Effect of α,α,α -trifluoro-2-methyl-4'-nitro-*m*-lactotoluidide (Sch 16423) on serum testosterone and LH in rats

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Summary. Administration of Sch 16423 to intact male rats (5 mg/day s.c. for 7 days) resulted in a rapid and dramatic rise in the serum concentrations of LH and testosterone. This suggests that this metabolite of flutamide is the active antiandrogenic agent.

Introduction

Flutamide (α,α,α -trifluoro-2-methyl-4'-nitro-*m*-propionotoluidide) is a potent non-steroidal antiandrogen which inhibits exogenous and endogenous androgenic effects at peripheral (Neri, Florance, Koziol, & Van Cleave, 1972) and central nervous (Clark & Nowell, 1980) target tissues. In man flutamide is rapidly and extensively metabolized to a related compound, α,α,α -trifluoro-2-methyl-4'-nitro-*m*-lactotoluidide (Sch 16423), by hydroxylation of the side chain (Katchen & Buxbaum, 1975), and Neri (1977) has suggested that flutamide may exert its antiandrogenic effect by means of its conversion *in vivo* to an active metabolite.

In view of the increasing usage of flutamide for examination of biochemical pathways we investigated whether the Sch 16423 metabolite exhibits central nervous antagonist activity.

Materials and Methods

Experiment 1. A total of 78 adult males of the Sprague–Dawley (CFY) strain of rats was used. On Day 0, 6 males were decapitated and blood was collected. The remaining animals were divided into 3 treatment groups: those in Group 1 were injected s.c. with 5 mg Sch 16423 (donated by Schering Co., Bloomfield, New Jersey, U.S.A.) suspended in 0.2 ml arachis oil every day for 7 days; those in Group 2 were injected s.c. with vehicle alone; and those in Group 3 were left untreated. On Days 1, 3, 5 and 7 of injection, 6 animals from each treatment group were decapitated and serum samples were prepared. Serum LH was measured by the double-antibody technique (Niswender, Midgley, Monroe & Reichert, 1968) using materials supplied by the NIAMDD, Bethesda. All samples were measured in duplicate within the same assay with an intra-assay variance of 7.7%. The standard was NIH-rat LH-RP1. Testosterone was assayed (Clark & Nowell, 1979) employing an antiserum raised in rabbits against testosterone-7 α -BSA (Miles Laboratories Ltd). The minimum detectable dose was 0.0025 ng/tube with an intra-assay variance of 5.1% and inter-assay variance of 12.9%. There was a 40% cross-reaction in the assay with 5 α -dihydrotestosterone (<0.05% with Sch 16423) but there was no significant difference between values obtained before and after column chromatography and this step was therefore omitted. Other steroids tested for cross-reactivity with this antibody were 5 α -

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androstane-3 α ,17 β -diol (3%), androstenedione (0.7%), dehydroepiandrosterone (0.5%), progesterone (<0.1%), cortisol (<0.1%) and oestradiol-17 β (<0.1%).

Experiment 2. Rats were orchidectomized 4 days before use: those in Group A (N = 6) were injected s.c. with Sch 16423 (5 mg/rat/day), while those in Group B (N = 6) were injected s.c. with 0.2 ml arachis oil/rat/day. Following treatment animals were decapitated and serum testosterone concentrations were measured.

The significance of differences between means was tested by Student's *t* test.

Results

Experiment 1

As shown in Text-fig. 1, serum levels of LH and testosterone were markedly elevated by Day 1 in Group 1 rats compared with those of rats in Group 2 (LH, $P = <0.05$; testosterone, $P = <0.001$), and values remained high for the duration of the experiment. There was a significant difference in LH values in rats in Groups 2 and 3 on Days 5 ($P < 0.05$) and 7 ($P < 0.05$) probably due to a 'stress' effect of injection.

Text-fig. 1. Mean $\pm$ s.e.m. (6 rats/group) serum concentrations of (a) testosterone and (b) LH in rats treated for 7 days with 5 mg Sch 16423/day (Group 1) or 0.2 ml arachis oil/day (Group 2). Group 3 rats were untreated.

Experiment 2

There was no significant difference ($P > 0.05$) in the serum levels of testosterone in the rats in Groups A (0.157 ± 0.03 pg/ml) and B (0.116 ± 0.02 pg/ml).

Discussion

The rapid and substantial increase in the serum concentrations of LH and testosterone after treatment with Sch 16423 demonstrates a potent central nervous antiandrogenic action of this major metabolite of flutamide. The antiandrogenic action of Sch 16423 is due to its ability to compete with androgens for binding sites on the cytoplasmic receptor (Neri, 1977). Hence the observed effect of Sch 16423 reported in this study is presumably by competitive inhibition with androgens at hypothalamic and/or pituitary receptor sites involved in the control of gonadotrophin secretion.

The potent central nervous antiandrogenic action of Sch 16423 makes this an ideal compound for use in future brain-steroid investigations.

References

- Clark, C.R. & Nowell, N.W. (1979) BOMT (6 α -bromo-17 β -hydroxy-17 α -methyl-4-oxa-5 α -androstan-3-one) is not an androgen antagonist within the central nervous system. *Steroids* **34**, 139–149.
- Clark, C.R. & Nowell, N.W. (1980) The effect of the non-steroidal antiandrogen flutamide on neural receptor binding of testosterone and intermale aggressive behaviour of mice. *Psychoneuroendocrinology* **5**, 39–45.
- Katchen, B. & Buxbaum, S. (1975) Disposition of a new, nonsteroid antiandrogen α,α,α -trifluoro-2-methyl-4'-nitro-m-propionoluidide (flutamide) in men following a single oral 200 mg dose. *J. clin. Endocr.* **41**, 373–379.
- Neri, R.O. (1977) Studies on the biology and mechanism of action of non-steroidal antiandrogens. In *Androgens and Antiandrogens*, pp. 179–190. Eds L. Martini & M. Motta. Raven Press, New York.
- Neri, R.O., Florance, K., Koziol, P. & Van Cleave, S. (1972) A biological profile of a nonsteroidal antiandrogen Sch 13521 (4'-nitro-3'-trifluor-methylisobutyranillide). *Endocrinology* **91**, 427–437.
- Niswender, G.D., Midgley, A.R., Monroe, S.E. & Reichert, L.E. (1968) Radioimmunoassay for rat luteinizing hormone with antiovine LH serum and ovine LH-137 I. *Proc. Soc. exp. Biol. Med.* **128**, 807–814.

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