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Tamoxifen produces conditioned taste avoidance in male rats: An analysis of microstructural licking patterns and taste reactivity

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ABSTRACT

Estrogen receptor activation has been shown to reduce body weight and produce a conditioned reduction in food intake in male rats that is putatively mediated by estradiol's suggested aversive effects. Evidence has shown that the selective estrogen receptor modulator tamoxifen used in the prevention and treatment of breast cancer may also produce changes in food intake and body weight, which are known to impact cancer development and survival. The purpose of the present study was to examine whether tamoxifen produces a conditioned reduction in intake similar to estradiol by producing a conditioned aversion. A one bottle lickometer test was used to examine conditioned changes in sucrose drinking, while the taste reactivity test was used to measure rejection reactions, which serve to index aversion in rats. A backward conditioning procedure that consisted of 3 conditioning days and one vehicle test day was used to examine conditioned changes in 0.3 M sucrose intake and taste reactivity. Our results show that tamoxifen produced a conditioned reduction in sucrose drinking in a one bottle fluid intake test that was similar to the effects produced by estradiol (positive control); however, no active rejection reactions were produced by either tamoxifen (1 and 10 mg/kg) or estradiol. The present results suggest that tamoxifen, at the doses used in the present study, acts as an estrogen receptor agonist to regulate food intake and that the conditioned reduction in intake produced by tamoxifen and estradiol reflects conditioned taste avoidance rather than conditioned taste aversion.

Introduction

Selective estrogen receptor modulators (SERMs) are a unique class of drugs that act as both estrogen receptor agonists and antagonists depending on the target tissue (Jordan, 2006). For example, the SERM tamoxifen has been shown to block the growth factor effects of estrogens on breast cancer cells (Forrest, 1971) while mimicking the effects of estrogens in the uterus (Gielen et al., 2008; Pole et al., 2005). Tamoxifen's anti-estrogenic effect in breast tissue makes it a useful therapeutic tool in both the prevention and treatment of breast cancer. However, due to the mixed agonist/antagonist effects, tamoxifen can produce a variety of effects throughout the body that involve both blocking and mimicking the effects of estrogens (see Fitzpatrick, 1999, for a review). For example, tamoxifen use has been associated with hot flashes, sweating, insomnia, anxiety and sexual dysfunction (Mourits et al., 2001). Even though tamoxifen is an effective pharmacological treatment option for male and female breast cancer patients, tamoxifen's effects outside of breast tissue are important as they may impact the clinical use of tamoxifen. For example, estrogen receptor activation has been shown to regulate food intake and body weight (Geary, 2004), which are two factors that can impact cancer development and survival (Camoriano et al., 1990; Eliassen et al., 2006; Enger et al., 2004; van den Brandt et al., 2000). It is, therefore, of interest to examine the effects of tamoxifen on food intake and body weight.

Results of a variety of animal studies have shown that estrogens act in an unconditioned manner to reduce both the amount of food consumed, as well as body weight, in both male and female rats and mice (Asarian and Geary, 2006; Eckel, 2004; Eckel and Geary, 2001; Geary et al., 2001). The anorexic effects of estrogens have been suggested to be mediated by both peripheral and central actions (Butera and Beikirch, 1989; Gandelman, 1983). Some of the brain areas implicated in mediating the anorexic effects of estrogens include portions of the hypothalamus such as the paraventricular hypothalamus (Butera and Beikirch, 1989), ventromedial hypothalamus (Nunez et al., 1980), medial preoptic area (Dagnault and Richard, 1997) and arcuate nucleus (Clegg et al., 2007).

There have been several clinical investigations that have reported limited or mixed results on the effects of tamoxifen on body weight. Some previous studies have reported that tamoxifen may increase weight gain, while others have reported no effect in female breast cancer patients (Demark-Wahnefried et al., 2001; Kumar et al., 1997;

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Rohatgi et al., 2002). To date, no reports on the effects of tamoxifen on body weight in male patients have been made available. Animal studies that have examined both food intake and body weight have also yielded conflicting evidence. Tamoxifen has been shown to reduce food intake and body weight in ovariectomized (OVX) female rats (Wade and Heller, 1993; Wallen et al., 2001). Tamoxifen was, however, reported to antagonize estradiol's suppressive effects on body weight in OVX Syrian hamsters (Wade and Powers, 1993). In orchidectomized male rats tamoxifen has been shown to inhibit body weight gain compared to controls (Fitts et al., 2004). Changes in food intake following tamoxifen administration have not been reported in males, to date.

In addition to estrogens' unconditioned regulation of food intake, the results of previous studies have also shown that estrogens produce conditioned changes in ingestion in both males and females as indexed by a conditioned taste avoidance. When given access to a sucrose solution that has been previously paired with estradiol administration, rats, mice, Mongolian gerbils, and humans will avoid consuming the estradiol-paired tastant during subsequent presentations (De Beun et al., 1991; Flanagan-Cato et al., 2001; Ganesan and Simpkins, 1990; Ganesan and Simpkins, 1991; Ganesan, 1994; Miele et al., 1988; Ossenkopp et al., 1996; Young et al., 1989). When examining the conditioned effects of tamoxifen on food intake in OVX female rats, Fudge et al. (2009) reported that tamoxifen produced a conditioned reduction in sucrose drinking comparable to the reduction in intake produced by estradiol. These findings suggest that tamoxifen acts as an estrogen receptor agonist to produce conditioned changes in ingestion in OVX female rats. To date, there has been only one report on the conditioned effects of tamoxifen on food intake in males, however, no significant effects were found (Lopez et al., 2006).

