Olanzapine Reduces Physical Activity in Rats Exposed to Activity-Based Anorexia: Possible Implications for Treatment of Anorexia Nervosa?

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Background: *Anorexia nervosa (AN) patients often show extreme hypophagia and excessive physical activity. Activity-based anorexia (ABA) is considered an animal model of AN and mimics food restriction and hyperactivity in rats. This study investigated whether treatment with olanzapine (Zyprexa) reduces the development of ABA in rats. The effect of olanzapine treatment in AN patients was also evaluated in a small open-label study.*

Methods: *Rats were chronically (1 week) infused with olanzapine (7.5 mg/kg) and exposed to the ABA model or ad libitum feeding. Hyperactive AN patients were followed for up to 3 months of olanzapine treatment (5 mg/kg).*

Results: *Olanzapine treatment reduced development of ABA in rats by reducing running wheel activity, starvation-induced hypothermia and activation of the hypothalamus-pituitary-adrenal axis. Olanzapine treatment reduced activity levels of AN patients compared with untreated AN patients, without affecting body weight and plasma leptin levels.*

Conclusions: *Olanzapine treatment reduced wheel running and thereby diminished development of ABA in rats. Olanzapine treatment also reduced physical activity in hyperactive AN patients in a small open-label study. These data support the need for controlled studies investigating the putative beneficial effects of olanzapine treatment in AN patients.*

Key Words: Hyperactivity, anorexia, antipsychotic, food restriction, running wheel

Anorexia nervosa (AN) is a psychiatric disorder that is often characterized by extreme hypophagia, obsessive fears of being fat, and hyperactivity (Casper et al 1991; Davis 1997; Kron et al 1978; Walsh et al 1998). Anorexia nervosa has the highest mortality rate of psychiatric disorders (Sullivan 1995).

Some studies show that serotonin (5-hydroxytryptamine, 5-HT) signaling is altered in ill AN patients as well as in recovered AN patients (Brewerton and Jimerson 1996). For example, AN patients show decreased cerebrospinal fluid (CSF) levels of 5-HT and 5-hydroxyindole acetic acid (5-HIAA) during disease and increased levels of 5-HIAA when recovered (Kaye et al 1991). Studies on the use of serotonin reuptake inhibitors (SSRIs) in malnourished AN patients showed no benefits of treatment (Kaye et al 1998); however, SSRIs seem to be effective in preventing relapse in recovered AN patients (Kaye et al 2001). Genetic association studies showed possible implications of polymorphisms in the 5-HT_{2A} receptor, 5-HT_{2C} receptor, and serotonin transporter in AN (Collier et al 1997; Di Bella et al 2000; Hu et al 2003; Nacmias et al 1999; Westberg et al 2002), although other studies could not confirm these findings (Ando et al 2001; Gorwood et al 2002; Hinney et al 1997; Nishiguchi et al 2001).

Trials on antipsychotic treatment in AN patients have been performed previously, but only to a limited extent (Hoffman and Halmi 1993; Johnson et al 1983; Pederson et al 2003). Controlled studies on chlorpromazine (Dally and Sargant 1966) and pimozide (Vandereycken and Pierloot 1982) showed increased weight gain and a more positive attitude of AN patients toward treatment, whereas the atypical antipsychotic sulpiride did not significantly influence body weight gain and eating and body attitudes in AN patients (Vandereycken 1984).

Olanzapine (Zyprexa) is an atypical antipsychotic with a broad pharmacologic profile. This thienobenzodiazepine compound has high affinity for 5-$HT_{2A/2C}$ receptors, histamine (H1) receptors, and adrenergic (a1) receptors and moderate affinity for dopamine (D_1–D_4) receptors (Bymaster et al 1996, 1997; Moore 1999; Schotte et al 1996). Olanzapine treatment in humans causes limited extrapyramidal effects and has been associated with body weight gain (Allison and Casey 2001). A few (uncontrolled) studies already reported beneficial effects of olanzapine treatment on food intake and anxiety levels of AN patients (Barbarich et al 2004; Boachie et al 2003; Malina et al 2003; Mehler et al 2001; Powers et al 2002), but no study has reported effects of olanzapine treatment on physical activity levels.

