei of adjacent cells (paracrine actions) and binds to RXR and RAR receptors, forming homo- and heterodimers that induce transcription after binding to retinoic acid response elements (RARE). Chronic treatment with RA or its 13-cis isomer isorotinoin can lead to severe depressive symptoms, whereas acute RA treatment exhibits antidepressant effects similar to those observed for fluoxetine (1). Polymorphisms of RA-catabolizing CYP450-isozymes (2) are associated with response to antidepressants as well as the risk for developing depression. RA-signaling controls dopamine d2 receptor (D2R) expression (4) with D2R-upregulation resulting in antidepressant effects. Moreover, RA directly affects synaptic plasticity via RAR\textalpha-mediated signaling (3), a process essentially involved in MDD pathogenesis. Furthermore, RA transcriptionally regulates CRH expression, with chronic RA exposure resulting in enhanced activity of the hypothalamic–pituitary–adrenal (HPA) axis. Finally, paracrine signaling (5) to adjacent cells results in anti-neuroinflammatory and neuroprotective actions.
potential downstream RA signaling) in fluoxetine’s mode of action. This pathway might explain fluoxetine’s capability to potently modulate neuronal plasticity as well as its neuroprotectant properties. Conversely, chronic RA treatment, which likely results in dysbalance of the homeostatically controlled endogenously RA signaling, has been shown to suppress hippocampal neurogenesis in adult rats, being associated with increased hippocampal RARα expression and occurrence of depressive-like symptoms.

Finally, MDD pathogenesis is strongly linked to chronic neuroinflammation, a process that is well inhibited by both, RA and fluoxetine, providing yet another important overlap of RA- and fluoxetine-mediated effects. Another overlap between RA’s and fluoxetine’s anti-inflammatory properties has been noted at the level of cytokine release from astrocytes and overall inhibition of microglial activation assessed by the release of the proinflammatory cytokine TNF-α or IL-6, demonstrating anti-inflammatory effects for both, fluoxetine and RA.

With respect to the canonical chronic stress model for MDD pathogenesis, RA also seems to be involved: While hyperactivity of the CRH-driven hypothalamic–pituitary–adrenal (HPA) system is a common feature in some depressive patients, RA-signaling has been shown to control CRH gene expression directly via RARα-dependent signal transduction. In a rat model it has been shown that RA-exposure can further result in altered glucocorticoid receptor (GR) expression and overall enhanced HPA axis activity. Fluoxetine is also known to alter GR expression and to change HPA activity via feedback–mechanisms, providing yet another overlap between the neurobiological effects of RA and of fluoxetine. Another direct, likely serotonin-independent effect of fluoxetine was shown for the acid sphingomyelinase-ceramide (ASM) system. ASM is a glycoprotein that catalyzes sphingomyelin degradation into ceramides that, in higher concentrations, are associated with depressive symptoms. While fluoxetine can reduce ASM activity and, consequently, lower hippocampal ceramide concentrations, RA has also been demonstrated to affect ASM-activity and lower ceramide levels, although lower ceramide levels have been attributed to increased ceramide degradation by ceramide kinase in RA treatment. This provides another common target for RA and fluoxetine. Against the background of the complex regulation underlying local RA signaling in the adult brain, it becomes clear that there is a pronounced difference between chronic RA-treatment, which can increase depressive symptoms, and acute RA exposure, which reduces depressive symptoms. This may point towards adaptational processes during long-term treatment.

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**Figure 2:** MDD-related neurobiological processes targeted by both, RA and fluoxetine. These processes include synaptic plasticity, that is directly affected by RA signaling at the level of homeostatic synaptic plasticity involving differential, calcium-triggered local RA synthesis, which essentially involves the calcium-dependent protein phosphatase calcineurin (CaN), a protein for which expression levels have been demonstrated to be affected by fluoxetine treatment and associated with fluoxetine response. Another common target of RA and fluoxetine is their anti-inflammatory property involving altered cytokine expression patterns. Moreover, both RA and fluoxetine alter HPA axis activity, while a precise mechanism involving RA-mediated CRH-transcription has been suggested. Finally, the canonical monoamine system is affected by both substances, with fluoxetine treatment resulting in an upregulation of the MDD-associated D2R, which is at the same time a known target of RA, bearing a retinoic acid response element in its promotor region. Taken together, numerous depression-related processes that are altered through fluoxetine treatment are equally classical targets of RA-signaling, strongly supporting a RA-dependent mechanism in fluoxetine’s pleiotropic mode of action (Figure 2).
involving the tightly regulated feedback machinery that is meant to keep local RA signaling in a physiological balance. Such adaptational processes may include downregulation of RA synthesizing-and upregulation of RA degrading enzymes in a temporo-spatial manner, which in the long term may lead to effects opposite to the effects seen for acute stimulation of RA signaling. Another highly relevant MDD-related target for RA signaling is the dopamine d2 receptor (D2R). RXRγ controls the expression of the canonical D2R, which is a relevant antidepressant target by numerous established antidepressant pharmacotherapies and known to be upregulated by chronic fluoxetine treatment16. Taken together, both fluoxetine and RA target common neuroprotective and pro-differentiative pathways, including key homeostatic and depression-related processes, suggesting a mechanism of action for fluoxetine that may potentially involve altered endogenous retinoid signaling (Figure 2).

Fluoxetine blocks RA degradation

Against the background of the common MDD-related pathways targeted by both, fluoxetine and RA, we recently hypothesized a direct interaction between fluoxetine and RA at a local pharmacokinetic level. Using traditional pharmacological approaches, we were able to show that degradation of RA can be potently inhibited by fluoxetine in a dose-dependent manner in brain tissue ex vivo, resulting in a dose-dependent increase of RA (tissue-) levels. At a functional level, we were able to demonstrate that fluoxetine was able to reduce glutamate excitotoxicity on primary neurons to a similar extent observed for RA degradation of RA can be potently inhibited by fluoxetine. Using traditional pharmacological approaches, we were able to show that RA signaling via RXR receptors may predominantly mediate the neuroprotective effects of fluoxetine9.

An interaction of RA and fluoxetine has also been suggested at the clinical level, where administration of fluoxetine has led to significant improvement of depressive symptoms after isotretinoin treatment in case reports18. Taken together, there are several independent lines of evidence pointing towards RA-involvement in fluoxetine’s pleiotropic actions, strongly supporting the experimentally demonstrated direct inhibition of RA degradation by fluoxetine to mediate its pleiotropic, neuroprotective and anti-inflammatory mode of action.

References


