Olanzapine Increases Allopregnanolone in the Rat Cerebral Cortex

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Background: The neurosteroid allopregnanolone (3α-hydroxy-5α-pregn-20-one) has anxiolytic and anticonvulsant properties, potentiating GABA_A receptor chloride channel function with 20-fold higher potency than benzodiazepines. Behavioral studies demonstrate that olanzapine has anxiolyticlike properties in animals, but the mechanism responsible for these effects is not clear. We examined the effect of acute olanzapine administration on cerebral cortical allopregnanolone and its relationship to serum progesterone and corticosterone levels in rats.

Methods: Male Sprague-Dawley rats were habituated to intraperitoneal (IP) saline injection for 5 days. On the day of the experiment, rats were injected with olanzapine (0, 2.5, 5.0, or 10.0 mg/kg IP, 10–11 rats per condition). Rats were sacrificed 1 hour later, and cerebral cortical allopregnanolone levels and serum progesterone and corticosterone levels were measured by radioimmunoassay.

Results: Olanzapine increases cerebral cortical allopregnanolone up to fourfold, depending on dose. Positive correlations were observed between cerebral cortical allopregnanolone and serum progesterone levels and between cerebral cortical allopregnanolone and serum corticosterone levels.

Conclusions: Olanzapine-induced increases in the potent GABA_A receptor modulator allopregnanolone may alter GABAergic neurotransmission, possibly contributing to antipsychotic efficacy. If allopregnanolone alterations are linked to psychotic symptom relief, neurosteroids may represent molecules for pharmacologic intervention. Biol Psychiatry 2000;47:1000–1004 © 2000 Society of Biological Psychiatry

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GABAergic neurotransmission and does not act at dopamine receptors. GABA<sub>A</sub> receptor activity may be particularly relevant to schizophrenia because converging evidence implicates altered GABAergic neurotransmission in the illness, and GABA<sub>A</sub> receptor agonists, such as benzodiazepines, are helpful in alleviating symptoms of the disorder in certain settings (Carpenter et al 1999). Finally, allopregnanolone levels in plasma and brain are present in greater quantities in women and may be important in the neurobiology of gender differences in schizophrenia.

Similar to allopregnanolone, the antipsychotic olanzapine also demonstrates anxiolyticlike effects in animals. For example, animals treated with allopregnanolone demonstrate anticonflict effects (Moore et al 1994; Nanry et al 1995), and olanzapine decreases the acquisition of conditioned freezing (Inoue et al 1996). These anxiolyticlike effects of olanzapine suggest that this agent may alter neurosteroid biosynthesis. Interestingly, the benzodiazepine antagonist flumazenil reduces the anticonflict effect of chloridiazepoxide but not allopregnanolone (Moore et al 1994), suggesting that the anxiolytic activity of olanzapine may not be mediated by the GABA<sub>A</sub> receptor benzodiazepine site. Because allopregnanolone does not act at the benzodiazepine recognition site on GABA<sub>A</sub> receptors (Morrow et al 1990), it is possible that the anxiolytic effects of olanzapine may be mediated by the induction of allopregnanolone in brain. The purpose of this study was to determine if olanzapine modulates the endogenous GABA<sub>A</sub> receptor-active neurosteroid allopregnanolone, possibly contributing to its clinical efficacy.

Methods and Materials

All animal care and use was conducted in accordance with IACUC guidelines. Male Sprague-Dawley rats weighing 200–250 g were obtained from Harlan (Indianapolis, IN). Rats were habituated to intraperitoneal saline injection for 5 days prior to the day of the experiment to minimize possible stress-induced increases in brain allopregnanolone. Rats were housed three per cage and placed on a 12-hour, light-dark cycle. Food and water were available ad libitum. To limit circadian variations in steroid levels, all animals were sacrificed between the hours of 9 AM and noon.

Olanzapine, kindly provided by Eli Lilly and Company (Indianapolis, IN), was dissolved in acetic acid then buffered with NaOH (pH = 6). On the day of the experiment, intraperitoneal olanzapine was administered at doses of 0, 2.5, 5.0, and 10.0 mg/kg, 10–11 rats per condition. Rats were sacrificed by decapitation 1 hour later. Cerebral cortex was rapidly dissected on ice and stored at −80°C until assayed for allopregnanolone. Trunk blood was collected for serum progesterone and corticosterone level determination and stored on ice until centrifugation for serum collection.

