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Olanzapine and fluoxetine administration and coadministration increase rat hippocampal pregnenolone, allopregnanolone and peripheral deoxycorticosterone: Implications for therapeutic actions

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Abstract

Olanzapine and fluoxetine elevate the GABAergic neuroactive steroid allopregnanolone to physiologically relevant concentrations in rodent cerebral cortex. It is unknown if these agents also alter pregnenolone or deoxycorticosterone. Since olanzapine and fluoxetine in combination have clinical utility and may demonstrate synergistic effects, we investigated neuroactive steroid alterations following olanzapine, fluoxetine or coadministration. Male rats received IP vehicle, olanzapine, fluoxetine or the combination of both agents in higher-dose (0, 10, 20 or 10/20 mg/kg, respectively) and lower-dose (0, 5, 10 or 5/10 mg/kg, respectively) experiments. Pregnenolone and allopregnanolone levels in hippocampus were determined by gas chromatography/mass spectrometry. Peripheral deoxycorticosterone and other steroid levels were determined by radioimmunoassay. Olanzapine, fluoxetine or the combination increased hippocampal pregnenolone and serum deoxycorticosterone in both higher- and lower-dose experiments, and elevated hippocampal allopregnanolone in higher-dose conditions. No synergistic effects on pregnenolone or allopregnanolone were observed following olanzapine and fluoxetine coadministration compared to either compound alone. Pregnenolone and its sulfate enhance learning and memory in rodent models, and therefore pregnenolone elevations may be relevant to cognitive changes in psychotic and affective disorders. Since pregnenolone decreases have been linked to depression, it is possible that olanzapine- and fluoxetine-induced pregnenolone elevations may contribute to the antidepressant actions of these agents.
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1. Introduction

Olanzapine and fluoxetine coadministration has been investigated in both preclinical models and clinical populations, yielding clues to potential synergistic effects of these agents in combination compared to monotherapy with either compound. In rodent models, olanzapine and fluoxetine coadministration results in synergistic increases in extracellular dopamine and norepinephrine concentrations in prefrontal cortex compared to either drug alone (Zhang et al., 2000). These synergistic effects on extracellular catecholamine concentrations following coadministration of both agents were also observed in hypothalamus, but not in nucleus accumbens or striatum (Koch et al., 2004). Olanzapine and fluoxetine in combination also demonstrate synergistic effects on fibroblast growth factor 2 expression (Maragnoli et al., 2004) and fluoxetine administration appears to potentiate the effects of olanzapine on locus coeruleus neuronal firing rate and burst firing (Seager et al., 2004). In contrast, olanzapine and fluoxetine in

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combination do not result in greater induction of cell proliferation in hippocampal dentate gyrus or prelimbic cortex compared to either agent alone (Kodama et al., 2004). Clinically, a randomized controlled double-blind trial ($n=833$) demonstrated that combined treatment with olanzapine and fluoxetine is more effective than olanzapine monotherapy or placebo in the treatment of bipolar depression (Tohen et al., 2003), and this olanzapine/fluoxetine combination was recently approved by the U.S. Food and Drug Administration for this indication. A randomized double-blind pilot investigation in patients with treatment-resistant depression without psychotic features ($n=28$) demonstrated that olanzapine plus fluoxetine resulted in superior efficacy compared to monotherapy with either agent (Shelton et al., 2001). In a larger subsequent clinical trial ($n=500$), however, olanzapine and fluoxetine in combination did not differ significantly from monotherapy with either agent or nortriptyline, although the combination produced a more rapid response that was sustained until treatment conclusion (Shelton et al., 2005). A recent investigation in treatment-resistant depression also demonstrated that the combination of olanzapine/fluoxetine was similarly effective at study endpoint compared to monotherapy with fluoxetine or venlafaxine, although patients receiving the combination of olanzapine/fluoxetine exhibited more rapid improvement by the first week of treatment (Corya et al., 2006). Hence, olanzapine/fluoxetine in combination appears to be clinically superior for bipolar depression compared to monotherapy with olanzapine (Tohen et al., 2003), but may not have preferred utility in other affective disorders.