It has been suggested that the conditioned reduction in food intake produced by estradiol may be due to aversive effects, possibly nausea, and thus has been historically referred to as conditioned taste aversion (Bernstein et al., 1986; De Beun et al., 1991; Gustavson et al., 1989). In humans, increased levels of estradiol have been reported to produce aversive effects such as nausea and vomiting (Goodman and Gilman, 1975; Young et al., 1989). Additional evidence for the aversive effects of estradiol has come from studies that examined the role of the area postrema (AP) in mediating estradiol-induced conditioned reductions in intake. The AP is a brain stem structure that is suggested to serve as a toxin-sensitive region that mediates nausea (Borison and Wang, 1953; Ossenkopp and Eckel, 1995). Bernstein et al. (1986) reported that AP lesions abolish estradiol-induced (0.9 mg/ml minipump infusion) conditioned reductions in food intake in male rats, thus further supporting the hypothesis that estradiol-induced illness mediates the observed conditioned reductions in ingestion. Other evidence that estradiol produces an aversive effect, at least in male rats, comes from place learning studies that have shown that estradiol produces conditioned place avoidance in males (De Beun et al., 1991).

The purpose of the present study was to examine and compare the effects of tamoxifen to the effects of estradiol on sucrose drinking patterns, gustatory conditioning (measured as conditioned taste avoidance) and body weight gain. As well, the present study examined whether aversive effects are a mediating factor in the effects of tamoxifen and estradiol on food intake in male rats. A lickometer apparatus was used to quantify the volume of fluid intake, as well as to examine several ingestive behaviors related to licking patterns, to measure conditioned taste avoidance and putative underlying mechanisms. Changes in anxiety related behaviors and locomotor activity, which could potentially influence intake measures in rats, were also examined. The taste reactivity test was used to index active oral rejection reactions in order to determine whether any observed conditioned reductions in intake, produced by tamoxifen and/or estradiol in the lickometer apparatus, are true taste aversions (conditioned rejection responses) or only conditioned taste avoidances (see Parker 2003).

Materials and methods

Animals

Naive male Long Evans rats (Charles River, St Constant, Quebec), weighing between 230–310 g at the beginning of the experiment, were pair housed in polypropylene cages (45×22×20 cm). Rats had continuous access to food (Prolab) and water throughout the entire experiment, unless otherwise stated. The colony room was kept on a 12:12 h-light/dark cycle (lights on at 0700 h) at a room temperature of 21±1 °C. All experimental procedures occurred during the light phase (0900–1630 h) and were approved by the institutional Animal Care Committee and carried out according to the guidelines set out by the Canadian Council on Animal Care.

Drugs and groups

Tamoxifen citrate (Sigma-Aldrich, Oakville, ON, Canada) was dissolved in 10% ethanol/90% saline and intraperitoneally (IP) injected (Young et al., 2001) at a dose of 1 and 10 mg/kg and a volume of 2 ml/kg. The doses of tamoxifen were chosen on the basis of the results of prior studies that examined food intake, body weight and anxiety (Gray et al., 1993; Walf and Frye, 2005). It is important to note that the 1 mg/kg dose of tamoxifen used in the present study is calculated to be just slightly below the equivalent dose that is prescribed to patients (20 mg) based on surface area, mg/m^2. Human equivalent doses were calculated using a standard conversion formula provided by the FDA (www.fda.gov/cder/cancer/animalframe.htm). Vehicle control injections (10% ethanol/90% saline, vehicle) were administered at a volume of 2 ml/kg, IP. The positive control 17β-Estradiol (estradiol, Sigma-Aldrich) was dissolved in 10% ethanol/90% saline and injected subcutaneously (SC) at a dose of 50 μg/kg and a volume of 1 ml/kg. The 50 μg/kg dose of estradiol was chosen based on previous evidence that demonstrated that this dose effectively produces conditioned taste avoidance in male rats (De Beun et al., 1991), as well as previous studies that suggested that pharmacological doses of estrogens are needed to produce conditioned taste avoidance (Ganesan and Simpkins, 1990; Ossenkopp et al., 1996; Roesch, 2006). Even though different routes of administration were used to inject tamoxifen (IP) and estradiol (SC), differential pharmacokinetics are unlikely as both compounds are lipid soluble. It has previously been shown that the release of fat soluble compounds does not differ if administered IP or SC (Goodman and Gilman, 1975).

Experiment 1: Sucrose intake and microstructure of lick patterns

Lickometer test chamber

The lickometer test chamber consisted of a clear Plexiglas box (31×41×31 cm) with a graduated glass cylinder and metal spout mounted on the front of the chamber. The end of the spout was mounted 8 cm above the chamber floor. The lickometer allowed for a microstructural analysis of drinking behavior by collecting the frequency and temporal correlates of licks on the spout (Davis and Smith, 1992). To record drinking measures, a non-invasive current (~60 nA) was passed through the drinking spout. When the animal's tongue contacted the spout, the circuit was completed and recorded by the Contact 108 lick analysis system (Dilog Instruments, Tallahassee, FL). The volume of sucrose solution consumed was quantified by reading fluid levels in the graduated glass cylinder. Custom made programs (Baird et al., 1999; Kaplan et al., 2001) were used to generate ingestive behavior variables from the frequency and temporal features of the licks. The microstructural variables of drinking that were analyzed are the number of licks (total number of tongue contacts