

## **The effects of fluoxetine on gene expression: two models for the antidepressant mechanism and a novel use in cancer therapy. A review.**

**Fluoxetine hydrochloride, commonly known by its brand name “Prozac,” is a popular antidepressant drug used to treat a variety of mental illnesses. Fluoxetine acts to block the synaptic reuptake of serotonin, a neurotransmitter required for normal mood stabilization. However, the exact mechanism of fluoxetine’s antidepressant effect remains unknown, and its use in the treatment of other conditions lacks sufficient research. Recent discoveries show that fluoxetine affects mRNA levels of cAMP phosphodiesterases and brain-derived neurotrophic factor, transcripts involved in signaling pathways. Additionally, a new study reveals that fluoxetine regulates genes involved in apoptosis of Burkitt lymphoma cells. Combined, these discoveries indicate that fluoxetine has diverse and unanticipated effects on gene expression, and that continued investigation of its action mechanism is essential.**

### **Introduction**

Introduced in the mid-1980s, fluoxetine was touted as a “miracle medicine,” treating mental illnesses such as depression, obsessive-compulsive disorder, and eating disorders. Fluoxetine is a selective serotonin reuptake inhibitor (SSRI). It prevents the degradation of the neurotransmitter serotonin by blocking its reuptake from the synapses between neurons (Anderson, 2004). As serotonin is required for normal mood regulation, it is believed that fluoxetine’s antidepressant properties result from its ability to elevate the intracellular concentration of serotonin. However, its exact molecular target remains unknown. Two new studies provide evidence that fluoxetine acts by up-regulating gene expression of a family of cAMP-specific phosphodiesterases (Miro et al., 2002) and brain-derived neurotrophic factor (De Foubert et al., 2004), both involved in neurological

signaling pathways. A third study involving the effect of fluoxetine on apoptosis of Burkitt lymphoma cells points to fluoxetine's varied effects, and its potential as a treatment for other diseases (Serafeim et al, 2003).

### **Effects on cAMP phosphodiesterase mRNA abundance**

Cyclic adenosine monophosphate (cAMP) is a molecule involved in signal transduction. Disruption of its pathway is associated with depression (Conti et al., 2002). Enzymes of the phosphodiesterase (PDE) family have the ability to hydrolyze cAMP molecules, thereby interrupting this signaling pathway (Rausch et al., 2002). Recently, PDE4 mRNA levels were measured in the brain tissue of rats treated with fluoxetine to determine the effect of this medication on PDE4 gene expression (Miro et al., 2002).

Wister rat treatment groups were injected with 3 mg/kg fluoxetine for 1 day or 14 days, a dosage approximately 3 times that prescribed for human depression treatment. Brain tissue samples were taken 2 hours after sacrifice and probed with labeled oligonucleotides complementary to PDE4 mRNA. Results revealed a 110-120% increase in PDE4 mRNA in several brain regions after acute fluoxetine treatment (Figure 1); a larger increase in the mRNA levels is seen with chronic treatment, such as the 150% increase as compared to control in the pontine nuclei (Figure 2). Interestingly, acute and chronic fluoxetine exposure had inconsistent effects on mRNA levels of 5 splice variants of PDE4D: change in mRNA levels depended upon the splice variant studied (Zhang et al., 2002).

That increased PDE4 mRNA levels were detected more notably with chronic than with acute fluoxetine exposure correlates with the time-delay observed in fluoxetine's antidepressant effect in humans. An increase in phosphodiesterase likely leads to cAMP

accumulation, indirectly resulting in the therapeutic benefits (Thome et al., 2000). Also supporting the hypothesis that fluoxetine acts by modulating PDE4 gene expression is the observed increase in PDE4 mRNA in the pontine nuclei following fluoxetine treatment. This region regulates sleep via the cAMP pathway in response to daily rhythmic decreases in serotonin. A common side-effect of fluoxetine is decreased REM sleep; the increased PDE4 expression in the pontine nuclei offers a biological explanation for this alteration in sleep patterns (Miro et al., 2002).

