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# RAPID COMMUNICATIONS

## IMIDAZOLE ANTIFUNGAL AGENTS REDUCE PRODUCTION OF 17β-OESTRADIOL BY RAT OVARIES IN VITRO

F. Latrille[1], C. Charuel[2], A.M. Monro[2*], J. Stadler[2] and B.Ch.J. Sutter[1]

[1] Université de Bordeaux I, Département d'Endocrinologie, UER de Biologie, Avenue des Facultés, 33405 Talence Cedex, France.

[2] Laboratoires Pfizer, Centre de Recherche, 37401 Amboise Cedex, France.

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### *Introduction*

Imidazole antifungal agents such as econazole, miconazole and tioconazole, when administered orally to pregnant rats from day 15 post-insemination (p.i.) delay the onset of parturition and cause a difficult labour (1, 2, 3). When tioconazole is administered from day 18 p.i., the onset of parturition is advanced. We have recently shown (4) that for tioconazole these perturbations of parturition may be caused by a modification of the circulating levels of 17β-oestradiol (ED) and progesterone.

Seeking an explanation of these effects we have examined whether tioconazole could affect directly the production of ED by the rat ovary. The imidazole antifungal agents, as a class, inhibit a variety of P-450 mediated reactions, including those involved in steroid synthesis (5, 6, 7). Indeed, their inhibition of the conversion of lanosterol to ergosterol is the basis of their antifungal action (2, 8, 9). Ketoconazole lowers circulating ED levels in humans (10) and after oral administration of ketoconazole to cycling rats it was found that the ovarian content of ED was reduced (11). Very recently, it was reported (12) that ketoconazole-treated rats showed reduced activities of ovarian microsomal 17,20-lyase, 17-ketoreductase and aromatase, three enzymes involved in the conversion of progesterone to oestrogen. Furthermore, Mason *et al.* have already shown that some imidazoles inhibit a semi-purified form of aromatase derived from human placental tissue (13). Due to the subtle manner in which the roles of the key hormones change in the late stages of pregnancy (14), and the critical relationship between the day of tioconazole administration and the subsequent effects on parturition in rats (4), we have measured production of ED *in vitro* from ovaries taken from rats on days 21 p.i. i.e. just prior to parturition. The effects of tioconazole on this ED production were compared with those of miconazole and ketoconazole.

### *Methods*

*Animals.* Female rats (Crl:COBS-CD(SD)BR), weights 180-200 g, were caged overnight with males of the same strain. The day on which vaginal plugs were detected was designated day 0 p.i. The environmental conditions were as described previously (4). There were 12 controls and 6 per concentration group of each drug.

* *For correspondence.*

*Incubation of ovaries.* Females were sacrificed by decapitation between 0900 and 1000 of day 21 p.i. Ovaries were removed, fat trimmed, and the whole organ including the corpora lutea cut into small pieces (*ca.* 1 $mm^3$). The tissue from individual ovaries was incubated in siliconized pots (10 ml) containing 2 ml of KRBA buffer (a Krebs-Ringer-Bicarbonate buffer containing 2% of bovine serum albumin and 0.1% of glucose) with or without test compound. Incubation was carried out in an agitating waterbath at 37°C (110 cycles/min.) for 4 consecutive periods of 1 hr each. Every hour the medium was removed and replaced with 2 ml of fresh solution. All samples were immediately frozen and stored at -20°C. Control ovaries were incubated in KRBA buffer containing the same final volume of absolute alcohol as that used for test compounds (0.5% of absolute alcohol).

*Compounds.* Tioconazole was obtained from Pfizer Central Research, Sandwich, Kent, U.K. and miconazole and ketoconazole from Janssen Pharmaceutica Research Laboratories, Beerse, Belgium. They were dissolved in 1 ml of absolute alcohol, then diluted with the KRBA buffer to final concentrations of 0.3, 3 and 30 µM in the incubation medium.

*Radioimmunoassay.* The ED concentration was measured by RIA using iodine-125 based kits from ORIS Industries (Saclay, France) according to the method of Abraham (15). Fifty µl samples of freshly thawed incubation media were analysed in duplicate; the concentration was determined by reference to a calibration curve established using 25 pg to 1,000 pg/ml of ED.

*Statistical analysis.* Data are presented as means (± standard errors of the means); statistical significance was tested with Student's t-test.

***Results and Discussion***

In control incubations (Fig 1) ED was produced at a rate of 1,070 pg/ml/hr. The constancy of the rate over 4 hr indicated that the synthesis and secretion of the steroid was stable under these experimental conditions.

