Neuroendocrinological effects of ketoconazole in rats

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Abstract The effect of ketoconazole on steroid synthesis was studied in intact (sham-operated) and castrated male and ovariectomized female rats. Rats were given 25 mg/kg ketoconazole twice a day im for 5 days. The influence of ketoconazole was also investigated on hormone release altered by GnRH, estradiol and haloperidol. The following hormones were measured: serum LH, PRL, testosterone, corticosterone, 17-OH-progesterone, estradiol, and dopamine content of the tubero-infundibular area. Ketoconazole treatment resulted in a significant decrease of testosterone level (from 7.93 ± 1.99 to 3.83 ± 0.94 nmol/l), whereas LH, PRL, corticosterone and 17-OH-progesterone remained unchanged in the male rat. The effect of castration on LH level was reduced by ketoconazole in male (from 590 ± 35 to 390 ± 25 µg/l) and female rats (from 468 ± 22 to 346 ± 39 µg/l), but the GnRH-stimulated LH release in castrated and ovariectomized animals was unchanged. The suppressive action of estradiol on LH in ovariectomized rats was enhanced (from 160 ± 41 to 64.6 ± 12.9 µg/l), and its priming effect on PRL release was diminished by ketoconazole (from 598 ± 81 to 281 ± 66 µg/l). Ketoconazole failed to modify the tubero-infundibular dopamine content and haloperidol-induced PRL release. It can be assumed that in addition to its inhibitory role of steroid biosynthesis ketoconazole has an influence on central mechanisms underlying LH and PRL release.

Ketoconazole (KZ) is an antifungal agent with a marked effect on steroidogenesis in humans and in animals, inhibiting the enzyme activity of the cholesterol side-chain cleavage enzyme, 17-hydroxylase, 11-hydroxylase, and mostly the 17-20-desmolase activity (1-6). Schurmeyer & Nieschlag (7) suggested that a phenylated side chain of the imidazole molecule is responsible for the inhibition of testosterone biosynthesis. Some data show that the substance is able to reduce ovarian aromatase activity in the rat (8,9). Basal aldosterone and cortisol levels are not necessarily altered by KZ, presumably because the inhibitory process is partial, and can be unequivocally demonstrated when the synthesis of these steroids is stimulated (10-13). The androgen steroid production is more sensitive to KZ suppression, and with long-term treatment a 50 per cent decrease of testosterone level can be achieved (14,15).

According to Santen et al. (16) and Bhasin et al. (17) the low serum testosterone concentrations were not accompanied by the expected rate of increase in LH and FSH release. It was assumed that KZ might have a direct effect at the pituitary level, but this has not been confirmed on castrated male rats (17).

The present work was devoted to studying the actions of KZ on hypothalamic and pituitary mechanisms in the rats, and to investigating the suppressive effect of the drug on the stimulated LH release in vivo.

Materials and Methods

Animals and treatment protocol
Adult male and regularly cycling female rats of the Wistar strain were used. The animals weighed 300-320 g and 200-220 g, respectively, at the start of the experiment. The rate of gain in body weight was similar in each treatment group during the experiments. Six rats were housed in a standard size cage. Food pellets and water were available ad libitum. The animal room was temperature con-
trolled, and a 14:10 h light-dark cycle was employed with lights on at 06.00 h (rat-time).

Male rats were castrated (CAST) and female rats were ovarioctomized (OVX) under ether anesthesia one week prior to the beginning of the injection procedure. Rats of both sexes were injected im with 25 mg per kg ketoconazole (Richer, Budapest, Hungary) suspended in oleum helianthi. The injections were administered every 12 h for five days. intact (sham-operated) control rats received the identical volume of Oil. The following hormones and substances were injected: GnRH (LHRH, Ferring, FRG) 500 ng per rat ip 2 h prior to sacrifice; estradiol propionate (Akrofollin®, Richer, Budapest, Hungary) 25 µg per rat im on days 3 to 5 on the experiment. Haloperidol (Richer, Budapest, Hungary) was given at a dose of 0.5 mg im per rat 90 min before decapitation.

The following groups were investigated and treated. Male rats: 1. intact (sham-operated), placebo; 2. intact, KZ; 3. CAST, placebo; 4. CAST, KZ; 5. CAST, placebo + GnRH; 6. CAST, KZ + GnRH. Female rats: 1. OVX, placebo; 2. OVX, KZ, 3. OVX, placebo + GnRH; 4. OVX, KZ + GnRH; 5. OVX, placebo + estradiol; 6. OVX, KZ + estradiol; 7. OVX, placebo + haloperidol; 8. OVX, KZ + haloperidol.

The rats were sacrificed at 9.50 h by decapitation at the end of the experiments. The trunk blood was collected, centrifuged and stored at −20°C until assayed.