Serum progesterone and corticosterone levels were measured in duplicate with radioimmunoassay kits using <sup>125</sup>I-progesterone and <sup>125</sup>I-cortisosterone (ICN Biomedicals, Inc. Costa Mesa, CA). The limits of detection for the progesterone and corticosterone assays were 0.1 and 10 ng/mL, respectively. Allopregnanolone levels were measured in cerebral cortex by radioimmunoassay as described by Janis et al (1998), with a modification of the methods of Purdy et al (1990) using a sheep polyclonal antibody kindly provided by CoCensys Inc. (Irvine, CA). Sample extracts in sodium phosphate-BSA buffer were incubated with antisemur and [³H]allopregnanolone for a minimum of 60 min. Bound steroids were separated from free steroids by the addition of ice-cold, dextran-coated charcoal, followed by centrifugation for 20 min at 2000 g. Supernatants were collected and counted by liquid scintillation spectroscopy. Sample values were compared with a concurrently run allopregnanolone standard curve produced using a one-site competition model (Prism2, GraphPad, San Diego, CA). Sample values were adjusted to account for the previously determined extraction efficiency. The sensitivity of the assay was 25 pg. The allopregnanolone antiserum used in this study has been shown to produce minimal cross-reactivity with other circulating steroids (Finn and Gee 1994).

Statistical analysis of the results was conducted by analyses of variance (ANOVA; with post-hoc Dunnett tests) or Linear Regression Analysis.

Results

Intraperitoneal olanzapine injection dose-dependently increased cerebral cortical allopregnanolone levels up to fourfold [Figure 1A, ANOVA F(3,38) = 3.828, p = .0172, post hoc Dunnett p < .05 for olanzapine 10 mg/kg dose]. Maximal allopregnanolone response to 4 ng/g was observed with 10 mg/kg olanzapine. Serum progesterone levels increased after acute olanzapine injection in a dose-dependent manner [Figure 1B, ANOVA F(3,38) = 8.020, p = .0003, post hoc Dunnett p < .01 for olanzapine doses 5 and 10 mg/kg]. A positive correlation was observed between cerebral cortical allopregnanolone levels and serum progesterone levels [Figure 2A, Linear Regression Analysis, Pearson correlation coefficient r = .8911, F(1,40) = 154.18, p < .0001]. Serum corticosterone levels also increased dose-dependently after acute olanzapine injection [Figure 1C, ANOVA F(3,38) = 13.608, p < .0001, post hoc Dunnett p < .01 for olanzapine doses 5 and 10 mg/kg]. A positive correlation was observed between cerebral cortical allopregnanolone levels and serum corticosterone levels [Figure 2B, Linear Regression Analysis, Pearson correlation coefficient r = .7503, F(1,40) = 51.527, p < .0001].

Discussion

Our results indicate that acute olanzapine administration dose-dependently increases cerebral cortical allopregnanolone levels. Olanzapine-induced allopregnanolone levels of 4 ng/g approach those known to potentiate
GABAergic neurotransmission (Morrow et al 1987). Comparable effects on allopregnanolone levels have been observed in hypothalamus using a swim-stress paradigm (Purdy et al 1991). It therefore is likely that allopregnanolone affects GABA neurotransmission at the concentrations observed in this study.

As a potent GABA<sub>A</sub> receptor modulator, allopregnanolone may be particularly relevant to the apparent GABAergic deficit observed in schizophrenia. Postmortem studies of patients with schizophrenia have demonstrated a reduction in GABA interneurons, an upregulation of GABA<sub>A</sub> receptor binding activity, and a decrease in the synthetic GABA enzyme GAD<sub>67</sub>, supporting the hypothesis that GABAergic neurotransmission is altered in schizophrenia (Akbarian et al 1995; Benes et al 1991, 1992, 1997). A deficit in GABAergic neurons expressing the calcium-binding protein parvalbumin (Beasley and Reynolds 1997; Reynolds et al 1999) and a decrease in the density of axon terminal “cartridges” for the GABA membrane transporter GAT-1 (Woo et al 1998) in the prefrontal cortex of schizophrenia have also been demonstrated. An acute olanzapine-induced increase in the potent GABA<sub>A</sub> receptor modulator allopregnanolone may represent a mechanism by which this antipsychotic alters GABAergic neurotransmission, possibly contributing to the efficacy of this drug.