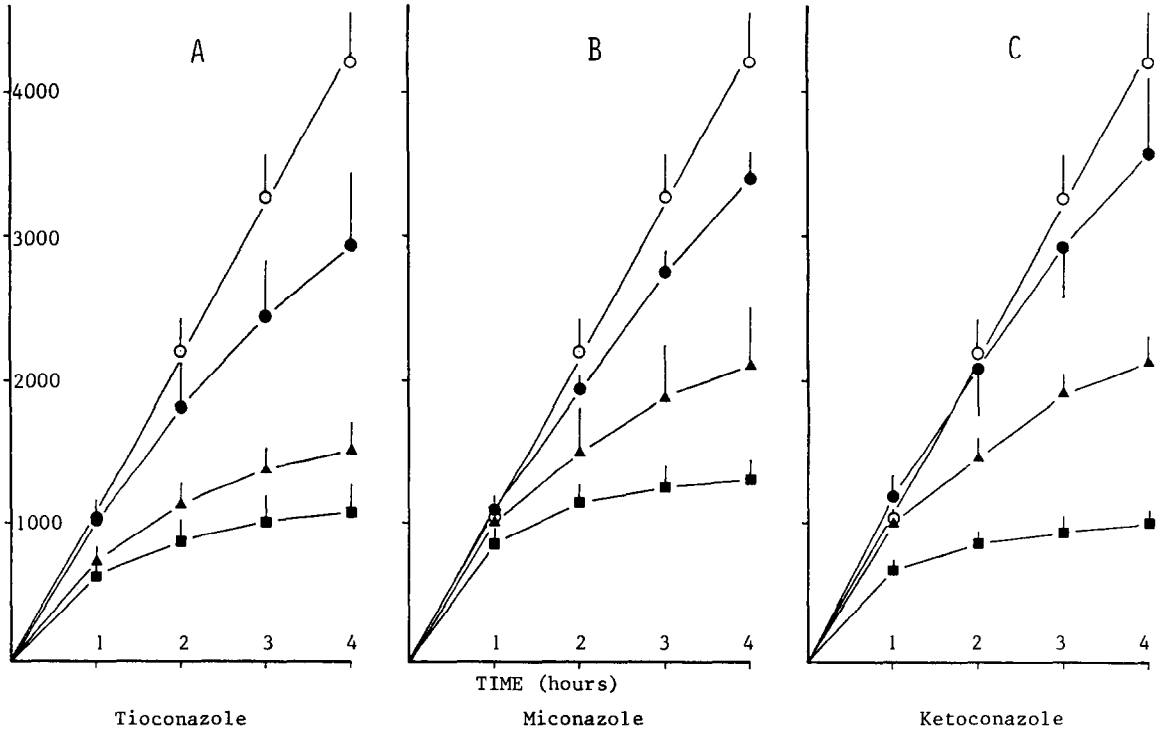


Figure 1 : 17β-oestradiol production (pg/ml) by ovaries from pregnant rats over 4 hours
Values are means ± S.E.M. for controls (N = 12) and test compounds (N = 6/concentration)
Key : o——o : Control ; ●——● : 0.3 µM ; ▲——▲ : 3 µM ; ■——■ : 30 µM

In the presence of the azole antifungals the rate of ED production was reduced in a dose-dependent manner (Fig 1A, B, C). The effect was slow in onset, with at 1 hr the only values significantly different ($p<0.05$) from controls being tioconazole (3 and 30 μM) and ketoconazole (30 μM). Thereafter the decreased rate of ED production was clearly dose-dependent: at 2, 3 and 4 hr the values for ED secretion in the presence of each of the three products at 3 and 30 μM were, with one exception (miconazole at 3 μM) highly significantly different ($p<0.01$) from controls. At the highest concentration the near horizontal nature of the line between 3 and 4 hr indicates that inhibition was virtually complete.

The inhibitory potencies of the 3 compounds were similar. This contrasts with the findings of Mason *et al* (13), who found that micoconazole was 100× more potent than ketoconazole as an inhibitor of aromatase derived from human placenta. While this may result from a true inter-species difference in sensitivity of the aromatase enzyme, it is equally important to note the differences in experimental conditions. Those of Mason *et al* would allow test compounds direct access to the enzyme; ours would reflect the net effect of uptake of the test compound by the ovarian cell and transport to the site or sites of ED synthesis, inhibition of one or more of the several enzymes involved in ED synthesis and subsequent secretion of ED. The profile we observed, with an initial "lag" phase, may be due, at least in part, to this sequence of steps. The particular enzymes inhibited were not investigated, but it is reasonable to suppose that tioconazole and miconazole inhibit, as has been reported for ketoconazole (12, 16, 17), several of the processes involved in the ovarian synthesis of progesterone, androsterone, testosterone and, finally, ED.

In conclusion, the present results allow us to rationalise our previous observations (4) on the modifications to parturition in rats induced by tioconazole. The delay in the onset of parturition associated with decreased serum levels of ED, may be the result of a direct action of tioconazole on the ovarian production of ED.

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