Analyses

Radioassay was used to determine serum corticosterone (18). Commercial RIA kits were used to determine serum testosterone (Serono, MAIA Clone), estradiol (Serono) and 17-OH-progesterone concentration (Institute of Isotopes, Hungarian Academy of Sciences, Budapest). Quality assurance methods revealed that in these kits the intra-assay variation coefficients ranged between 3.63 and 5.56%; and the inter-assay variation coefficients ranged between 7.25 and 11.45%. Rat prolactin and LH were determined with rat RIA technique (NIDDK). The intra-assay coefficients in these cases ranged between 3.73 and 4.92%, whereas the inter-assay variation coefficients were less than 10%.

Brains were removed, dissected and the dopamine content of the hypothalamus was assayed with the method of Chang (19).

ANOVA was used to evaluate data and was followed by unpaired t-test (20).

Results

Experiments on male rats

The effect of ketoconazole on steroid synthesis. Ketoconazole administration for 5 days resulted in a significant decrease of serum testosterone concentration (from 7.93 ± 1.19 to 3.83 ± 0.94 nmol/l; p<0.02). The change of the basal 17-OH-progesterone level (1.1 ± 0.37 nmol/l) was not statistically apparent. The serum corticosterone basal levels (85.5 ± 40.9 nmol/l) also failed to diminish. The serum testosterone concentration of castrated rats was as low as 0.23 ± 0.05 nmol/l at the time of sacrifice, and the postoperative corticosterone and 17-OH-progesterone values were also in the control range.

Ketoconazole administration and the stimulated LH release. Ketoconazole injections failed to increase LH levels in intact rats. However, the induced LH release caused by castration was significantly suppressed in the KZ-treated rats as compared with the corresponding controls. The effect of GnRH on pituitary LH release was tested in castrated animals: KZ failed to modify the LH release following GnRH injection (Table 1).

Ketoconazole administration and prolactin release. Ketoconazole administration did not influence serum prolactin levels, neither in sham-operated control or in castrated rats. However, GnRH injection resulted in a statistically significant but moderate increase in prolactin level both in placebo and in KZ-treated castrated rats (Table 1). These hormonal changes presumably lack any biological significance.

Changes in hypothalamic dopamine content following ketoconazole administration. Castration or KZ administration failed to result in any change of dopamine content in the tubero-infundibular area, as compared with the control placebo-treated rats. The dopamine values were as follows: control + placebo: 1.66 ± 0.03; control + KZ: 1.52 ± 0.05; castrated + placebo: 1.53 ± 0.03; castrated + KZ: 1.65 ± 0.07 ng/mg wet weight.

Experiments on female ovarioctomized rats

The effect of ketoconazole on the LH level. A significant reduction of the LH concentration was observed in KZ-treated rats. GnRH injection increased the LH levels, but KZ failed to modify this action (Table 2).

The action of estradiol and haloperidol on LH release of ketoconazole treated rats. Estradiol treatment resulted in a significant reduction of LH release, which was further reduced by KZ administration. The serum estradiol concentrations were measured in each group; obviously estradiol could be detected in Groups 5 and 6, only (see Table 2). The values were: 343 ± 76 and 373 ± 38 ng/l, respectively.
Table 1.
The influence of ketoconazole (KZ), 2×25 mg/kg for 5 days, modifying the effects of GnRH, 500 ng/rat, estradiol, 25 μg/rat, and haloperidol, 0.5 mg/rat, on the serum LH and PRL levels in intact and castrated (CAST) male rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of rats</th>
<th>Serum LH (mean±SEM) μg/l</th>
<th>Serum PRL (mean±SEM) μg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Intact/placebo</td>
<td>6</td>
<td>36.2±7.1</td>
<td>0.45±0.14</td>
</tr>
<tr>
<td>2. Intact/KZ</td>
<td>6</td>
<td>33.1±5.96</td>
<td>0.82±0.68</td>
</tr>
<tr>
<td>3. CAST/placebo</td>
<td>12</td>
<td>590±35</td>
<td>5.32±1.49</td>
</tr>
<tr>
<td>4. CAST/KZ</td>
<td>12</td>
<td>390±25</td>
<td>3.97±1.04</td>
</tr>
<tr>
<td>5. CAST/placebo+GnRH</td>
<td>6</td>
<td>711±89.1</td>
<td>24.9±7.36</td>
</tr>
<tr>
<td>6. CAST/KZ+GnRH</td>
<td>6</td>
<td>749.0±72.0</td>
<td>50.4±7.79</td>
</tr>
</tbody>
</table>

1 vs 3 = a, 2 vs 4 = a, 1 vs 5 = b, 2 vs 4 = c, 3 vs 4 = a, 4 vs 6 = a, 5 vs 6 = c

a = p < 0.001 b = p < 0.01 c = p < 0.05

Haloperidol administration also significantly diminished the LH concentration, and KZ failed to influence this action (Table 2).

The effect of ketoconazole on the prolactin level. There was no remarkable difference between prolactin levels of placebo- and/or KZ-treated rats. Moreover, GnRH injection had no further modifying effect on this hormone (Table 2).

