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Flutamide treatment induces anxiolytic-like behavior in adult castrated rats

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Abstract:
Background: It has previously been speculated that the androgen receptor antagonist flutamide produces behavioral effects that are not mediated by androgen receptors. These earlier studies were performed in intact rodents and thus, flutamide may have interfered with endogenous testosterone produced by the testes. The main objective of the present study was to examine whether flutamide induces anxiolytic-like behavior in castrated rats.
Methods: Male Wistar rats (8–9 weeks old) were castrated and thereafter, in the same operation, the rats received silastic capsules subcutaneously (*sc*) that were filled with dihydrotestosterone (DHT) or were left empty. Three weeks later, rats were *sc* administered flutamide 50 mg/kg/day or vehicle for seven days. Four hours after the last injection, anxiolytic-like behavior was studied in a modified Vogel's drinking conflict model. In a separate experiment, shock threshold and drinking motivation were estimated.
Results: Flutamide induced anxiolytic-like behavior in castrated rats irrespective of administration of DHT. Treatment with DHT alone did not induce a significant behavioral effect. Shock threshold and drinking motivation were not affected by flutamide and/or DHT treatment.
Conclusions: This study demonstrates that flutamide induces anxiolytic-like behavior in a modified Vogel's conflict model in castrated rats, which indicates that flutamide has anxiolytic-like properties that are not dependent on testes-produced testosterone.

Key words:
dihydrotestosterone, brain, testosterone, androgen receptor, flutamide, anxiety, behavioral disinhibition, conflict behavior

Introduction

Testosterone produces anxiolytic-like behavior in rodents in several models of anxiety [2, 4, 6, 17, 20, 33–36]. The mechanisms by which testosterone produces anxiolytic-like behavior are not well known. Testosterone may either be converted to dihydrotestosterone by 5α-reductase or aromatized to estradiol by the enzyme aromatase [28]. Estradiol stimulates estradiol receptor α and β [31] and produces anxiolytic-like behavior in rats [23, 25, 39]. The non-aromatizable androgen receptor agonist dihydrotestosterone also exerts anxiolytic-like properties [14–16, 19]. Moreover, dihydrotestosterone can be metabolized by the enzyme 3α-hydroxysteroid dehydrogenase to 3α-diol (5α-androstane-3α,17β-diol), which induces anxiolytic-like behavior [18]. The non-steroidal androgen receptor antagonist flutamide, antagonizes the anxiolytic-like effect of dihydrotestosterone [14] and testosterone [17] in castrated rats, indicating that androgen receptors may be involved in the underlying mechanisms. Flutamide did not display any behavioral effect *per se* in castrated rats in these studies.

Other studies on intact rodents have shown that flutamide administration induces a different behavioral response. Systemically administered flutamide induced anxiolytic-like behavior (behavioral disinhibition) in a modified Vogel's drinking conflict model in intact male rats [33], and flutamide exerted anticonvulsant effects in intact male mice [1]. The latter effect was blocked by the benzodiazepine receptor antagonist flumazenil [1]. The mechanisms underlying these anxiolytic-like and anticonvulsant effects can only be hypothesized upon. Testosterone metabolites may be involved in intact rats since it has been demonstrated that flutamide treatment increases serum levels of estradiol and dihydrotestosterone in intact rats [26]. As mentioned, estradiol and the dihydrotestosterone metabolite 3α-diol exert anxiolytic-like effects and 3α-diol has anticonvulsant properties [29]. Another possibility, also possible in castrated rats, is that flutamide interacts with receptors other than androgen receptors. Indeed, flutamide has been found to displace [^{3}H]Ro 5-4864 from translocator protein (TSPO), previously known as the peripheral-type benzodiazepine receptor [7].

The primary objective of this study was to investigate whether flutamide treatment of castrated rats induces anxiolytic-like behavior in a modified Vogel's conflict model. A second objective was to examine whether dihydrotestosterone administration to castrated rats produces anxiolytic-like effects in this model. A third objective was to examine if the postulated anxiolytic-like effect of flutamide was displayed also in castrated rats treated with dihydrotestosterone.

Materials and Methods

Animals

A total of 74 male Wistar rats (Taconic, Ry, Denmark) weighing 230–310 g. (8–9 weeks old) were used. The animals were kept in controlled light-dark conditions (light on at 7:00 a.m. and off at 7:00 p.m.) and at constant cage temperature (20°C) and cage humidity (40–50%). The animals were adapted to the animal maintenance facilities for at least seven days prior to the start of the experiments. The rats, housed four to each cage (55 × 35 × 20 cm), had free access to water and food (rat standard feed, Harlan Teklad Europe, UK) except during experiments. All experiments were approved by the Ethics Committee for Animal Experiments, Gothenburg, Sweden and conducted in a manner complying with the European Community guidelines for the use of experimental animals.

Chemicals and drugs

Flutamide (Sigma, St. Louis, MO, USA) was dissolved in 10% ethanol in propylene glycol (Sigma, St. Louis, MO, USA) in a similar manner as described earlier [13]. Isoflurane (Baxter, Apoteksbolaget AB, Sweden) was used as an anesthetic. Silastic capsules (effective length: 40 mm, inner diameter: 1.57 mm, SIKEMA) were filled with crystalline dihydrotestosterone (4-androsten-17β-ol-3-one, Sigma, cat. number A-8380, St. Louis, MO, USA).

Castration and capsule implantation

Silastic capsules were filled with crystalline dihydrotestosterone (see "Drugs" above) or were left empty. Capsules were incubated in 0.9% NaCl for 24 h. They were washed first in 70% ethanol for 30 min and then in saline for 30 min prior to implantation. The rats were anesthetized with isoflurane (3.5–4.0% in air) and were castrated or sham-operated as follows. Scrotal incisions were performed, and the main arteries and veins as well as the ductus deferens were located and ligated, after which the testes were removed. Sham-operated rats were exposed to similar scrotal incisions. Thereafter, in the same operation, one silastic capsule containing dihydrotestosterone or one empty capsule was implanted subcutaneously (*sc*) in the back. Behavioral experiments were initiated four weeks after operation to ensure the lack of endogenous androgens from the testes.

Shock-induced behavioral inhibition

In order to study shock-induced behavioral inhibition, a modified Vogel's drinking conflict model was used. On the first day of the experiment, the animals were adapted for 20 min to a Plexiglas box (inner dimensions 30 × 24 × 20 cm) enclosed in a soundproof cage. The box was equipped with a grid floor of stainless steel bars and a drinking bottle containing a 5.5% (w/v) glucose solution. A 24-h period of water deprivation then followed in their home cages. Thereafter,