### **Effects on BDNF mRNA abundance**

A second hypothesis surrounding the action mechanism of fluoxetine involves gene expression of brain-derived neurotrophic factor (BDNF). This neurotrophin is required for neuronal survival and plasticity, such that damage to BDNF due to chronic stress has been cited as a risk factor for depression (Tsai et al., 2003). Patients taking fluoxetine display heightened blood serum levels of BDNF, and increased BDNF in animals has been shown to alleviate symptoms of depression (Dias et al., 2003). A new study expands on this knowledge of BDNF's role in depression by evaluating BDNF mRNA levels in rats treated with fluoxetine from 1 day to 3 weeks (De Foubert et al., 2004).

Adult male Sprague-Dawley rats were orally administered fluoxetine at 10 mg/kg ranging from 1 to 21 days, with tissue samples taken 48 hours after sacrifice. *In situ* hybridization results detected BDNF mRNA in uneven expression patterns throughout the brain, with the highest density present in the hippocampus (De Foubert et al., 2004). Expression patterns also differed based on length of treatment. A detectable difference in BDNF mRNA levels between the treatment group and the control was not observed until

4 days, with mRNA levels differing significantly after 14 days (Figure 3). A change in protein levels was not observed until 21 days.

The observation that BDNF mRNA levels took several days to change following fluoxetine exposure suggests a neuroadaptive mechanism, whereby gene expression in the brain is altered only after serotonin levels have stabilized for several days (Altieria et al., 2004). Additionally, BDNF mRNA levels rose in the medial habenular nucleus of the fluoxetine-treated rats. This observation offers implications for treatment of depressive symptoms, since the decreased serotonin associated with depression causes a breakdown in the signaling pathway between the raphe nucleus and the medial habenular nucleus. The presence of fluoxetine may help to restore neurotransmission in this region (De Foubert et al., 2004).

### **Effects on apoptosis**

Insights in to how fluoxetine acts on signaling pathways to treat depression allows one to ask what other pathways this medicine effects. A new study resulted from the surprising discovery that fluoxetine may signal for apoptosis in certain cancer cells (Serafeim et al., 2003). Burkitt lymphoma (BL) is a cancer characterized by malignant tumors with exceedingly high mitotic rates (Blum et al., 2004). The molecular feature defining BL is the translocation of *c-myc* allele to an immunoglobulin locus on chromosome 14, followed by constitutive overexpression of *c-myc* upon binding nm23-H, a regulatory factor (Serafeim et al., 2003). BL cells carry serotonin transporters, and fluoxetine is able to prevent the reuptake of serotonin in to these cells. This observation suggests that fluoxetine itself can directly signal for apoptosis in BL cells.

To test this idea, control and fluoxetine-treated BL cells were cultured in a growth

medium and labeled thymidine incorporation was used as a measurement of DNA synthesis. Cells were stained and chromosome staining patterns analyzed to determine whether the cells were viable or had undergone apoptosis. Additionally, real-time PCR of cDNA reverse-transcribed from *c-myc* and *nm32-H* genes was carried out to assess differences in expression of these two genes between the control and treatment groups (Serafeim et al., 2003).

Cells exposed to fluoxetine displayed inhibited DNA synthesis in a concentration-dependent fashion (Figure 4). The number of viable cells in the fluoxetine-treated cultures was significantly reduced, and a correlation was noted between fluoxetine treatment and the characteristic DNA strand breaks associated with apoptosis (method described by Gavrieli et al., 1992). Lending the most support to the fluoxetine-induced apoptosis hypothesis was the 60-75% down-regulation of *c-myc* and *nm23-H* genes in cells exposed to fluoxetine (Serafeim et al., 2003).

Based on these observations, it follows that fluoxetine does affect apoptosis in BL cells. Interestingly, although BL cells possess serotonin receptors, this alone is not enough to target fluoxetine to the BL cells: BL cell cultures display the same apoptotic results even when additional serotonin is added. Furthermore, dosages 75 times those administered to human patients were used to achieve the results (Serafeim et al., 2003). Further studies on the efficacy of lower fluoxetine doses and human safety factors are required before utilizing fluoxetine as a cancer treatment.