The precise mechanisms mediating the synergistic effects of olanzapine and fluoxetine coadministration in specific clinical settings and rodent experimental paradigms remain to be determined. Both of these agents elevate levels of the neuroactive steroid 3α-hydroxy-5α-pregnan-20-one (allopregnanolone) in rodent brain to physiologically relevant concentrations individually (Uzunov et al., 1996; Serra et al., 2001; Pinna et al., 2003, 2004; Marx et al., 2000, 2003). Clinically, fluoxetine also appears to elevate allopregnanolone levels in plasma (Romeo et al., 1998) and cerebrospinal fluid (Uzunova et al., 1998) in patients with major depression. We therefore hypothesize that the combination of olanzapine and fluoxetine may result in enhanced elevations of neuroactive steroids in rat brain. If these changes also occur in humans, it is possible that potential synergistic effects on neuroactive steroids following olanzapine and fluoxetine in combination could contribute to their therapeutic effects in bipolar depression (Tohen et al., 2003). We thus investigated possible synergistic effects on neuroactive steroid levels in rodent hippocampus following olanzapine, fluoxetine or the combination utilizing both higher-dose and lower-dose drug administration strategies.

Since some studies suggest that olanzapine may improve cognitive symptoms in patients with schizophrenia (Keefe et al., 2004; Sharma et al., 2003), we were particularly interested in characterizing the potential effects of olanzapine administration on pregnenolone levels in hippocampus. Pregnenolone and its sulfated derivative enhance learning and memory in rodent models, and pregnenolone sulfate administration in aged rats transiently reverses age-related cognitive decline (Flood et al., 1992, 1995; Akwa et al., 2001; Vallee et al., 1997, 2001). In addition, decreased levels of pregnenolone have been linked to depressive symptoms (George et al., 1994). Potential olanzapine- and fluoxetine-induced increases in central pregnenolone levels could therefore theoretically contribute to their efficacy in the treatment of depressive symptoms, including decreased concentration. Prior reports that fluoxetine potentiates the antidepressant-like effects of the GABAergic neuroactive steroid allopregnanolone (Khisti and Chopde, 2000), and that allopregnanolone potentiates olanzapine actions on dopamine-mediated behaviors in rodents (Ugale et al., 2004) support the possibility that neuroactive steroid induction may be relevant to the therapeutic actions of these agents.

Our prior investigations have demonstrated that olanzapine dose-dependently increases the GABAergic neuroactive steroid allopregnanolone in rodent cerebral cortex (Marx et al., 2000, 2003). Allotetrahydrodeoxycorticosterone (THDOC) is a neuroactive steroid with potent modulatory $GABA_A$ receptor activity comparable to allopregnanolone (Morrow et al., 1990). We thus examined peripheral serum levels of deoxycorticosterone, a precursor molecule to THDOC. Since we have recently determined that a number of serum steroids are highly interrelated in male subjects with nicotine dependence (Marx et al., 2006a), we also examined a panel of peripheral steroids in these rodent experiments to determine if interrelationships among steroids are also present in our animal model.

2. Methods

2.1. Animals

Animals were purchased, housed and euthanized in accordance with approved IACUC protocols at the University of North Carolina at Chapel Hill. Male rats (200–250 mg/kg, Sprague–Dawley) were obtained from Harlan (Indianapolis, IN), group housed and permitted free access to food and water.

2.2. Experimental design

Male rats ($n=8$–11 per condition) were injected i.p. with vehicle, olanzapine, fluoxetine or the combination of both agents in higher-dose (0, 10, 20 or 10/20 mg/kg, respectively) and lower-dose (0, 5, 10 or 5/10 mg/kg, respectively) experiments after habituation to i.p. saline injection for 5 consecutive days. Rats were sacrificed by decapitation 1 h following i.p. drug administration. Hippocampus was rapidly dissected on ice and stored at −80 °C for pregnenolone and allopregnanolone analyses. Trunk blood was collected for serum deoxycorticosterone, progesterone, corticosterone, dehydroepiandrosterone (DHEA) and estradiol analyses, kept on ice until centrifugation for serum collection and stored at −80 °C. The neuroactive steroids pregnenolone and allopregnanolone were determined by gas chromatography/mass spectrometry, preceded by high performance liquid chromatography (HPLC). Peripheral deoxycorticosterone and other steroid levels were determined by radioimmunoassay. Statistical analyses were performed by ANOVA with post-hoc Dunnett tests. Associations between steroids were also investigated and Pearson correlation