Animal models of AN might contribute to the understanding of AN and subsequently improve AN treatment. The activity-based anorexia (ABA) model is used to study anorectic behavior in rodents and serves as an animal model of AN (Routtenberg and Kuznesof 1967). In the ABA model, scheduled feeding in combination with voluntary access to running wheels leads to a paradoxical increase in running wheel activity (RWA) and decrease in food intake (compared with food-restricted control animals; Routtenberg and Kuznesof 1967). This results in activation of the hypothalamus-pituitary-adrenal (HPA) axis and substantial body weight loss. Few studies already reported increased feeding behavior and reduced locomotor activity in rats following acute olanzapine treatment (Prinssen et al 2000; Thornton-Jones et al 2002). These effects suggest that olanzapine treatment might influence pathophysiologic processes in anorexia. Data on chronic (instead of acute) administration of olanzapine in rodents is rare (Pouzet et al 2003), however, although it is necessary for clinical comparisons (Kapur et al 2003).

In this study, the effects of chronic olanzapine treatment on development of ABA in rats as well as the putative positive

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effects of olanzapine treatment on body weight and physical activity in AN patients were investigated.

Materials and Methods

Rats

Female outbred Wistar WU rats (n = 30; Harlan, Horst, The Netherlands) weighing 160 g upon arrival were individually housed in a temperature- and humidity-controlled room (21 ± 2°C) under a 12-hour light–dark cycle (Zeitgeber [ZT]12: lights off). The University of Utrecht ethical committee on use and care of animals approved all described procedures.

Drugs

The olanzapine for the animal studies was kindly provided by Eli Lilly (Indianapolis, Indiana). Olanzapine was dissolved in a minimum quantity of acetic acid, made up to volume with sterile isotonic saline and adjusted to pH 6 with 5 M NaOH, and continuously infused (subcutaneously [sc]) during 1 week at a concentration of 7.5 mg/kg/day using osmotic minipumps (Alzet, model 2001, DURECT, Cupertino, California; Kapur et al 2003). In the human study, AN patients received olanzapine (5 mg–15 mg) tablets (Eli Lilly).

Surgical Procedures Animal Studies

Transmitters (TA10TA-F40 Data Sciences International, St. Paul, Minnesota) were placed in the abdominal cavity under fentanyl/fluanisone (Hypnorm, Janssen Pharmaceutica, Beerse, Belgium, .1 mL/100 g, intramuscular) and midazolam (Dormicum, Hoffman-LaRoche, Mijdrecht, The Netherlands, .05 mL/100 g, intraperitoneal) anesthesia. After surgery, rats were treated with buprenorphin (Temgesic, Schering-Plough, Maarssen, The Netherlands, .05 mL/100 g sc) and saline (1 mL sc) and were allowed to recover for 2 weeks.

For chronic infusions, osmotic minipumps were filled with olanzapine or vehicle and were activated by overnight incubation at 37°C. The pumps were positioned subcutaneously into the flank of the rats under Hypnorm anesthesia at the end of day –1 (ZT10). After surgery, rats were treated with Temgesic and saline as indicated earlier.