## **Conclusion**

Indeed, fluoxetine has discernable yet unrelated effects on gene expression. Shown to affect mRNA levels of cAMP-specific phosphodiesterases and brain-derived

neurotrophic factor, we are offered insights as to its antidepressant mechanism. Based on fluoxetine's role in apoptosis of Burkitt lymphoma cells, it is logical to assume that this medication has effects on a collection of other genes throughout the body. Further human study in specific brain regions and at specific doses will shed new light on how fluoxetine affects gene expression, how this gene expression is involved in the drug's action, and what novel and beneficial uses may be discovered for this drug in the future.

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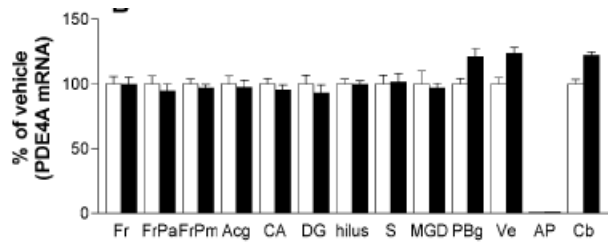
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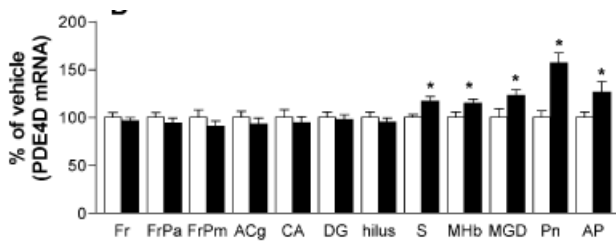
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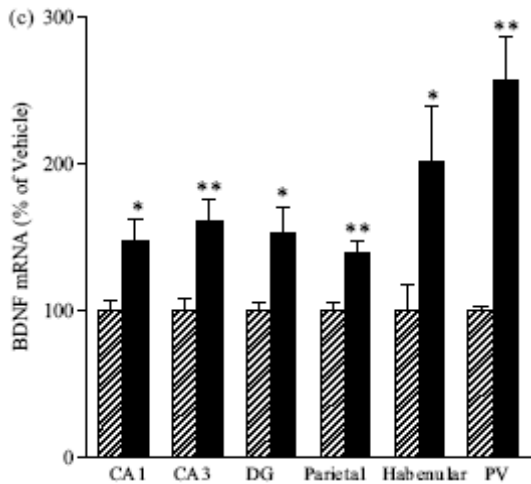
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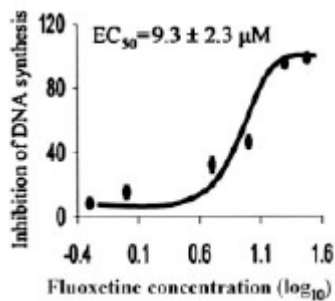
**Figure 1.** Effect of acute fluoxetine treatment on PDE4A mRNA levels in several brain regions. Note the increased mRNA levels in the PBg (parabigeminal nucleus), Ve (vestibular nuclei), and Cb (cerebellum) following fluoxetine exposure. Figure from Miro et al., 2002.



**Figure 2.** Effect of chronic fluoxetine treatment on PDE4D mRNA levels in several brain regions. Asterisks (\*) indicate significant differences ( $p < 0.05$ ) between control and treatment groups. Abbreviations are as follows: S (cubiculum), MHb (medial habenular nucleus), Pn (pontine nuclei), and AP (area postrema). Figure from Miro et al., 2002.



**Figure 3.** Effect of 14 day fluoxetine exposure on BDNF mRNA levels in several brain regions. A single asterisk (\*) indicates  $p < 0.05$  and a double asterisk (\*\*) indicates  $p < 0.01$  between control and treatment groups. Abbreviations are as follows: CA1-3 (hippocampus), DG (dentate gyrus), and PV (paraventricular thalamic nuclei). Figure from De Foubert et al., 2004.



**Figure 4.** Inhibition of DNA synthesis as compared to log fluoxetine concentration in BL cell cultures following 24 hours of fluoxetine exposure. Inhibition of DNA synthesis appears to correlate with increased fluoxetine concentrations. Figure from Serafeim et al., 2003.