Clinically, studies examining GABA<sub>A</sub> modulators, such as benzodiazepines, in the treatment of schizophrenia symptoms have yielded mixed results. A recent study, however, found that diazepam was superior to placebo and comparable to fluphenazine in preventing symptom progress during early symptom exacerbation in schizophrenia (Carpenter et al 1999). Because allopregnanolone,
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similar to benzodiazepines, diminishes dopaminergic neurotransmission by modulation of dopamine release (Grobin et al 1992; Motto et al 1996; van Kammen 1977), it is possible that these GABAergic effects could have therapeutic actions. Allopregnanolone may have more pronounced antidopaminergic effects than benzodiazepines because it induces catalepsy in mice (Khisti et al 1998). Allopregnanolone may also have a broader spectrum of action than benzodiazepines because benzodiazepines bind a limited population of GABA\textsubscript{A} receptors that contain \( \alpha \) and \( \gamma \) subunits (Pritchett et al 1989). Allopregnanolone, in contrast, demonstrates equal potency and efficacy on several GABA\textsubscript{A} receptor subtypes assembled only with \( \beta \), with \( \alpha \) or \( \beta \), or with \( \alpha \), \( \beta \), and \( \gamma \) subunits (Puia et al 1990). Given evidence suggesting a GABAergic deficit in the pathophysiology of schizophrenia, it is particularly interesting that an antipsychotic such as olanzapine increases cerebral cortical allopregnanolone, which potentiates GABAergic neurotransmission. Future experiments utilizing antipsychotics other than olanzapine, both typical and atypical, will be required to determine the specificity of this effect.

Our data suggest that olanzapine-induced increases in cerebral cortical allopregnanolone levels are correlated with increases in serum progesterone \((r = .89)\). The degree to which serum progesterone serves as a precursor to olanzapine-induced allopregnanolone biosynthesis in brain is currently not known and will require further study using adrenalectomized animals. It is possible that serum progesterone is a peripheral indicator of altered cerebral cortical allopregnanolone levels in response to acute olanzapine administration.

The neurochemical mechanisms responsible for olanzapine-induced HPA axis activation resulting in dose-dependent serum progesterone and corticosterone elevations are currently unknown. Because brain allopregnanolone and serum corticosterone levels were correlated in our experiments, the potential actions of allopregnanolone on the HPA axis may be important. It is known that corticotropin-releasing factor (CRF) release from the hypothalamus is decreased by activation of GABA\textsubscript{A} receptors in the hypothalamus (Calogero et al 1988). Allopregnanolone decreases CRF (Patchev et al 1994), adrenocorticotropic hormone (ACTH; Patchev et al 1996), and corticosterone (Guo et al 1995; Patchev et al 1996) release in rats. Stress-induced increases in brain allopregnanolone therefore would ultimately suppress the HPA axis. This process may represent an endogenous autoregulatory mechanism to restore homeostasis after a stressful event (Morrow et al 1995). It is possible that a similar autoregulatory mechanism involving allopregnanolone may be activated by treatment with olanzapine (or other antipsychotics) and that this mechanism is important in the clinical actions of olanzapine. This action may be particularly relevant to schizophrenia pathophysiology because recent evidence indicates that suppression of glucocorticoid secretion and antipsychotic drugs have similar effects on mesolimbic dopaminergic transmission (Piazza et al 1996).

In summary, these preclinical results demonstrating that olanzapine alters cerebral cortical allopregnanolone may be clinically relevant, and it is possible that allopregnanolone may play a role in mediating the therapeutic actions of olanzapine and its consequent symptom reduction. Olanzapine also may alter other neurosteroids in addition to allopregnanolone, and this possibility will require further investigation. Clinical studies will be needed to elucidate a possible relationship between olanzapine-induced changes in neurosteroids and the pathophysiology of schizophrenia. If neurosteroid alterations are linked to psychotic symptom relief, neuroactive steroids may represent molecules for pharmacologic intervention.

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