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Dopamine antagonism inhibits anorectic behavior in an animal model for anorexia nervosa

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Abstract

Excessive physical activity is commonly described as symptom of Anorexia Nervosa (AN). Activity-based anorexia (ABA) is considered an animal model for AN. The ABA model mimics severe body weight loss and increased physical activity. Suppression of hyperactivity by olanzapine in anorectic patients as well as in ABA rats suggested a role of dopamine and/or serotonin in this trait. Here, we investigated the effect of a non-selective dopamine antagonist in the ABA model. A dose–response curve of chronic treatment with the non-selective dopaminergic antagonist *cis*-flupenthixol was determined in the ABA model. Treatment reduced activity levels in both *ad libitum* fed and food-restricted rats. Treated ABA rats reduced body weight loss and increased food intake. These data support a role for dopamine in anorexia associated hyperactivity. Interestingly, in contrast to leptin treatment, food-anticipatory activity still persists in treated ABA rats.

1. Introduction

Anorexia nervosa (AN) is a psychiatric disorder with a high mortality rate (reviewed in Bulik et al., 2007). AN is characterized by a dramatic reduction in caloric intake by excessive dieting, which is accompanied by physiological, biochemical, and behavioral disturbances (Casper et al., 1991; Davis, 1997). Excessive physical activity is commonly described as symptom of AN (Kron et al., 1978). Between 31% and 80% of anorectic patients display abnormally high levels of physical activity and overexercise (Hebebrand et al., 2003). Furthermore, excessive physical activity and caloric restriction reinforce each other in the development of severe weight loss.

Although the exact cause of AN is still unknown, serotonin and dopamine have been implicated in the etiology of AN

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(reviewed in Bulik et al., 2007). Human genetic research demonstrated an association between the dopamine D2 receptor polymorphism with AN (Bergen et al., 2005; reviewed in Bulik et al., 2007). Regarding the serotonergic receptors, Ricca and researchers found an association with the 5-HT_{2A} receptor gene polymorphism (Ricca et al., 2002). In another study, a functional polymorphism of the serotonergic transporter gene was found in anorectic patients compared to normal weight controls (Fumeron et al., 2001). Besides genetic studies, positron emission tomography imaging studies with selective neurotransmitter radioligands confirmed altered serotonergic and dopaminergic neuronal pathway activities. Altered brain serotonin 5-HT_{1A} and 5-HT_{2A} receptor binding, and increased dopamine D2/D3 receptor binding were found in patients after recovery of AN (Bailer et al., 2005; Frank et al., 2002, 2005). Taken together, these studies support an involvement of serotonin and dopamine in AN.

The serotonergic/dopaminergic system has been targeted therapeutically in AN; antipsychotics were among the first agents studied for the treatment of AN. For example, the typical antipsychotic chlorpromazine induces body weight gain in anorectic patients, but long-term treatment provides significant adverse effects (Dally and Sargant, 1966). Another typical antipsychotic, pimozide, has been shown to increase body weight, but did not improve patient's attitude or behavior (Vandereycken and Pierloot, 1982). Both these drugs antagonize central dopaminergic receptors (Reilly, 1978). Typical antipsychotics as described above have been replaced by newer atypical antipsychotics, such as olanzapine. Olanzapine is prescribed to anorectic patients to reduce agitation and to reduce anxiety about refeeding (Barbarich et al., 2004; Malina et al., 2003; Powers et al., 2002). Additionally, it has been associated with body weight gain (Allison and Casey, 2001). In animal studies, acute injections of olanzapine increase food intake and reduce locomotor activity (Thornton-Jones et al., 2002). In food-restricted running rats, chronic infusion of olanzapine significantly reduced running wheel activity (Hillebrand et al., 2005b). These reduced activity levels were also observed in anorectic patients when treated with olanzapine (Hillebrand et al., 2005b). Olanzapine has a mixed receptor pharmacology. It has high affinity for 5-HT_2 serotonin receptors and for dopamine receptors, and a lower affinity is apparent for most cholinergic and α-adrenergic receptors (reviewed in Roth et al., 2004). Although it is unclear via which receptors olanzapine reduces hyperactivity, the serotonergic and dopaminergic receptors are good candidates.