Experimental Setup Animal Studies

Olanzapine Treatment in Activity-Based Anorexia. Transmitters were implanted 1 week after arrival of the rats. After 2 weeks of recovery (day –10), the rats (n = 14, synchronized for estrous cycle) were placed in cages with running wheels for adaptation to the running wheel. During this 10-day period, food and water were available ad libitum. RWA was continuously registered using a Cage Registration Program (Department Biomedical Engineering, UMC Utrecht, The Netherlands). At day –2, transmitters were activated for baseline recordings of body temperature. At day –1 (ZT11), rats were divided into two groups, matched for body weight (vehicle: 214.7 ± 2.5 g, olanzapine: 215.0 ± 2.7 g, *ns*) and baseline RWA. Baseline RWA was determined as average RWA during the 4 days before the start of infusion (day –4 to day –1; vehicle: 5281 ± 1409 revolutions, olanzapine: 5694 ± 1496 revolutions, *ns*). Osmotic minipumps containing olanzapine or vehicle were implanted as indicated above. Immediately after surgery, food was removed (day 0, ZT12). The next days (day 1–6), rats had 1 hour access to food (ZT12–ZT13), and water was available ad libitum. Body weight and food intake were measured daily. At day 6 (ZT11), rats were decapitated, and trunk blood was collected into lithium-heparin (Sarstedt, Nümbrecht, Germany) containing tubes with 83 mmol ethylenediamine tetraacetatic acid and 1 mg aprotonin. Plasma was stored at –20°C. Retroperitoneal white adipose tissue (rWAT), interscapular brown adipose tissue (iBAT), and adrenals were collected and weighed.

Olanzapine Treatment in Ad Libitum–Fed Running Rats. This experiment was performed similarly to the previous experiment, with the only difference being that rats had ad libitum access to food throughout the experiment. Sixteen rats were divided into two groups, matched for body weight (vehicle: 236.2 ± 4.7 g, olanzapine: 231.2 ± 5.9 g, *ns*) and baseline RWA (vehicle: 6686 ± 1124 revolutions, olanzapine: 6514 ± 1590 revolutions, *ns*).

Patients

In the open-label trial AN patients were studied in a specialized treatment setting. At their entrance to the hospital, activity levels of AN patients were scored by trained nurses on a scale from 0 to 100 (score 0 = inactive, score 100 = extremely active), which has recently been validated by the use of Actiwatches (manuscript in preparation).

From a cohort of 27 AN patients, 18 patients (67%) displayed activity scores greater than 50 at their entrance to the hospital and were entitled hyperactive and included in this study. Of these 18 female AN patients, 7 received olanzapine treatment (5 mg, except for 1 patient who was treated with 15 mg), and 11 received no medication (age: olanzapine,16.0 ± 1.0 years; no medication, 17.3 ± .7 years, *ns*). Patients were assigned to the olanzapine treatment group because of anxious behavior toward eating and weight gain. All AN patients were free from other forms of pharmacotherapy. Additional treatment was generally the same for all patients and was aimed at body weight gain (.75 kg/week) followed by further normalizing of cognition and body image and treatment of possible comorbid problems. Both groups contained inpatients as well as day-treatment patients (who visited the hospital twice weekly). Every week activity levels were scored. Body weight (z scores to control for age) was measured, and blood was collected once in 2 weeks. All AN patients and parents (in case of minors) gave informed consent for participation in a clinical study, which was approved by the Ethical Committee of the University Medical Center of Utrecht.

Radioimmunoassay

In rats, plasma levels of corticosterone, adrenocorticotropic hormone (ACTH), and leptin were measured by radioimmunoassays (RIA). Plasma levels of corticosterone were measured using a commercially available rat corticosterone RIA kit (ICN Biochemicals, Costa Mesa, California). Plasma ACTH was measured using a specific rabbit antiserum directed to the midportion of ACTH, which was kindly provided by Dr. G.B. Makara (Budapest, Hungary). Synthetic human $ACTH_{(1–39)}$ (Peninsula Laboratories, Belmont, California) was labeled with ^{125}I and used as a tracer (Nijsen et al 2000). Plasma levels of leptin were measured using a commercially available rat leptin RIA kit (detection limit .5 ng/mL), according to the manufacturer's protocol (Linco Research, St. Charles, Missouri). In patients, plasma levels of leptin were analyzed by the DLR-Institute of Aerospace Medicine, Space Physiology in Cologne, Germany using a sensitive human leptin RIA assay (Mediagnost Reutlingen, Germany).

Data Analysis

Data are presented as mean ± standard error. For all measurements (in the animal and human studies), baseline levels