The action of estradiol and haloperidol on prolactin release of ketoconazole-treated rats. Estradiol administration led to an increase in prolactin release both in placebo- and KZ-treated ovariectomized rats. However, this action of estradiol was significantly reduced in the KZ-treated group.

Haloperidol resulted in a marked increase in prolactin levels, but this effect could not be modified by KZ administration. The estradiol-induced prolactin release was significantly higher than that of the haloperidol-treated rats (Table 2).

Table 2.
Influence of ketoconazole (KZ), 2×25 mg/kg for 5 days, modifying the effects of GnRH, 500 ng/rat, estradiol (E2), 25 μg/rat, and haloperidol, 0.5 mg/rat, on the serum LH and PRL levels in ovariectomized (OVX) rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of rats</th>
<th>Serum LH (mean ± sem) μg/l</th>
<th>Serum PRL (mean ± sem) μg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Placebo</td>
<td>6</td>
<td>468±22</td>
<td>1.81±0.45</td>
</tr>
<tr>
<td>2. KZ</td>
<td>6</td>
<td>346±39</td>
<td>5.35±1.26</td>
</tr>
<tr>
<td>3. Placebo + GnRH</td>
<td>6</td>
<td>1190±124</td>
<td>8.14±1.68</td>
</tr>
<tr>
<td>4. KZ + GnRH</td>
<td>6</td>
<td>1106±105</td>
<td>8.54±1.04</td>
</tr>
<tr>
<td>5. Placebo + E2</td>
<td>6</td>
<td>160±41</td>
<td>598±81</td>
</tr>
<tr>
<td>6. KZ + E2</td>
<td>6</td>
<td>64.6±12.9</td>
<td>281±66</td>
</tr>
<tr>
<td>7. Placebo + haloperidol</td>
<td>6</td>
<td>347±28</td>
<td>206±19</td>
</tr>
<tr>
<td>8. KZ + haloperidol</td>
<td>6</td>
<td>346±49</td>
<td>212±32</td>
</tr>
</tbody>
</table>

1 vs 2 = c, 1 vs 3 = a, 1 vs 5 = d, 1 vs 3 = b, 1 vs 5 = a, 2 vs 6 = a, 2 vs 6 = a, 5 vs 6 = c, 1 vs 7 = a, 1 vs 7 = b, 5 vs 7 = a

a = p < 0.001 b = p < 0.01 c = p < 0.02 d = p < 0.05
Discussion

Male rats were used to avoid the hormonal variability of the ovarian cycle. The decrease of serum testosterone indicated that the administered dose of KZ was properly chosen (21). To alter 17-OH-progesterone and corticosterone levels, a higher dose would be required in rats, since Bhasin et al. (17) found a decrease of corticosterone following the administration 3 × 25 mg/rat per day.

No change of LH level was found in intact male rats after KZ injections, and this finding was in accordance with previous experiments (17,21). The question was raised how KZ influenced the stimulated LH release. The substance reduced the LH in castrated male and female rats. In ovariectomized animals KZ further increased the suppressive action of estradiol on the LH release. We found, however, that the action of GnRH on the pituitary was left unchanged. Therefore, it can be suggested that KZ modified the stimulated LH release at the hypothalamic level, although Biswanath et al. (22) reported that KZ exhibited a weak but competitive binding affinity for the pituitary GnRH receptor.

The role of the dopaminergic mechanism was also in question. We could not demonstrate a direct evidence that the role of the dopaminergic mechanisms are underlying the KZ effect, since it failed to influence the dopamine content of the tubero-infundibular region in male rats. Moreover, the haloperidol-induced prolactin release also could not be modified by KZ pretreatment.

Estradiol priming in itself significantly increased the prolactin content in the blood of ovariectomized rats (23), but this effect was diminished by KZ pretreatment. It is known that the estrogens exert a direct effect on lactotrope cells, influencing both the basal and the stimulated prolactin release.

There are several options for the explanation of the present findings. It can be suggested that KZ influences estrogen receptor sites in the hypothalamus and in the lactotrope cells. Loose et al. (24) reported that KZ antagonized glucocorticoid action on the hepatoma cell receptors. Moreover, a competition between KZ and estradiol and/or dihydrotestosterone on the sex hormone binding protein has been also demonstrated (25), however, it is rather a poor inhibitor of androgen binding to sex hormone binding globulin (26,27). Further experiments are required for direct evidences of binding and alteration of the estrogen receptor by the drug.

Obviously, the weak but competitive binding affinity of KZ to the pituitary GnRH receptors, in vitro, may also play a role in the phenomenon (22). The possibility also cannot be excluded that it may reduce the metabolic and renal clearance of estradiol, as it has been shown for prednisolone by Zürcher et al. (28).

Acknowledgments

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References


18. Murphy BEP. Some studies on the protein binding of steroids and their application to the routine micro and ultramicro measurement of various steroids in body fluids by competitive protein binding radioassay. J Clin Endocrinol Metab 1967;27:979-90.