Activity-based anorexia (ABA) is an animal model which mimics a subset of important characteristics of AN, in particular excessive exercise and reduced food consumption (Routtenberg and Kuznesof, 1967). In this model, rats are given free access to a running wheel and fed once per day for a limited period of time (1–2 h). These food-restricted animals increase their activity levels and decrease their food intake, whereas *ad lib* fed animals regain their body weight after a short period of body weight loss when given access to a running wheel. The hyperactive behavior observed upon exposure to the ABA model has been explained in terms of foraging behavior, anticipation, reward and stress (reviewed in Casper et al., 2008; Mistlberger, 1994; Watanabe et al., 1992). Hyperactivity and reduction of food intake have also been associated with dopamine function (Barry and Klawans, 1976; Leibowitz and Brown, 1980). This is in agreement with animal experiments. Dopamine-deficient mice (DD) lacking the dopamine synthesizing enzyme tyrosine hydroxylase (TH) in dopaminergic neurons become hypoactive, hypophagic, and will die of starvation (Zhou and Palmiter, 1995). DD mice become more active and immediately eat following L-dopa administration (Szczypka et al., 1999b; Zhou and Palmiter, 1995). Furthermore, restoring dopamine production in DD mice by viral-mediated gene transfer results in feeding (Szczypka et al., 1999a).

In the present study, we determined whether antagonism of dopamine receptors counteracts anorectic behavior. A dose–response of chronic non-selective dopaminergic antagonist *cis*-flupenthixol treatment on development of ABA in rats was determined. We discuss the effect of dopamine antagonism in comparison to the effect of antipsychotics and leptin in the ABA model, in order to determine whether hyperactivity and other anorectic behaviors were affected in a similar manner.

2. Experimental procedures

2.1. Animals

Fifty-six female outbred Wistar WU rats (Harlan, Horst, The Netherlands) weighing 155–165 g upon arrival were individually housed in a ambient temperature- and humidity-controlled room (21 °C ± 2 °C) under a 12-hour dark-light cycle, lights on at 2 am. All described procedures were approved by the ethical committee on the use and care of animals of the University of Utrecht, The Netherlands. For ethical reasons, it was decided that rats were to be removed from the experiment when their body temperature was lower than 33 °C, or when rats lost more than 25% of their initial body weight.

2.2. Drugs

The non-selective dopaminergic D1/D2 receptor antagonist *cis*-flupenthixol (Sigma-Aldrich, Zwijndrecht, The Netherlands) was dissolved in sterile isotonic saline and was chronically infused (continuous for 7 days, 12 μl/day, s.c.) with osmotic pumps (Alzet model 1007D, DURECT, Cupertino California). A dose–response curve was determined using doses ranging from 0.03–1.0 mg/day. Experimental food-restricted groups treated with different dose of *cis*-flupenthixol are displayed as follows; saline, FLU 0.03, FLU 0.1, FLU 0.25, and FLU 1.0. Each experimental group consisted of 8 animals.

2.3. Surgical procedures

One week after arrival, all rats (n = 56) received transmitters (TA10TA-F40 Data Sciences International, St. Paul, Minnesota) in the abdominal cavity under fentanyl/fluanisone (0.1 ml/100 g body weight, IM; Hypnorm, Janssen Pharmaceutica, Beerse, Belgium) and midazolam (0.05 ml/100 g body weight, IP; Dormicum, Hoffman-LaRoche, Mijdrecht, The Netherlands) anesthesia. After surgery, rats were treated with carprofen (0.01 ml/100 g body weight, s.c.; Rimadyl, Pfizer Animal Health, Capelle a/d Ijssel, The Netherlands) and saline (3 ml, s.c.) and allowed to recover for two weeks. After recovery, rats were anesthetized by isoflurane, and osmotic minipumps containing vehicle or *cis*-flupenthixol were placed s.c. into the flank region of the rat after overnight incubation in saline at 37 °C.

2.4. Experimental set-up

Animals were individually housed in cages with running wheels for a training period of ten days (from day-10 to day 0). Running wheel